

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

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¹ Posted at www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing . ² See file labelled □chemicals-in-food-FOIA-Main.pdf□ for information on other GRNs.				



NATURAL RESOURCES DEFENSE COUNCIL

October 11, 2013

VIA FAX AND CERTIFIED MAIL

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

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

Dear FOIA Officer:

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

I. Description of Records Sought

Please produce 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
Natural Resources Defense Council, Inc.
1152 15th Street NW, Suite 300
Washington, DC 20005
202-513-6252
(202) 289-1060 FAX
tneltner@nrdc.org

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,

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



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
College Park, MD 20740

January 16, 2014

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

Re: FOI Request No. 2013-8042

Dear Mr. Neltner:

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

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

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

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

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

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

Sincerely Yours,

Sharon R. Dodson

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

Enclosure



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
College Park, MD 20740

March 19, 2014

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

Re: FOI Request No. 2013-8042

Dear Mr. Neltner:

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

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

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.

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

Signature required by Standard & Standard
204 4075, 4076, 4077, 4078, 4079, 4080, 4081, 4082, 4083, 4084, 4085, 4086, 4087, 4088, 4089, 4090, 4091, 4092, 4093, 4094, 4095, 4096, 4097, 4098, 4099, 4100, 4101, 4102, 4103, 4104, 4105, 4106, 4107, 4108, 4109, 4110, 4111, 4112, 4113, 4114, 4115, 4116, 4117, 4118, 4119, 4120, 4121, 4122, 4123, 4124, 4125, 4126, 4127, 4128, 4129, 4130, 4131, 4132, 4133, 4134, 4135, 4136, 4137, 4138, 4139, 4140, 4141, 4142, 4143, 4144, 4145, 4146, 4147, 4148, 4149, 4150, 4151, 4152, 4153, 4154, 4155, 4156, 4157, 4158, 4159, 4160, 4161, 4162, 4163, 4164, 4165, 4166, 4167, 4168, 4169, 4170, 4171, 4172, 4173, 4174, 4175, 4176, 4177, 4178, 4179, 4180, 4181, 4182, 4183, 4184, 4185, 4186, 4187, 4188, 4189, 4190, 4191, 4192, 4193, 4194, 4195, 4196, 4197, 4198, 4199, 4200, 4201, 4202, 4203, 4204, 4205, 4206, 4207, 4208, 4209, 4210, 4211, 4212, 4213, 4214, 4215, 4216, 4217, 4218, 4219, 4220, 4221, 4222, 4223, 4224, 4225, 4226, 4227, 4228, 4229, 4230, 4231, 4232, 4233, 4234, 4235, 4236, 4237, 4238, 4239, 4240, 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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



June 22, 2010

Stanley M. Tarka, Ph.D.
The Tarka Group Inc.
210 N. Old Stonehouse Road
Carlisle, PA 17015-8517

Re: GRAS Notice No. GRN 000340

Dear Dr. Tarka:

The Food and Drug Administration (FDA) has received the notice, dated May 10, 2010, that you submitted on behalf of Theocorp Holding Company, LLC (Theocorp) 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 12, 2010, filed it on May 17, 2010, and designated it as GRN No. 000340.

The subject of the notice is theobromine. The notice informs FDA of the view of Theocorp that theobromine is GRAS, through scientific procedures, for use as an ingredient in bread, ready-to-eat instant and regular oatmeal breakfast cereals, sports and isotonic drinks, vitamin and enhanced bottled waters, chewing gums, bottled tea, soy milk, gelatins, hard mint candy, non-chocolate meal replacement beverages, yogurt (non-chocolate and yogurt drinks), fruit smoothies, and powdered fruit flavored drinks.


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 Natalia.Weinsetel@fda.hhs.gov or 301-436-2907.

Sincerely yours,


Natalia Weinsetel, Ph.D.

Division of Biotechnology and
GRAS Notice Review
Center for Food Safety
and Applied Nutrition

(b) (5)





Weinsetel, Natalia

From: Weinsetel, Natalia
Sent: Tuesday, July 20, 2010 1:22 PM
To: 'tarkagroup@comcast.net'
Subject: RE: Theobromine GRAS Notification Submission

Dear Dr. Tarka,

The review team for GRN340 has completed their preliminary assessment, and the reviewers would like clarification on issues that they identified on the notice. I have provided the reviewers' questions for you below. So that we can complete our review and respond to your notice in a timely manner, we would appreciate receiving your responses to the reviewers' comments by close of business on Friday, August 6, 2010. If you will be unable to respond by this date, or if you have questions regarding the reviewers' comments, please feel free to contact me.

Best regards,
 Natalia

1. The notifier (Theocorp) mentions that: "TINOS LLC submitted a letter of intent to market theobromine as a New Dietary Ingredient and FDA filed this letter as an official filing on January 22, 2004." However, it also states that FDA issued a supplemental letter dated April 6, 1996. Please confirm if this is an error or explain the date discrepancy (page 20).
2. Please spell out the acronym PADI (page 21).
3. The percentage of unchanged theobromine (1-18%) found in the human urine as noted on page 36 is inconsistent with that presented in Table 5 (page 37).
4. In the calculations provided for total methylxanthine intake from cocoa powder on page 60, there appears to be some miscalculations (see below).
 - a. The notifier calculated that "cocoa powder provided 25.8 (mg/g) theobromine + 0.19 (mg/g) caffeine = 25.99 mg total methylxanthines/g cocoa powder". However, based on their previous statement that cocoa powder is comprised of 93% theobromine and 7% caffeine, the amount of caffeine would be 1.94 mg/g resulting in 27.74 mg total methylxanthines/g cocoa powder.
 - b. The total methylxanthine intake (mg/day) would be 21.64 mg/d instead of 20.27 mg/day.
 - c. That translates to 161.49 mg/kg/day (instead of 151 mg/kg/day) of methylxanthines in rats consuming 5% cocoa powder.

Please re-calculate and correct or explain how you derived your numbers.

- d. The notifier states that "The high dose level provided a mean methylxanthine intake of approximately 57 mg/kg bw/d for males and 74 mg/kg bw /d for females from wk 26-104 of the study. Again, the next paragraph provides different numbers for the same period. Moreover, it is not specified what 2.1g/kg bw/d and 2.7g/kg bw/d represent.

The description of the same study on pages 58, 60, and 78 are not identical.

5. Please explain how you derived that the lowest theobromine dose tested was approximately 300 mg/kg b.w. per day in the goat study? (Aregheore, 2002), page 70.
6. We were unable to find the NOAEL and LOAEL for young chickens and older broiler chickens and laying hens in the references you have provided. Please clarify.
7. We noticed a typographical error in the reference. Hostetler et al. is listed as 1991 on page 77 while the same reference is listed as 1990 in the subsequent paragraph. Please confirm which is the correct one.

8. The notifier states that the acceptable daily intake (ADI) of ~30 mg/person/day is significantly lower than the estimated daily intake (EDI) of 319 mg/person/day (90th percentile) from the intended uses of theobromine in the specified foods. The notifier discusses that the safety and exposure assessments of caffeine by Health Canada can be applied/considered to evaluate theobromine which is structurally similar to caffeine and is one of its metabolites. However, the notifier does not provide any comparative exposure estimate of theobromine derived from caffeine metabolism. Although the notifier notes that theobromine is consumed in large quantities by humans in various forms, the notifier does not clearly justify why the proposed 10x excess use on a daily basis can be considered as safe.
9. Using data from NHANES 2003-2006 and the food codes reported, FDA calculated that 261 mg/p/d of theobromine is consumed at the mean and 367 mg/p/d at the 90th percentile for users. This is twice as high as 150 mg/person/day at the mean and 319 mg/person/day at the 90th percentile that was reported on page 19. In addition, FDA calculated a higher number of users (89.6%) vs. the 65.1% found in the Cantox assessment report (page 8 of Appendix 1). Please explain this discrepancy.
10. The 90th percentile of consumption of powdered fruit-flavored drinks is nearly 11x larger than the reported mean on page 26. Typically, the amount consumed at the 90th percentile is 2x greater than the mean. Please explain this difference.
11. The description of the manufacturing process on page 15 states that sodium bicarbonate is added to 3-methylxanthine in acetone and water before the addition of dimethyl sulfate. On page 16, the figure and written description shows that 3-methylxanthine is converted to the potassium salt before the addition of dimethylsulfate. Are there two methods for preparation of theobromine, or is one method preferred over the other?

Natalia Weinsetel, Ph.D.
 Consumer Safety Officer
 Division of Biotechnology and GRAS Notice Review
 Office of Food Additive Safety
 Center for Food Safety and Applied Nutrition
 U.S. Food and Drug Administration
 Tel: (301) 436-2907/Fax: (301) 436-2965
 E-mail: Natalia.Weinsetel@fda.hhs.gov

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From: Weinsetel, Natalia
Sent: Tuesday, June 22, 2010 5:10 PM
To: 'Stanley M Tarka Jr, PhD'
Subject: RE: Theobromine GRAS Notification Submission

Hello Dr. Tarka,
Just wanted to let you know that I placed the acknowledgement letter for GRN340 on the mail for you this afternoon.
Best regards,
Natalia

Natalia Weinsetel, Ph.D.
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Tel: (301) 436-2907/Fax: (301) 436-2965
E-mail: Natalia.Weinsetel@fda.hhs.gov

From: Stanley M Tarka Jr, PhD [<mailto:tarkagroup@comcast.net>]
Sent: Friday, June 18, 2010 2:31 PM
To: Weinsetel, Natalia
Subject: RE: Theobromine GRAS Notification Submission

Dear Dr. Weinsetel,
Thank you very much for your quick response. I greatly appreciate this information and look forward to working with you. Please do not hesitate to contact me at any time with any questions that may arise during FDA's evaluation of this GRAS Notification.

Sincerely,

Stanley Tarka

Stanley M. Tarka, Jr., Ph.D.
President
The Tarka Group, Inc.
210 N. Old Stone House Road
Carlisle, PA 17015-8517
(Ph): 717-243-9216
(Fax): 702-993-5458
tarkagroup@comcast.net
website: www.tarkagroup.com

From: Weinsetel, Natalia [<mailto:Natalia.Weinsetel@fda.hhs.gov>]
Sent: Friday, June 18, 2010 9:54 AM
To: tarkagroup@comcast.net
Subject: RE: Theobromine GRAS Notification Submission

Dr. Tarka,
I am the CSO assigned to this notice. The acknowledgement letter is under internal review. You will be receiving a copy of it within a week or so.
Thank you,

Natalia Weinsetel, Ph.D.
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety

Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Tel: (301) 436-2907/Fax: (301) 436-2965
E-mail: Natalia.Weinsetel@fda.hhs.gov

From: Stanley M Tarka Jr, PhD [<mailto:tarkagroup@comcast.net>]
Sent: Thursday, June 17, 2010 2:25 PM
To: Martin, Robert L
Subject: RE: Theobromine GRAS Notification Submission

Dear Dr. Martin,
I am writing to follow-up with you regarding the Theobromine GRAS Notification that I submitted on behalf of Theocorp Holding Co and which was filed by FDA on May 17 as GRN 340. I had not received any official letter indicating that this was filed but do appreciate knowing that it has been filed based on FDA's website for pending GRAS Notices and is under review.

Could you please let me know who has been assigned as the official Consumer Safety Officer with the responsibility for this filing?

Thanks in advance.

Best regards,

Stanley Tarka

Stanley M. Tarka, Jr., Ph.D.
President
The Tarka Group, Inc.
210 N. Old Stone House Road
Carlisle, PA 17015-8517
(Ph): 717-243-9216
(Fax): 702-993-5458
tarkagroup@comcast.net
website: www.tarkagroup.com

From: Martin, Robert L [<mailto:Robert.Martin@fda.hhs.gov>]
Sent: Monday, May 17, 2010 7:20 AM
To: Stanley M Tarka Jr, PhD
Subject: RE: Confirmation of Receipt of Theobromine GRAS Notification Submission

Dr. Tarka, this is to confirm that we have received your submission. You can expect to hear from us soon.

Thanks.
Robert L. Martin
301-436-1219

From: Stanley M Tarka Jr, PhD [<mailto:tarkagroup@comcast.net>]
Sent: Friday, May 14, 2010 8:12 AM

To: Martin, Robert L; Gaynor, Paulette M
Subject: RE: Confirmation of Receipt of Theobromine GRAS Notification Submission

Dear Dr. Martin,

I am writing you to confirm that you did indeed receive the theobromine GRAS Notification submission that I sent by overnight delivery on behalf of Theocorp Holding Co, LLC. It was sent to your attention and delivered on Tuesday morning, May 10 and signed for by S. Johnson.

Thanks in advance and I look forward to hearing from you that the package was indeed received. After it is reviewed for completeness, I will look forward to the next step of in the process of receiving a written confirmation that is has been accepted for filing.

Sincerely,

Stanley Tarka

Stanley M. Tarka, Jr., Ph.D.

President

The Tarka Group, Inc.

210 N. Old Stone House Road

Carlisle, PA 17015-8517

(Ph): 717-243-9216

(Fax): 702-993-5458

tarkagroup@comcast.net

website: www.tarkagroup.com

From: Martin, Robert L [mailto:Robert.Martin@fda.hhs.gov]
Sent: Friday, March 19, 2010 6:48 AM
To: Stanley M Tarka Jr, PhD; Gaynor, Paulette M
Subject: RE: Request to Schedule Pre-GRAS Consultation Meeting

Dr. Tarka, 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: Stanley M Tarka Jr, PhD [mailto:tarkagroup@comcast.net]
Sent: Thursday, March 18, 2010 12:21 PM
To: Martin, Robert L
Subject: Request to Schedule Pre-GRAS Consultation Meeting
Importance: High

Dear Dr. Martin,

As a follow-up to the voice message I left you this morning, we are formally requesting on behalf of our client Theocorp Holding Company, LLC, a pre-GRAS consultation meeting with the FDA for a

proposed food additive, theobromine (3,7-dimethylxanthine). The intended use of theobromine is as a nutrient to improve dentition. We would like to request a one hour meeting with the FDA with the primary objective of providing a review of our completed safety assessment and conclusions regarding the safety of the intended uses of theobromine in specified foods prior to submitting a GRAS Notification.

Company Name:

Theocorp Holding Company, LLC
3512 8th Street
Metairie, LA 70002

Date Preferred by Requestor:

April 28-PM

Alternatively, April 27 or 29-PM.

NOTE: Due to travel logistics of participants, it would be greatly appreciated if this meeting could be scheduled in the early PM if at all possible.

Names of Theocorp Holding Company Attendees at Meeting:

Dr. Arman Sadeghpour, President & CEO, Joseph Fuselier, Dr. Tetsuo Nakamoto

Names of Consultant Attendees at Meeting:

Dr. Stanley M. Tarka, Jr., The Tarka Group, Inc. Carlisle, PA
Professor Joseph F. Borzelleca, Virginia Commonwealth University School of Medicine
Professor John A. Thomas, Indiana University School of Medicine

Name of substance:

Theobromine (3,7-dimethylxanthine)
IUPAC Nomenclature: 3,7-dimethyl-2,3,6,7-tetrahydro-1*H*-purine-2,6-dione
EINECS number: 201-494-2
CAS No: 83-67-0
FEMA Number 3591

Objectives of the Meeting:

The primary objective of the meeting is to review the safety assessment work completed on theobromine, and our conclusions regarding the safety of its intended use in specified foods prior to submitting a GRAS Notification. The secondary objective is to solicit FDA's comments and advice on this or any other matters that may need to be addressed.

Proposed Agenda

1. Brief introduction of Attendees-All

2. Overview of Briefing Information- Dr. Tarka
3. Power Point presentation overview of comprehensive safety evaluation of theobromine for its intended uses- Dr. Tarka
4. FDA comments and recommendations- FDA
5. Any other matters

Thank you for the opportunity to consult with the Agency and OFAS on this matter. I look forward to hearing from you regarding confirmation of a date and time for this meeting. Please feel free to e-mail at tarkagroup@comcast.net or call me at 717-243-9216 if you have any questions. I will be traveling from March 20-25 and will have limited email or telephone access.

Thanks in advance.

Sincerely,

Stanley Tarka

Stanley M. Tarka, Jr., Ph.D.
President
The Tarka Group, Inc.
210 N. Old Stone House Road
Carlisle, PA 17015-8517
(Ph): 717-243-9216
(Fax): 702-993-5458
tarkagroup@comcast.net
website: www.tarkagroup.com

AM



Weinsetel, Natalia

Subject: FW: Theobromine GRAS Notification Submission : RESPONSE TO REVIEWERS QUESTIONS ON GRN340

From: Stanley M Tarka Jr, PhD [mailto:tarkagroup@comcast.net]

Sent: Friday, July 30, 2010 11:31 AM

To: Weinsetel, Natalia; Gaynor, Paulette M

Subject: RE: Theobromine GRAS Notification Submission : RESPONSE TO REVIEWERS QUESTIONS ON GRN340

Importance: High

Dear Dr. Weinsetel:

Thank you very much for your email of July 20 indicating that the review team has completed their preliminary assessment of the GRAS Notification for theobromine and requesting clarification on issues that they identified in the notice. I apologize for the slight delay in responding but had been out of town. The reviewers have been very thorough and I have prepared responses to all of the questions below. I also spoke with Dr. Gaynor yesterday and she asked me to also copy her on this response so that she can send it on to the reviewers in your absence.

RESPONSE TO FDA REVIEWERS OF GRN340

1. The notifier (Theocorp) mentions that: "TINOS LLC submitted a letter of intent to market theobromine as a New Dietary Ingredient and FDA filed this letter as an official filing on January 22, 2004." However, it also states that FDA issued a supplemental letter dated April 6, 1996. Please confirm if this is an error or explain the date discrepancy (page 20).

RESPONSE: The reviewers are correct; this is an error and the correct date is January 22, 1996 not January 22, 2004.

2. Please spell out the acronym PADI (page 21).

RESPONSE: Possible average daily intake (PADI) is spelled out.

3. The percentage of unchanged theobromine (1-18%) found in the human urine as noted on page 36 is inconsistent with that presented in Table 5 (page 37).

RESPONSE: This is a typographical error and should be 10-18% on page 36 and not 1-18%. Table 5 is correct.

4. In the calculations provided for total methylxanthine intake from cocoa powder on page 60, there appears to be some miscalculations (see below).
 - a. The notifier calculated that "cocoa powder provided 25.8 (mg/g) theobromine + 0.19 (mg/g) caffeine = 25.99 mg total methylxanthines/g cocoa powder". However, based on their previous statement that cocoa powder is comprised of 93% theobromine and 7% caffeine, the amount of caffeine would be 1.94 mg/g resulting in 27.74 mg total methylxanthines/g cocoa powder.

RESPONSE: The reviewer is correct; there is a miscalculation and the reviewer's calculated numbers are correct (27.74 mg total methylxanthines/g cocoa powder).

- b. The total methylxanthine intake (mg/day) would be 21.64 mg/d instead of 20.27 mg/day.

RESPONSE: The reviewer is correct; there is a miscalculation and the correct number is 21.64 mg/d.

- c. That translates to 161.49 mg/kg/day (instead of 151 mg/kg/day) of methylxanthines in rats consuming 5% cocoa powder.

Please re-calculate and correct or explain how you derived your numbers

RESPONSE: The reviewer is correct; there is a miscalculation and their calculated number (161.49 mg/kg/day) is correct.

- d. The notifier states that "The high dose level provided a mean methylxanthine intake of approximately 57 mg/kg bw/d for males and 74 mg/kg bw /d for females from wk 26- 104 of the study. Again, the next paragraph provides different numbers for the same period. Moreover, it is not specified what 2.1g/kg bw/d and 2.7g/kg bw/d represent.

The description of the same study on pages 58, 60, and 78 are not identical.

RESPONSE: The reviewer is correct; there is a miscalculation in the notification where 57 mg/kg bw/day should be 58 mg/kg bw/day where this occurs in the text. The 2.1g/kg bw/d and 2.7g/kg bw/d represent cocoa powder intake in the text on pages 58, 60, and 78.

5. Please explain how you derived that the lowest theobromine dose tested was approximately 300 mg/kg b.w. per day in the goat study? (Aregheore, 2002), page 70.

RESPONSE: This is a good question as there were few specific details about theobromine content of the material fed and thus several assumptions were made relative to its concentration. Thus, the data provided should be considered at best an estimate. Aregheore (2002) evaluated the inclusion of cocoa shell or cocoa dust (a waste byproduct in the manufacture of chocolate) into goat feed. Goats 18-20 months of age and weighing ~20.5-21.3 kg b.w. were fed a diet containing up to 50% of a cocoa product (unknown theobromine content) for 56 days. Goats fed cocoa dust (from cocoa powder production) in particular, but also those fed cocoa shell had significantly reduced voluntary dry matter intake compared to the controls fed brewer's yeast which resulted in correspondingly reduced body weight gain. The author indicated that the effect could be due to occurrence of theobromine in the cocoa material but no concentration levels were provided in the publication. In order to derive an approximate exposure to theobromine, theoretical estimates of theobromine concentrations were used for these materials based on what is known from the literature for cocoa shell and cocoa dust waste to arrive at approximate exposure concentrations from intake. For cocoa shell, based on what is known from the literature, theobromine content of shell was estimated to be 13 g/kg and the theobromine content of cocoa dust was considered to be similar to that of cocoa bean meal, 20 g/kg. From these data and based on food intake estimates, the theobromine intake was calculated to be 6.9 and 9.7 g/animal per day for the cocoa shell and cocoa dust rations respectively, corresponding to roughly 323 and 465 mg/kg b.w. per day of theobromine. Since in goats consuming these diets there was both reduced dry matter intake and body weight gain at the lowest theobromine level tested (cocoa shell diet), the amount of theobromine was estimated and rounded to 300 mg/kg b.w. per day.

6. We were unable to find the NOAEL and LOAEL for young chickens and older broiler chickens and laying hens in the references you have provided. Please clarify.

RESPONSE: I understand that it is difficult to determine the exact NOAEL and LOAEL from these studies. These studies were included for the sake of completeness even though data have limitations based on assumptions and calculated values. The European Food Safety Authority Expert Panel report (2008) examined the use of theobromine in animal feed and made a number of assumptions based on typical chicken weights at certain ages in order to arrive at an approximate numbers for the NOAEL and LOAELs in these poultry studies. EFSA's detailed analysis of these studies and derivations of estimated NOAELs and LOAELs are presented below.

Day and Dilworth (1984) fed broiler chickens starter diets with 0, 1, 2, 4, or 6% cocoa shell meal (at the expense of maize) from day 1 to 21 of age. By analysis, cocoa shell meal contained 13g theobromine per kg. The cocoa shell meal did not significantly affect 3 week body weights, but feed conversion at 3 weeks was significantly affected by feeding 6% cocoa shell meal. The investigators claimed that performance tended to be depressed over 1% cocoa shell meal. The addition of pure theobromine to four additional diets at levels identical to those provided by 1, 2, 4 and 6% cocoa shell meal depressed performance somewhat more than did cocoa shell meal and reached significance at the two highest doses. The highest dietary theobromine concentration without significant adverse effects (NOAEL) was estimated by EFSA to be 260 mg theobromine/kg diet (corresponding to 2% cocoa shell meal), and this was further estimated to correspond to a theobromine dose of 26-39 mg/kg b.w. per day.

Odunsi and Longe (1995a) fed six groups of day-old chickens (Isa Brown pullet type) isonitrogenous (but not isocaloric) diets with 0, 5, 10, 20, 30 or 40% cocoa bean cake for 9 weeks. As the theobromine content of the cocoa bean cake was 22.4 g/kg, the diets contained 1.1, 2.2, 4.5, 6.7, and 9.0 g theobromine per kg feed, respectively. At 4 weeks of age, 2 chickens were randomly selected for blood collection, and at 8 weeks of age, 2 chickens were sacrificed to evaluate the influence of the feed on the relative weights of liver, kidney and heart to the body weight. The experiment was ended after 9 weeks. Feed intake and weight gain were depressed at 20% inclusion of cocoa bean cake and above. As the metabolizable energy of the cocoa bean cake-containing feed was reduced, an increased feed consumption was expected. Feed intake was however reduced at 20% cocoa bean cake and above. The reduced weight gain was correlated to the reduced feed consumption (and reduced protein intake). Inclusion rates of 10% or more cocoa bean cake resulted in reduced kidney and heart weights and increased liver weights. No effects were observed at the 5% cocoa bean cake level, estimated to correspond to a theobromine dose of 110 mg/kg b.w. per day after the first week. Due to the relatively higher feed intake in the first week of life, the estimated theobromine dose during their first days was 165 mg/kg b.w. per day. Mortality was low and not related to treatment. Hematological parameters such as hemoglobin concentration, packed cell volume and red blood cell count were reduced with increases in dietary cocoa bean cake. The authors interpreted these findings as possibly a consequence of the reduced feed intake.

Odunsi and Longe (1998) fed 28-day old broiler chickens a standard maize-groundnut based diet, or diets with 15 or 30% cocoa bean meal (22.4 g theobromine/kg) for 14 days. Broiler chickens that received the cocoa meal had reduced feed intake and weight gain, and increased mortality with dose. The lowest inclusion of cocoa bean meal, 15%, was estimated to correspond to 3.4 g theobromine/kg diet (estimated to be 340 mg theobromine/kg b.w. per day). The experiment also included diets with cocoa bean meal that had been pretreated to reduce the content of theobromine. Hot water-extracted cocoa bean meal contained 9.8 g theobromine/kg and cocoa pod ash-treated cocoa bean meal 3.3-17 g theobromine/kg. However, the pretreatment also changed the nutritional composition of the meal. The theobromine-reduced cocoa bean meals were mixed in diets at 15, 30 or 45% levels and fed to the 28-day old broilers for 14 days. The pretreatment of the feeding material reduced the adverse effects but also reduced feed intake and weight gain was observed at the lowest theobromine concentration; 15% cocoa pod ash treated cocoa bean meal with a theobromine concentration of 0.95

g/kg diet, estimated to be 95 mg theobromine /kg b.w. per day. In another experiment, Odunsi et al. (1999) fed hot water pretreated cocoa pod ash, alkali treated and untreated cocoa bean meal to 28-days old Anak 180 broiler chickens for four weeks. The theobromine concentrations in the hot water extracted cocoa bean meal, the alkali treated cocoa bean meal, and the non-treated cocoa bean meal were 9.8, 6.3 and 22.4 g/kg. The three types of cocoa bean meal were included in separate diets at levels of 15% and 30%, respectively. Chickens receiving 15% of the untreated cocoa bean meal or more, corresponding to an intake of 3.4 g theobromine or more per kg diet, performed less well than chickens on the control diet. The most pronounced effects were reduced feed intake, reduced daily weight gain, reduced hemoglobin levels and increased creatinine levels. These negative effects were not observed in chickens given the hot-water or alkali-treated cocoa bean meal feeds at an inclusion rate of 15% of the diet, reducing theobromine exposure to 1.5 and 0.95 g theobromine per kg feed (estimated to be 150 and 95 mg/kg b.w. per day). However, the higher inclusion rate of pretreated cocoa bean meal, at 30%, resulted in those adverse effects.

In conclusion, EFSA estimated that the NOAEL of theobromine in young chickens was found to vary between 260 and 1100 mg/kg diet (approximately 26-110 mg theobromine/kg b.w. per day). In older broiler chickens, a LOAEL of 950 mg/kg (approximately 95 mg theobromine/kg b.w. per day) was calculated.

Laying hens

Fangauf and Haenfel (1938) reported that substituting 20% of laying Leghorn hens feed with cacao shell meal for four months resulted in a decreased feed consumption, reduced weight gain, reduced egg production and lower egg weight than in fowls given normal hen diets. Assuming the theobromine concentration in cocoa shell meal to be 13 g/kg, the diet contained 2.6 g theobromine/kg, corresponding to 160 mg theobromine/kg b.w. per day.

Black and Barron (1943) reported on a poisoning episode in laying hens. Among 300 hens that were fed a diet including 15% cacao shell 80 birds died suddenly in convulsions. The cacao shell contained 17 g theobromine/kg. The only organ changes observed post mortem were a color change of the liver, and mottled appearance of kidneys that, coupled with histological changes, indicated subacute glomerulo-nephritis. During the feeding period of 15% cacao shell, egg-production was reduced by around 80%. When the cacao shell ration in the feed was reduced to 7.5%, egg production rose again. The cause of the poisoning episode mentioned above was tested in a small feeding experiment in which groups of three fowls were fed for 200 days diets with 0, 7.5, 15 or 30% cacao shell (contained 17 g theobromine/kg). All hens in the highest dose group died. In the 15% dose group two hens died. Hens in the 7.5% dose group (1.3 g theobromine/kg diet, estimated to be around 80 mg/kg b.w. per day) survived and consumed a normal amount of feed but the droppings were looser than normal.

Black and Barron (1943) concluded that feeding cocoa meal containing 15 g of theobromine per kg may be lethal to hens. The authors concluded that feeding 15% and upwards of cocoa meal to laying birds is extremely harmful; it decreased appetite and egg production, and caused scouring and high mortality.

Four groups of 20-week old layers (Isa Brown pullet type) were supplied isonitrogenous (but not isocaloric) diets with 0, 5, 10 or 20% cocoa bean meal for 25 weeks (Odunsi and Longe, 1995b). Assuming the same feed was used as in the study of Odunsi and Longe (1995a), the pullets were given diets containing 1.1, 2.2, 4.5, 6.7, and 9.0 g theobromine per kg feed. Egg production was followed carefully. Delayed start of egg production was observed in all groups fed cocoa bean meal. It was not known whether the effect was caused by theobromine. Otherwise, there were no adverse effects on layers and laying performance. During the second half of the laying period, no influence of the diets was observed. Thus, the diet with 5% cocoa bean meal assumed to contain 1.1 g theobromine/kg

feed (estimated to correspond to 66 mg theobromine/kg b.w. per day), was the lowest observed effect level.

In conclusion, EFSA concluded that for laying hens the LOAEL is 1100 mg cocoa shell/kg diet (corresponding to 66 mg theobromine/kg b.w. per day). No NOAEL was identified.

ADDITIONAL REFERENCES CITED BY EFSA:

Black, D.J.G. and Barron, N.S. 1943. Observations on the feeding of a cacao waste product to poultry. *The Veterinary Record* 55: 166-167.

Fangauf, R. and Haenfel, A. 1938. Bericht über einen Fütterungsversuch mit Kakaoschalenmehl bei Legehühnern. *Archiv für Geflügelkunde* 12:183-187.

7. We noticed a typographical error in the reference. Hostetler et al. is listed as 1991 on page 77 while the same reference is listed as 1990 in the subsequent paragraph. Please confirm which is the correct one.

RESPONSE: The correct date is 1990.

8. The notifier states that the acceptable daily intake (ADI) of ~30 mg/person/day is significantly lower than the estimated daily intake (EDI) of 319 mg/person/day (90th percentile) from the intended uses of theobromine in the specified foods. The notifier discusses that the safety and exposure assessments of caffeine by Health Canada can be applied/considered to evaluate theobromine which is structurally similar to caffeine and is one of its metabolites. However, the notifier does not provide any comparative exposure estimate of theobromine derived from caffeine metabolism. Although the notifier notes that theobromine is consumed in large quantities by humans in various forms, the notifier does not clearly justify why the proposed 10x excess use on a daily basis can be considered as safe.

RESPONSE: The following explanation provides a clear justification for the safety of theobromine at the proposed use levels.

Various reports in the scientific literature have reported on the estimated contribution of theobromine from caffeine metabolism. Gu *et al.* 1992, Rodopoulos and Norman (1996), Lelo *et al.* 1986, all reported that the metabolic profile of caffeine biotransformation averaged 81.5% for paraxanthine, 10.8% for theobromine and 5.4% for theophylline formation. Assuming 10.8% theobromine is produced from caffeine metabolism and that caffeine consumption estimates range from a mean of approximately 200 mg/p/d up to 400 mg p/d at higher intake levels from various dietary sources, then it follows that 21.8 to 43.2 mg per person per day of theobromine could be generated endogenously as part of caffeine metabolism from various sources (coffee, tea, kola nut flavored beverages, guarana, mate and cocoa and chocolate products). Additionally, based on what is known about the metabolic pathways of caffeine, the same metabolic end products produced from consumption of theobromine when administered or consumed as the parent compound are also produced from these other sources of dietary caffeine when it is metabolized to theobromine. Toxicological evaluations of caffeine have consistently concluded that there are no safety concerns with reasonable levels of consumption. Similarly, it has been shown that no safety concerns have been reported for theobromine from normal intakes (61-147 mg/p/d) regardless of dietary source.

Theobromine is recognized as being less toxic than caffeine. This is clearly demonstrated in the table below particularly in regard to reproductive or developmental toxicity of theobromine versus caffeine

which experts agree is the primary concern with regard to the safety of caffeine and where much research has been focused. Any conclusions regarding caffeine's safety can be equally applied to conclusions regarding theobromine's safety while taking into account its lower order of toxicity.

Comparison of Caffeine and Theobromine in Rats

Methylxanthine	Oral LD50 mg/kg	Developmental NOEL mg/kg/day	Reproductive NOEL mg/kg/day
Caffeine	200	30	80-120
Theobromine (as theobromine sodium acetate)-oral gavage	950		
Theobromine (oral gavage)		50	
via diet		~100	250

Christian and Brent (2001) evaluated the developmental and reproductive effects of caffeine and reported that the developmental NOEL for caffeine in rodents is approximately 30 mg/kg/day, the teratogenic NOEL is 80 to 100 mg/kg/day, and the reproductive NOEL approximately 80 to 120 mg/kg/day. They noted that the probable blood level of caffeine required to produce teratogenic effects in rats is in excess of 60 µg/mL, which can only be reached in rodents by administration of large bolus dosages achieving peak short-term exposure. As shown in the table for theobromine, a higher dose is required to achieve the same observation.

Neither rodents nor humans can attain a 60 µg/mL peak exposure by consuming solutions of caffeine over several hours, the usual mode of human caffeine consumption. Christian and Brent (2001) hypothesized that this blood peak plasma concentration of 60 µg/mL might be achieved in the rodent by an 800 mg/kg/day dosage of caffeine in the drinking water; however, this is a dose equivalent to a 60 kg human consuming an enormous amount of caffeine. Again, the same analogy would hold true for theobromine consumption and additionally, this would not be achievable based on the excessive and unrealistic caloric intake required from foods naturally containing theobromine or combined with the specified food uses identified in this Notification.

Under normal conditions of oral consumption, humans cannot achieve blood levels of caffeine that are within the range of those that affect reproductive performance or development of offspring in the most sensitive animal species (Christian and Brent, 2001). The same would hold true for theobromine consumption.

While critical reviews of available animal studies demonstrate that caffeine can produce adverse effects in some species when given at a sufficiently high dose by gavage or injection, Christian and Brent (2001) also demonstrate that caffeine does not affect reproductive performance or development of the offspring of any animal species, unless given at a maternally toxic dosage that exceeds normal levels of human dietary consumption. They conclude that the usual range of human exposures to caffeine from food and beverages is well below the threshold dose that would result in developmental/teratogenic or reproductive effects in experimental animals. Klebanoff et al. (1999) cited in the Notification is the only study that dealt with actual blood levels of caffeine metabolites in humans and they reached the conclusion with regard to caffeine consumption and spontaneous abortion: "that moderate consumption of caffeine is unlikely to increase the risk of spontaneous abortion." These observations and conclusions are equally applicable to theobromine whether as a metabolite of caffeine or from natural background sources in the diet or combined with the proposed food uses as specified in the Notification.

Recent support for the analysis and conclusions of Christian and Brent (2001) regarding the safety of

caffeine which are summarized above can be found in the critical analysis by Peck *et al.* (in press and available online) who have published a critical review of the literature of the epidemiologic evidence concerning the consumption of caffeine-containing products and any association with potential reproductive effects in humans. Humans must be regarded as the most sensitive target species in the safety evaluation of caffeine and theobromine. This review is an update of the comprehensive critical report previously published by Leviton and Cowan (2002). As such, this review is restricted to human studies of caffeine and reproductive health published in English between January 2000 and December 2009. From their review, the authors concluded that the evidence for an effect of caffeine on reproductive health and fetal development is limited by the inability to rule out plausible alternative explanations for the observed associations, namely, confounding by pregnancy symptoms and smoking, and by exposure measurement error. Further, because of these limitations, the weight of evidence does not support a positive relationship between caffeine consumption and adverse reproductive or perinatal outcomes. The authors concluded that the studies available from January 2000 through December 2009 do not provide convincing evidence that caffeine consumption increases risk of any reproductive adversity. Future studies addressing the methodological limitations of current research may alter this conclusion. This is consistent with the earlier conclusions of Leviton and Cowan (2002) regarding caffeine and reproductive and perinatal outcomes in humans who also concluded from their review that no convincing evidence had been presented to show that caffeine consumption increases the risk of any reproductive adversity. Additionally, as discussed above, this is also applicable to theobromine which is a metabolite of caffeine but with a lower order of toxicity. The Expert Panel critically reviewed information and data on the safety of theobromine and caffeine and concluded that the proposed use of theobromine as an ingredient in certain selected foods and beverages as described in the Notification is Generally Recognized as Safe (GRAS) based on scientific procedures.

Health Canada (referenced in the Notification) indicated that humans can safely tolerate (no adverse effects reported) caffeine at a level of up to 400 mg/day. Since theobromine has been shown to be less toxic than caffeine, this conclusion of safety derived from the evaluation of human studies on caffeine should be equally applicable to theobromine consumption at intake levels up to 400 mg/day which is below the 90th percentile of the combined natural dietary background sources and proposed food use levels in the Notification.

Finally, it should also be noted that the intake estimates for theobromine in this Notification assume a 100 percent market penetration of the proposed uses of theobromine listed in Table 4 combined with background dietary intake estimates. Because 100 percent market penetration of the specified theobromine use in these products is highly unlikely, this estimate almost certainly overstates actual intake, which is likely to be much lower.

REFERENCES FOR THIS QUESTION:

Christian MS and Brent RL. (2001). Teratogen Update: Evaluation of the reproductive and developmental risks of caffeine. *Teratology* 64(1): 51-78. (Cited in the Notification.)

Gu L, Gonzalez FJ, Kalow W, Tang BK. (1992). Biotransformation of caffeine, paraxanthine, theobromine and theophylline by cDNA-expressed human CYP1A2 and CYP2E1. *Pharmacogenetics*; 2 (2): 73-77.

Klebanoff MA, Levine RJ, DerSimonian R, Clemens JD, Wilkins DG (1999). Maternal serum paraxanthine, a caffeine metabolite, and spontaneous abortion. *New England Journal of Medicine* 34(22): 1639-1644.

Lelo A, Birkett DJ, Robson RA and Miners JO. (1986) Comparative pharmacokinetics of caffeine and its primary demethylated metabolites paraxanthine, theobromine and theophylline in man. *Br J Clin Pharmacol*; **22**: 177-182. (Cited in the Notification.)

Leviton A and Cowan L. (2002). A review of the literature relating caffeine consumption by women to their risk of reproductive hazards. Review. *Food and Chemical Toxicology*; 40:1271-1310.

Peck JD, Leviton A, Cowan LD. (in press). A review of the epidemiologic evidence concerning the reproductive health effects of caffeine consumption: A 2000–2009 update. *Food and Chemical Toxicology* doi:10.1016/j.fct.2010.06.019.

Rodopoulos, N, Hojvall, L and Norman, A. (1996). Elimination of theobromine metabolites in healthy adults. *Scandinavian Journal of Clinical and Laboratory Investigation* 56(4):373-383. (Cited in the Notification.)

9. Using data from NHANES 2003-2006 and the food codes reported, FDA calculated that 261 mg/p/d of theobromine is consumed at the mean and 367 mg/p/d at the 90th percentile for users. This is twice as high as 150 mg/person/day at the mean and 319 mg/person/day at the 90th percentile that was reported on page 19. In addition, FDA calculated a higher number of users (89.6%) vs. the 65.1% found in the Cantox assessment report (page 8 of Appendix 1). Please explain this discrepancy.

RESPONSE: It is difficult to explain this discrepancy without more information concerning the intake assessment conducted by the FDA and due to some confusion as to what values specifically the FDA is calling into question. The 65.1% users identified by Cantox refers only to the background intake of theobromine, and this was associated with intake of 61 and 147 mg/person/day at the mean and 90th percentile. This was calculated using all foodcodes in the NHANES database and by employing the USDA measured levels of theobromine. When the proposed food uses were added to the background levels, the intake was reported to be 150 and 319 mg/person/day, the numbers listed in the FDA question; however, this assessment was associated with 94.6% users, a number higher than that identified by the FDA. The FDA question does not detail whether the numbers referenced refer to calculations completed with the proposed food uses only or the proposed food uses and the background levels. It would be extremely helpful if this information could be provided and if the discrepancy between the scenarios being employed for comparison could be explained. In any case, the discrepancy in the levels of intake reported (150 and 319 mg/person/day as compared to the FDA derived 261 and 367 mg/person/day) likely result from differing approaches in food code selection with the powdered fruit flavored drinks being the most likely food use impacting the different estimates. Cantox limited the foodcodes chosen to represent this category to those identified as being produced from powders. If the FDA included all foodcodes from this category, which are widely consumed, this could explain the difference in the intake estimates.

10. The 90th percentile of consumption of powdered fruit-flavored drinks is nearly 11x larger than the reported mean on page 26. Typically, the amount consumed at the 90th percentile is 2x greater than the mean. Please explain this difference.

RESPONSE: The mean and 90th percentile all-person intakes of powdered fruit flavored drinks are reported on page 12 as being 12 and 135 mg/person/day, respectively. This is a typographical

error as this is an error in the insertion of the values from table A-7. The mean intake of powdered fruit flavored drinks is indeed equivalent to 12 mg/person/day; however, there is no 90th percentile value for this intake as insufficient data were available and so there is no value for the 90th percentile intake. Instead the mean all-user intake of 135 mg/person/day was mistakenly inserted in place of that information. The correct values were present in Table A-7; however, please find attached a version of the report with the correct values also reported on page 12.

11. The description of the manufacturing process on page 15 states that sodium bicarbonate is added to 3-methylxanthine in acetone and water before the addition of dimethyl sulfate. On page 16, the figure and written description shows that 3-methylxanthine is converted to the potassium salt before the addition of dimethylsulfate. Are there two methods for preparation of theobromine, or is one method preferred over the other?

RESPONSE: The method that is preferred and ascribed to the theobromine material that is the subject of the Notification requires 3-methylxanthine to be converted to the potassium salt before the addition of dimethylsulfate.

In addition to the response to the questions raised, I am attaching an electronic copy of the revised CanTox intake exposure document as referenced above as well as the four publications cited in the response to Question #8 for completeness. A hard copy of this information will also be sent to your attention by regular mail. We trust that these responses have addressed the questions raised by the review team.

Should you have any additional questions regarding this GRAS Notice, please contact me at 717-243-9216 or by email. We look forward to FDA's completion of their review on this submission.

Sincerely,

Stanley M. Tarka, Jr., Ph.D.
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From: Weinsetel, Natalia [<mailto:Natalia.Weinsetel@fda.hhs.gov>]
Sent: Tuesday, July 20, 2010 1:22 PM
To: 'tarkagroup@comcast.net'
Subject: RE: Theobromine GRAS Notification Submission

Dear Dr. Tarka,

The review team for GRN340 has completed their preliminary assessment, and the reviewers would like clarification on issues that they identified on the notice. I have provided the reviewers' questions for you below. So that we can complete our review and respond to your notice in a timely manner, we would appreciate receiving your responses to the reviewers' comments by close of business on Friday, August 6, 2010. If you will be unable to respond by this date, or if you have questions regarding the reviewers' comments, please feel free to contact me. Best regards,

Natalia

1. The notifier (Theocorp) mentions that: "TINOS LLC submitted a letter of intent to market theobromine as a New Dietary Ingredient and FDA filed this letter as an official filing on January 22, 2004." However, it also states that FDA issued a supplemental letter dated April 6, 1996. Please confirm if this is an error or explain the date discrepancy (page 20).
2. Please spell out the acronym PADI (page 21).
3. The percentage of unchanged theobromine (1-18%) found in the human urine as noted on page 36 is inconsistent with that presented in Table 5 (page 37).
4. In the calculations provided for total methylxanthine intake from cocoa powder on page 60, there appears to be some miscalculations (see below).
 - a. The notifier calculated that "cocoa powder provided 25.8 (mg/g) theobromine + 0.19 (mg/g) caffeine = 25.99 mg total methylxanthines/g cocoa powder". However, based on their previous statement that cocoa powder is comprised of 93% theobromine and 7% caffeine, the amount of caffeine would be 1.94 mg/g resulting in 27.74 mg total methylxanthines/g cocoa powder.
 - b. The total methylxanthine intake (mg/day) would be 21.64 mg/d instead of 20.27 mg/day.
 - c. That translates to 161.49 mg/kg/day (instead of 151 mg/kg/day) of methylxanthines in rats consuming 5% cocoa powder.
Please re-calculate and correct or explain how you derived your numbers.
 - d. The notifier states that "The high dose level provided a mean methylxanthine intake of approximately 57 mg/kg bw/d for males and 74 mg/kg bw /d for females from wk 26-104 of the study. Again, the next paragraph provides different numbers for the same period. Moreover, it is not specified what 2.1g/kg bw/d and 2.7g/kg bw/d represent.
The description of the same study on pages 58, 60, and 78 are not identical.
5. Please explain how you derived that the lowest theobromine dose tested was approximately 300 mg/kg b.w. per day in the goat study? (Aregheore, 2002), page 70.
6. We were unable to find the NOAEL and LOAEL for young chickens and older broiler chickens and laying hens in the references you have provided. Please clarify.
7. We noticed a typographical error in the reference. Hostetler et al. is listed as 1991 on page 77 while the same reference is listed as 1990 in the subsequent paragraph. Please confirm which is the correct one.
8. The notifier states that the acceptable daily intake (ADI) of ~30 mg/person/day is significantly lower than the estimated daily intake (EDI) of 319 mg/person/day (90th percentile) from the intended uses of theobromine in the specified foods. The notifier discusses that the safety and exposure assessments of caffeine by Health Canada can be applied/considered to evaluate theobromine which is structurally similar to caffeine and is one of its metabolites. However, the notifier does not provide any comparative exposure estimate of theobromine derived from caffeine metabolism. Although the notifier notes that theobromine is consumed in large quantities by humans in various forms, the notifier does not clearly justify why the proposed 10x excess use on a daily basis can be considered as safe.
9. Using data from NHANES 2003-2006 and the food codes reported, FDA calculated that 261 mg/p/d of theobromine is consumed at the mean and 367 mg/p/d at the 90th percentile for users. This is twice as high as 150 mg/person/day at the mean and 319 mg/person/day at the 90th percentile that was reported on page 19. In addition, FDA calculated a higher number of users (89.6%) vs. the 65.1% found in the Cantox assessment report (page 8 of Appendix 1). Please explain this discrepancy.
10. The 90th percentile of consumption of powdered fruit-flavored drinks is nearly 11x larger than the reported mean on page 26. Typically, the amount consumed at the 90th percentile is 2x greater than

the mean. Please explain this difference.

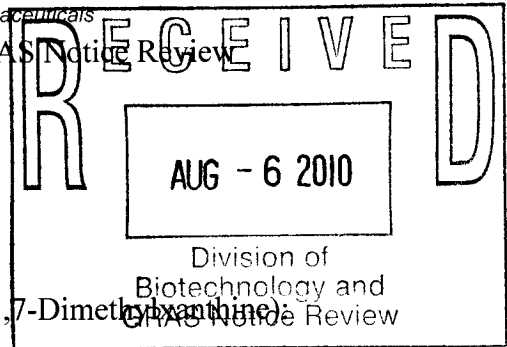
11. The description of the manufacturing process on page 15 states that sodium bicarbonate is added to 3-methylxanthine in acetone and water before the addition of dimethyl sulfate. On page 16, the figure and written description shows that 3-methylxanthine is converted to the potassium salt before the addition of dimethylsulfate. Are there two methods for preparation of theobromine, or is one method preferred over the other?

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July 30, 2010

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RE: Notification of GRAS Determination for Theobromine (3,7-Dimethylxanthine)
Response to Reviewers Questions on GRN340

Dear Dr. Weinsetel:

Thank you very much for your email of July 20 indicating that the review team has completed their preliminary assessment of the GRAS Notification for theobromine and requesting clarification on issues that they identified in the notice. I apologize for the slight delay in responding but had been out of town. The reviewers have been very thorough and I have prepared responses to all of the questions below.

RESPONSE TO FDA REVIEWERS OF GRN340

1. The notifier (Theocorp) mentions that: "TINOS LLC submitted a letter of intent to market theobromine as a New Dietary Ingredient and FDA filed this letter as an official filing on January 22, 2004." However, it also states that FDA issued a supplemental letter dated April 6, 1996. Please confirm if this is an error or explain the date discrepancy (page 20).

RESPONSE: The reviewers are correct; this is an error and the correct date is January 22, 1996 not January 22, 2004.

2. Please spell out the acronym PADI (page 21).

RESPONSE: Possible average daily intake (PADI) is spelled out.

3. The percentage of unchanged theobromine (1-18%) found in the human urine as noted on page 36 is inconsistent with that presented in Table 5 (page 37).

RESPONSE: This is a typographical error and should be 10-18% on page 36 and not 1-18%. Table 5 is correct.

4. In the calculations provided for total methylxanthine intake from cocoa powder on page 60, there appears to be some miscalculations (see below).
 - a. The notifier calculated that "cocoa powder provided 25.8 (mg/g) theobromine + 0.19 (mg/g) caffeine = 25.99 mg total methylxanthines/g cocoa powder". However, based on their previous statement that cocoa

powder is comprised of 93% theobromine and 7% caffeine, the amount of caffeine would be 1.94 mg/g resulting in 27.74 mg total methylxanthines/g cocoa powder.

RESPONSE: The reviewer is correct; there is a miscalculation and the reviewer's calculated numbers are correct (27.74 mg total methylxanthines/g cocoa powder).

- b. The total methylxanthine intake (mg/day) would be 21.64 mg/d instead of 20.27 mg/day.

RESPONSE: The reviewer is correct; there is a miscalculation and the correct number is 21.64 mg/d.

- c. That translates to 161.49 mg/kg/day (instead of 151 mg/kg/day) of methylxanthines in rats consuming 5% cocoa powder.
Please re-calculate and correct or explain how you derived your numbers

RESPONSE: The reviewer is correct; there is a miscalculation and their calculated number (161.49 mg/kg/day) is correct.

- d. The notifier states that "The high dose level provided a mean methylxanthine intake of approximately 57 mg/kg bw/d for males and 74 mg/kg bw /d for females from wk 26-104 of the study. Again, the next paragraph provides different numbers for the same period. Moreover, it is not specified what 2.1g/kg bw/d and 2.7g/kg bw/d represent. The description of the same study on pages 58, 60, and 78 are not identical.

RESPONSE: The reviewer is correct; there is a miscalculation in the notification where 57 mg/kg bw/day should be 58 mg/kg bw/day where this occurs in the text. The 2.1g/kg bw/d and 2.7g/kg bw/d represent cocoa powder intake in the text on pages 58, 60, and 78.

- 5. Please explain how you derived that the lowest theobromine dose tested was approximately 300 mg/kg b.w. per day in the goat study? (Aregheore, 2002), page 70.

RESPONSE: This is a good question as there were few specific details about theobromine content of the material fed and thus several assumptions were made relative to its concentration. Thus, the data provided should be considered at best an estimate. Aregheore (2002) evaluated the inclusion of cocoa shell or cocoa dust (a waste byproduct in the manufacture of chocolate) into goat feed. Goats 18-20 months of age and weighing ~20.5-21.3 kg b.w. were fed a diet containing up to 50% of a cocoa product (unknown theobromine content) for 56 days. Goats fed cocoa dust (from cocoa powder production) in particular, but also those fed cocoa

shell had significantly reduced voluntary dry matter intake compared to the controls fed brewer's yeast which resulted in correspondingly reduced body weight gain. The author indicated that the effect could be due to occurrence of theobromine in the cocoa material but no concentration levels were provided in the publication. In order to derive an approximate exposure to theobromine, theoretical estimates of theobromine concentrations were used for these materials based on what is known from the literature for cocoa shell and cocoa dust waste to arrive at approximate exposure concentrations from intake. For cocoa shell, based on what is known from the literature, theobromine content of shell was estimated to be 13 g/kg and the theobromine content of cocoa dust was considered to be similar to that of cocoa bean meal, 20 g/kg. From these data and based on food intake estimates, the theobromine intake was calculated to be 6.9 and 9.7 g/animal per day for the cocoa shell and cocoa dust rations respectively, corresponding to roughly 323 and 465 mg/kg b.w. per day of theobromine. Since in goats consuming these diets there was both reduced dry matter intake and body weight gain at the lowest theobromine level tested (cocoa shell diet), the amount of theobromine was estimated and rounded to 300 mg/kg b.w. per day.

6. We were unable to find the NOAEL and LOAEL for young chickens and older broiler chickens and laying hens in the references you have provided. Please clarify.

RESPONSE: I understand that it is difficult to determine the exact NOAEL and LOAEL from these studies. These studies were included for the sake of completeness even though data have limitations based on assumptions and calculated values. The European Food Safety Authority Expert Panel report (2008) examined the use of theobromine in animal feed and made a number of assumptions based on typical chicken weights at certain ages in order to arrive at an approximate numbers for the NOAEL and LOAELs in these poultry studies. EFSA's detailed analysis of these studies and derivations of estimated NOAELS and LOAELS are presented below.

Day and Dilworth (1984) fed broiler chickens starter diets with 0, 1, 2, 4, or 6% cocoa shell meal (at the expense of maize) from day 1 to 21 of age. By analysis, cocoa shell meal contained 13g theobromine per kg. The cocoa shell meal did not significantly affect 3 week body weights, but feed conversion at 3 weeks was significantly affected by feeding 6% cocoa shell meal. The investigators claimed that performance tended to be depressed over 1% cocoa shell meal. The addition of pure theobromine to four additional diets at levels identical to those provided by 1, 2, 4 and 6% cocoa shell meal depressed performance somewhat more than did cocoa shell meal and reached significance at the two highest doses. The highest dietary theobromine concentration without significant adverse effects (NOAEL) was estimated by EFSA to be 260 mg theobromine/kg diet (corresponding to 2% cocoa shell meal), and this was further estimated to correspond to a theobromine dose of 26-39 mg/kg b.w. per day.

Odunsi and Longe (1995a) fed six groups of day-old chickens (Isa Brown pullet type) isonitrogenous (but not isocaloric) diets with 0, 5, 10, 20, 30 or 40% cocoa bean cake for 9 weeks. As the theobromine content of the cocoa bean cake was 22.4 g/kg, the diets contained 1.1, 2.2, 4.5, 6.7, and 9.0 g theobromine per kg feed, respectively. At 4 weeks of age, 2 chickens were randomly selected for blood collection, and at 8 weeks of age, 2 chickens were sacrificed to evaluate the influence of the feed on the relative weights of liver, kidney and heart to the body weight. The experiment was ended after 9 weeks. Feed intake and weight gain were depressed at 20% inclusion of cocoa bean cake and above. As the metabolizable energy of the cocoa bean cake-containing feed was reduced, an increased feed consumption was expected. Feed intake was however reduced at 20% cocoa bean cake and above. The reduced weight gain was correlated to the reduced feed consumption (and reduced protein intake). Inclusion rates of 10% or more cocoa bean cake resulted in reduced kidney and heart weights and increased liver weights. No effects were observed at the 5% cocoa bean cake level, estimated to correspond to a theobromine dose of 110 mg/kg b.w. per day after the first week. Due to the relatively higher feed intake in the first week of life, the estimated theobromine dose during their first days was 165 mg/kg b.w. per day. Mortality was low and not related to treatment. Hematological parameters such as hemoglobin concentration, packed cell volume and red blood cell count were reduced with increases in dietary cocoa bean cake. The authors interpreted these findings as possibly a consequence of the reduced feed intake.

Odunsi and Longe (1998) fed 28-day old broiler chickens a standard maize-groundnut based diet, or diets with 15 or 30% cocoa bean meal (22.4 g theobromine/kg) for 14 days. Broiler chickens that received the cocoa meal had reduced feed intake and weight gain, and increased mortality with dose. The lowest inclusion of cocoa bean meal, 15%, was estimated to correspond to 3.4 g theobromine/kg diet (estimated to be 340 mg theobromine/kg b.w. per day). The experiment also included diets with cocoa bean meal that had been pretreated to reduce the content of theobromine. Hot water-extracted cocoa bean meal contained 9.8 g theobromine/kg and cocoa pod ash-treated cocoa bean meal 3.3-17 g theobromine/kg. However, the pretreatment also changed the nutritional composition of the meal. The theobromine-reduced cocoa bean meals were mixed in diets at 15, 30 or 45% levels and fed to the 28-day old broilers for 14 days. The pretreatment of the feeding material reduced the adverse effects but also reduced feed intake and weight gain was observed at the lowest theobromine concentration; 15% cocoa pod ash treated cocoa bean meal with a theobromine concentration of 0.95 g/kg diet, estimated to be 95 mg theobromine /kg b.w. per day. In another experiment, Odunsi et al. (1999) fed hot water pretreated cocoa pod ash, alkali treated and untreated cocoa bean meal to 28-days old Anak 180 broiler chickens for four weeks. The theobromine concentrations in the hot water extracted cocoa bean meal, the alkali treated cocoa bean meal, and the non-treated cocoa bean meal were 9.8, 6.3 and 22.4 g/kg. The three types of cocoa bean meal were included in separate diets at levels of 15% and 30%, respectively. Chickens receiving 15% of the untreated cocoa bean meal or more, corresponding to an

intake of 3.4 g theobromine or more per kg diet, performed less well than chickens on the control diet. The most pronounced effects were reduced feed intake, reduced daily weight gain, reduced hemoglobin levels and increased creatinine levels. These negative effects were not observed in chickens given the hot-water or alkali-treated cocoa bean meal feeds at an inclusion rate of 15% of the diet, reducing theobromine exposure to 1.5 and 0.95 g theobromine per kg feed (estimated to be 150 and 95 mg/kg b.w. per day). However, the higher inclusion rate of pretreated cocoa bean meal, at 30%, resulted in those adverse effects.

In conclusion, EFSA estimated that the NOAEL of theobromine in young chickens was found to vary between 260 and 1100 mg/kg diet (approximately 26-110 mg theobromine/kg b.w. per day). In older broiler chickens, a LOAEL of 950 mg/kg (approximately 95 mg theobromine/kg b.w. per day) was calculated.

Laying hens

Fangauf and Haenfel (1938) reported that substituting 20% of laying Leghorn hens feed with cacao shell meal for four months resulted in a decreased feed consumption, reduced weight gain, reduced egg production and lower egg weight than in fowls given normal hen diets. Assuming the theobromine concentration in cocoa shell meal to be 13 g/kg, the diet contained 2.6 g theobromine/kg, corresponding to 160 mg theobromine/kg b.w. per day.

Black and Barron (1943) reported on a poisoning episode in laying hens. Among 300 hens that were fed a diet including 15% cacao shell 80 birds died suddenly in convulsions. The cocoa shell contained 17 g theobromine/kg. The only organ changes observed post mortem were a color change of the liver, and mottled appearance of kidneys that, coupled with histological changes, indicated subacute glomerulo-nephritis. During the feeding period of 15% cacao shell, egg-production was reduced by around 80%. When the cacao shell ration in the feed was reduced to 7.5%, egg production rose again. The cause of the poisoning episode mentioned above was tested in a small feeding experiment in which groups of three fowls were fed for 200 days diets with 0, 7.5, 15 or 30% cacao shell (contained 17 g theobromine/kg). All hens in the highest dose group died. In the 15% dose group two hens died. Hens in the 7.5% dose group (1.3 g theobromine/kg diet, estimated to be around 80 mg/kg b.w. per day) survived and consumed a normal amount of feed but the droppings were looser than normal.

Black and Barron (1943) concluded that feeding cocoa meal containing 15 g of theobromine per kg may be lethal to hens. The authors concluded that feeding 15% and upwards of cocoa meal to laying birds is extremely harmful; it decreased appetite and egg production, and caused scouring and high mortality.

Four groups of 20-week old layers (Isa Brown pullet type) were supplied isonitrogenous (but not isocaloric) diets with 0, 5, 10 or 20% cocoa bean meal for 25 weeks (Odunsi and Longe, 1995b). Assuming the same feed was used as in the study of Odunsi and Longe (1995a), the pullets were given diets containing 1.1, 2.2,

4.5, 6.7, and 9.0 g theobromine per kg feed. Egg production was followed carefully. Delayed start of egg production was observed in all groups fed cocoa bean meal. It was not known whether the effect was caused by theobromine. Otherwise, there were no adverse effects on layers and laying performance. During the second half of the laying period, no influence of the diets was observed. Thus, the diet with 5% cocoa bean meal assumed to contain 1.1 g theobromine/kg feed (estimated to correspond to 66 mg theobromine/kg b.w. per day), was the lowest observed effect level.

In conclusion, EFSA concluded that for laying hens the LOAEL is 1100 mg cocoa shell/kg diet (corresponding to 66 mg theobromine/kg b.w. per day). No NOAEL was identified.

ADDITIONAL REFERENCES CITED BY EFSA:

Black, D.J.G. and Barron, N.S. 1943. Observations on the feeding of a cacao waste product to poultry. The Veterinary Record 55: 166-167.

Fangauf, R. and Haenfel, A. 1938. Bericht über einen Fütterungsversuch mit Kakaoschalenmehl bei Legehühnern. Archiv für Geflügelkunde 12:183-187.

7. We noticed a typographical error in the reference. Hostetler et al. is listed as 1991 on page 77 while the same reference is listed as 1990 in the subsequent paragraph. Please confirm which is the correct one.

RESPONSE: The correct date is 1990.

8. The notifier states that the acceptable daily intake (ADI) of ~30 mg/person/day is significantly lower than the estimated daily intake (EDI) of 319 mg/person/day (90th percentile) from the intended uses of theobromine in the specified foods. The notifier discusses that the safety and exposure assessments of caffeine by Health Canada can be applied/considered to evaluate theobromine which is structurally similar to caffeine and is one of its metabolites. However, the notifier does not provide any comparative exposure estimate of theobromine derived from caffeine metabolism. Although the notifier notes that theobromine is consumed in large quantities by humans in various forms, the notifier does not clearly justify why the proposed 10x excess use on a daily basis can be considered as safe.

RESPONSE: The following explanation provides a clear justification for the safety of theobromine at the proposed use levels.

Various reports in the scientific literature have reported on the estimated contribution of theobromine from caffeine metabolism. Gu *et al.* 1992, Rodopoulos and Norman (1996), Lelo *et al.*, 1986, all reported that the metabolic profile of caffeine biotransformation averaged 81.5% for paraxanthine, 10.8% for theobromine and 5.4% for theophylline formation. Assuming 10.8% theobromine is produced from caffeine metabolism and that

caffeine consumption estimates range from a mean of approximately 200 mg/p/d up to 400 mg p/d at higher intake levels from various dietary sources, then it follows that 21.8 to 43.2 mg per person per day of theobromine could be generated endogenously as part of caffeine metabolism from various sources (coffee, tea, kola nut flavored beverages, guarana, mate and cocoa and chocolate products). Additionally, based on what is known about the metabolic pathways of caffeine, the same metabolic end products produced from consumption of theobromine when administered or consumed as the parent compound are also produced from these other sources of dietary caffeine when it is metabolized to theobromine. Toxicological evaluations of caffeine have consistently concluded that there are no safety concerns with reasonable levels of consumption. Similarly, it has been shown that no safety concerns have been reported for theobromine from normal intakes (61-147 mg/p/d) regardless of dietary source.

Theobromine is recognized as being less toxic than caffeine. This is clearly demonstrated in the table below particularly in regard to reproductive or developmental toxicity of theobromine versus caffeine which experts agree is the primary concern with regard to the safety of caffeine and where much research has been focused. Any conclusions regarding caffeine's safety can be equally applied to conclusions regarding theobromine's safety while taking into account its lower order of toxicity.

Comparison of Caffeine and Theobromine in Rats

Methylxanthine	Oral LD50 mg/kg	Developmental NOEL mg/kg/day	Reproductive NOEL mg/kg/day
Caffeine	200	30	80-120
Theobromine (as theobromine sodium acetate)- oral gavage	950	50	
Theobromine (oral gavage) via diet		~100	250

Christian and Brent (2001) evaluated the developmental and reproductive effects of caffeine and reported that the developmental NOEL for caffeine in rodents is approximately 30 mg/kg/day, the teratogenic NOEL is 80 to 100 mg/kg/day, and the reproductive NOEL approximately 80 to 120 mg/kg/day. They noted that the probable blood level of caffeine required to produce teratogenic effects in rats is in excess of 60 µg/mL, which can only be reached in rodents by administration of large bolus dosages achieving peak short-term exposure. As shown in the table for theobromine, a higher dose is required to achieve the same observation.

Neither rodents nor humans can attain a 60 µg/mL peak exposure by consuming solutions of caffeine over several hours, the usual mode of human caffeine consumption. Christian and Brent (2001) hypothesized that this blood peak plasma concentration of 60 µg/mL might be achieved in the rodent by an 800 mg/kg/day dosage of caffeine in the drinking water; however, this is a dose equivalent to a 60 kg human consuming an enormous amount of caffeine. Again, the same analogy would hold true for theobromine consumption and additionally, this would not be achievable based on the excessive and unrealistic caloric intake required from foods naturally containing theobromine or

combined with the specified food uses identified in this Notification.

Under normal conditions of oral consumption, humans cannot achieve blood levels of caffeine that are within the range of those that affect reproductive performance or development of offspring in the most sensitive animal species (Christian and Brent ,2001). The same would hold true for theobromine consumption.

While critical reviews of available animal studies demonstrate that caffeine can produce adverse effects in some species when given at a sufficiently high dose by gavage or injection, Christian and Brent (2001) also demonstrate that caffeine does not affect reproductive performance or development of the offspring of any animal species, unless given at a maternally toxic dosage that exceeds normal levels of human dietary consumption. They conclude that the usual range of human exposures to caffeine from food and beverages is well below the threshold dose that would result in developmental/teratogenic or reproductive effects in experimental animals. Klebanoff et al. (1999) cited in the Notification is the only study that dealt with actual blood levels of caffeine metabolites in humans and they reached the conclusion with regard to caffeine consumption and spontaneous abortion: "that moderate consumption of caffeine is unlikely to increase the risk of spontaneous abortion." These observations and conclusions are equally applicable to theobromine whether as a metabolite of caffeine or from natural background sources in the diet or combined with the proposed food uses as specified in the Notification.

Recent support for the analysis and conclusions of Christian and Brent (2001) regarding the safety of caffeine which are summarized above can be found in the critical analysis by Peck *et al.* (in press and available online) who have published a critical review of the literature of the epidemiologic evidence concerning the consumption of caffeine-containing products and any association with potential reproductive effects in humans. Humans must be regarded as the most sensitive target species in the safety evaluation of caffeine and theobromine. This review is an update of the comprehensive critical report previously published by Leviton and Cowan (2002). As such, this review is restricted to human studies of caffeine and reproductive health published in English between January 2000 and December 2009. From their review, the authors concluded that the evidence for an effect of caffeine on reproductive health and fetal development is limited by the inability to rule out plausible alternative explanations for the observed associations, namely, confounding by pregnancy symptoms and smoking, and by exposure measurement error. Further, because of these limitations, the weight of evidence does not support a positive relationship between caffeine consumption and adverse reproductive or perinatal outcomes. The authors concluded that the studies available from January 2000 through December 2009 do not provide convincing evidence that caffeine consumption increases risk of any reproductive adversity. Future studies addressing the methodological limitations of current research may alter this conclusion. This is consistent with the earlier conclusions of Leviton and Cowan (2002) regarding caffeine and reproductive and perinatal outcomes in humans who also concluded from their review that no convincing evidence had been presented to show that caffeine consumption increases the risk of any reproductive adversity. Additionally, as discussed above, this is also applicable to

theobromine which is a metabolite of caffeine but with a lower order of toxicity. The Expert Panel critically reviewed information and data on the safety of theobromine and caffeine and concluded that the proposed use of theobromine as an ingredient in certain selected foods and beverages as described in the Notification is Generally Recognized as Safe (GRAS) based on scientific procedures.

Health Canada (referenced in the Notification) indicated that humans can safely tolerate (no adverse effects reported) caffeine at a level of up to 400 mg/day. Since theobromine has been shown to be less toxic than caffeine, this conclusion of safety derived from the evaluation of human studies on caffeine should be equally applicable to theobromine consumption at intake levels up to 400 mg/day which is below the 90th percentile of the combined natural dietary background sources and proposed food use levels in the Notification.

Finally, it should also be noted that the intake estimates for theobromine in this Notification assume a 100 percent market penetration of the proposed uses of theobromine listed in Table 4 combined with background dietary intake estimates. Because 100 percent market penetration of the specified theobromine use in these products is highly unlikely, this estimate almost certainly overstates actual intake, which is likely to be much lower.

REFERENCES FOR THIS SECTION:

Christian MS and Brent RL. (2001). Teratogen Update: Evaluation of the reproductive and developmental risks of caffeine. *Teratology* 64(1): 51-78. (Cited in the Notification.)

Gu L, Gonzalez FJ, Kalow W, Tang BK. (1992). Biotransformation of caffeine, paraxanthine, theobromine and theophylline by cDNA-expressed human CYP1A2 and CYP2E1. *Pharmacogenetics*; 2(2): 73-77.

Klebanoff MA, Levine RJ, DerSimonian R, Clemens JD, Wilkins DG (1999). Maternal serum paraxanthine, a caffeine metabolite, and spontaneous abortion. *New England Journal of Medicine* 34(22): 1639-1644.

Lelo A, Birkett DJ, Robson RA and Miners JO. (1986) Comparative pharmacokinetics of caffeine and its primary demethylated metabolites paraxanthine, theobromine and theophylline in man. *Br J Clin Pharmacol*; **22**: 177-182. (Cited in the Notification.)

Leviton A and Cowan L. (2002). A review of the literature relating caffeine consumption by women to their risk of reproductive hazards. Review. *Food and Chemical Toxicology*; 40:1271-1310.

Peck JD, Leviton A, Cowan LD. (in press). A review of the epidemiologic evidence concerning the reproductive health effects of caffeine consumption: A 2000–2009 update. *Food and Chemical Toxicology* doi:10.1016/j.fct.2010.06.019.

Rodopoulos, N, Hojvall, L and Norman, A. (1996). Elimination of theobromine metabolites in healthy adults. *Scandinavian Journal of Clinical and Laboratory Investigation* 56(4):373-383. (Cited in the Notification.)

9. Using data from NHANES 2003-2006 and the food codes reported, FDA calculated that 261 mg/p/d of theobromine is consumed at the mean and 367 mg/p/d at the 90th percentile for users. This is twice as high as 150 mg/person/day at the mean and 319 mg/person/day at the 90th percentile that was reported on page 19. In addition, FDA calculated a higher number of users (89.6%) vs. the 65.1% found in the Cantox assessment report (page 8 of Appendix 1). Please explain this discrepancy.

RESPONSE: It is difficult to explain this discrepancy without more information concerning the intake assessment conducted by the FDA and due to some confusion as to what values specifically the FDA is calling into question. The 65.1% users identified by Cantox refers only to the background intake of theobromine, and this was associated with intake of 61 and 147 mg/person/day at the mean and 90th percentile. This was calculated using all foodcodes in the NHANES database and by employing the USDA measured levels of theobromine. When the proposed food uses were added to the background levels, the intake was reported to be 150 and 319 mg/person/day, the numbers listed in the FDA question; however, this assessment was associated with 94.6% users, a number higher than that identified by the FDA. The FDA question does not detail whether the numbers referenced refer to calculations completed with the proposed food uses only or the proposed food uses and the background levels. It would be extremely helpful if this information could be provided and if the discrepancy between the scenarios being employed for comparison could be explained. In any case, the discrepancy in the levels of intake reported (150 and 319 mg/person/day as compared to the FDA derived 261 and 367 mg/person/day) likely result from differing approaches in food code selection with the powdered fruit flavored drinks being the most likely food use impacting the different estimates. Cantox limited the foodcodes chosen to represent this category to those identified as being produced from powders. If the FDA included all foodcodes from this category, which are widely consumed, this could explain the difference in the intake estimates.

10. The 90th percentile of consumption of powdered fruit-flavored drinks is nearly 11x larger than the reported mean on page 26. Typically, the amount consumed at the 90th percentile is 2x greater than the mean. Please explain this difference.

RESPONSE: The mean and 90th percentile all-person intakes of powdered fruit flavored drinks are reported on page 12 as being 12 and 135 mg/person/day, respectively. This is a typographical error as this is an error in the insertion of the

values from table A-7. The mean intake of powdered fruit flavored drinks is indeed equivalent to 12 mg/person/day; however, there is no 90th percentile value for this intake as insufficient data were available and so there is no value for the 90th percentile intake. Instead the mean all-user intake of 135 mg/person/day was mistakenly inserted in place of that information. The correct values were present in Table A-7; however, please find attached a version of the report with the correct values also reported on page 12.

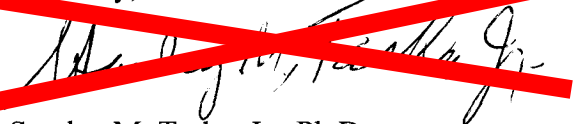
11. The description of the manufacturing process on page 15 states that sodium bicarbonate is added to 3-methylxanthine in acetone and water before the addition of dimethyl sulfate. On page 16, the figure and written description shows that 3-methylxanthine is converted to the potassium salt before the addition of dimethylsulfate. Are there two methods for preparation of theobromine, or is one method preferred over the other?

RESPONSE: The method that is preferred and ascribed to the theobromine material that is the subject of the Notification requires 3-methylxanthine to be converted to the potassium salt before the addition of dimethylsulfate.

In addition to the response to the questions raised, I am enclosing an electronic copy of the revised CanTox intake exposure document as referenced above as well as the three publications cited in the response to Question #8 for completeness. We trust that these responses have addressed the questions raised by the review team.

Should you have any additional questions regarding this GRAS Notice, please contact me at 717-243-9216 or by email. We look forward to FDA's completion of their review on this submission.

Sincerely,



Stanley M. Tarka, Jr., Ph.D.
President
The Tarka Group, Inc.

cc: Arman Sadeghpour, Ph.D., Theocorp Holding Company, LLC, Metairie, LA

Enclosures: Revised Intake Assessment Report prepared by CanTox Health Sciences;
Publications by Gu *et al.* (1992), Klebanoff *et al.* (1999), Peck *et al.* (in press),
and Leviton and Cowan (2002).

CANTOX

HEALTH SCIENCES INTERNATIONAL

Estimated Daily Intake of Theobromine by the U.S. Population from Proposed Food-Uses

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Estimated Daily Intake of Theobromine by the U.S. Population from Proposed Food-Uses

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Estimated Daily Intake of Theobromine by the U.S. Population from Proposed Food-Uses

1.0 INTRODUCTION

Cantox Health Sciences International has completed an assessment of the consumption of theobromine by the United States (U.S.) population as proposed for use in baked goods and baking mixes, breakfast cereals, beverages and beverage bases, bottled water, chewing gum, coffee and tea, dairy product analogs, gelatins, puddings, and custard, hard candy, milk products, processed fruits and fruit juices, and vitamin and mineral supplements. In addition to the intake solely from all proposed uses, the overall intake of theobromine based on all naturally occurring levels also was estimated.

Estimates for the intake of theobromine were based on the proposed food-uses and use-levels in conjunction with food consumption data included in the National Center for Health Statistics' (NCHS) National Health and Nutrition Examination Surveys (NHANES) (CDC, 2006; USDA, 2009a,b). The data from the 2003-2004 and 2005-2006 cycles of the NHANES survey were combined to provide a larger population from which to estimate theobromine consumption. Calculations for the mean and 90th percentile all-person and all-user intakes, and percent consuming were performed for each of the individual proposed food-uses of theobromine. Similar calculations were used to determine the estimated total intake of theobromine resulting from all proposed food-uses of theobromine combined. In both cases, the per person and per kilogram body weight intakes were reported for the following population groups:

- infants, ages 0 to 2;
- children, ages 3 to 11;
- female teenagers, ages 12 to 19;
- male teenagers, ages 12 to 19;
- female adults, ages 20 and up;
- male adults, ages 20 and up; and
- total population (all age and gender groups combined).

2.0 NHANES SURVEY DATA

2.1 Survey Description

National Health and Nutrition Examination Surveys (NHANES) for the years 2003-2004 and 2005-2006 are available for public use. NHANES are conducted as a continuous, annual survey, and are released in 2-year cycles. Each year about 7,000 people from 15 different locations across the U.S. are interviewed, and approximately 5,000 complete the health

examination component of the survey. Any combination of consecutive years of data collection is a nationally representative sample of the U.S. population. It is well established that the length of a dietary survey affects the estimated consumption of individual users and that short-term surveys, such as the typical 1-day dietary survey, overestimate consumption over longer time periods (Anderson, 1988). Because two 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) are available from the NHANES 2003-2004, 2005-2006 survey, these data were used to generate estimates for the current intake analysis.

NHANES 2003-2004, 2005-2006 survey data were collected from individuals and households *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) throughout all 4 seasons of the year. Day 1 data were collected in-person, and Day 2 data were collected by telephone in the following 3 to 10 days, on different days of the week, to achieve the desired degree of statistical independence. The data were collected by first selecting Primary Sampling Units (PSUs), which were counties throughout the U.S, of which 15 PSUs are visited per year. Small counties were combined to attain a minimum population size. These PSUs were segmented and households were chosen within each segment. One or more participants within a household were interviewed. For NHANES 2003-2004 12,761 individuals were selected for the sample, 10,122 were interviewed (79.3%), and 9,643 were sampled (75.6%). For NHANES 2005-2006 12,862 individuals were selected for the sample, 10,348 were interviewed (80.4%), and 9,950 were sampled (77.4%).

In addition to collecting information on the types and quantities of foods being consumed, NHANES 2003-2004 and 2005-2006 collected socioeconomic, physiological and demographic information from individual participants in the survey, such as sex, age, height and weight, and other variables useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total population. Sample weights were incorporated with NHANES 2003-2004 and 2005-2006 data to compensate for the potential under-representation of intakes from specific population groups as a result of sample variability due to survey design, differential non-response rates, or other factors, such as deficiencies in the sampling frame (CDC, 2006; USDA, 2009b).

2.2 Statistical Methods

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of theobromine by the U.S. population. Estimates for the daily intake of theobromine represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2005-2006 data; these average amounts comprised the distribution from which mean and percentile intake estimates were produced. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. All-person

intake refers to the estimated intake of theobromine averaged over all individuals surveyed, regardless of whether they potentially consumed food products containing theobromine, and therefore includes “zero” consumers (those who reported no intake of food products containing theobromine during the 2 survey days). All-user intake refers to the estimated intake of theobromine by those individuals potentially consuming food products containing theobromine, hence the “all-user” designation. Individuals were considered users if they consumed 1 or more food products containing theobromine on either Day 1 or Day 2 of the survey.

2.3 Statistical Reliability

Mean or percentile intake estimates based on small sample sizes or with high variability relative to the mean [assessed using the coefficient of variation (CV)] may be less statistically reliable than estimates based on adequate sample sizes or low variability relative to the mean (LSRO, 1995). Data presented herein for the estimated daily intake of theobromine follow the guidelines proposed by the Human Nutrition Information Service/National Center for Health Statistics Analytic Working Group for evaluating the reliability of statistical estimates adopted in the “Third Report on Nutrition Monitoring in the United States”, whereby an estimated mean may be unreliable if the CV is equal to or greater than 30% (LSRO, 1995). The CV is the ratio of the estimated standard error of the mean to the estimated mean, expressed as a percentage (LSRO, 1995). Therefore, for the estimated intakes of theobromine presented herein, values were considered statistically unreliable if the CV was equal to or greater than 30% or the sample size is less than 30 respondents. These values were not considered when assessing the relative contribution of specific food-uses to total theobromine consumption and are marked with an asterisk.

3.0 FOOD USAGE DATA

The individual proposed food-uses and use-levels for theobromine employed in the current intake analysis are summarized in Table 3-1. Food codes representative of each proposed food-use, with the exception of calcium chews, were chosen from the NHANES 2005-2006 (CDC, 2006; USDA, 2009b). Food codes were grouped in food-use categories according to Title 21, Section §170.3 of the Code of Federal Regulations (CFR, 2009a). Product-specific adjustment factors were developed based on data provided in the standard recipe file for the CSFII 1994-1996, 1998 survey (USDA, 2000). All food codes included in the current intake assessment are listed in Appendix C.

The background intakes of theobromine were calculated using the USDA’s Food and Nutrient Database for Dietary Studies 3.0 (FNDDS 3.0) (USDA, 2009a,b). This database contains information pertaining to the content of 63 nutrients/food components, including theobromine, for all food codes employed in the NHANES intake assessment. As such the calculation of the background intakes was completed employing the theobromine variable from the FNDDS 3.0 as

the daily food amount for each food code in the NHANES survey. The same statistical methodology described above was then employed to generate mean and 90th percentile intake estimates for the all-person and all-user designations.

For calcium chews, the dietary supplement surveys from the 2003-2004 and 2005-2006 were employed in the selection of the representative supplement codes. The dietary supplement survey is conducted in conjunction with the NHANES dietary survey and documents a variety of information concerning the nature and frequency of dietary supplement consumption. The survey is conducted during the household interview and the questions included in the Dietary Supplement Questionnaire are designed to reflect the consumption of dietary supplements over the past 30 days. As such the data has been adjusted to allow the average daily intake of theobromine from this proposed use (based on the reported consumption of the 30 day period) to be incorporated into the estimated intake from foods. To select codes reflective of the proposed inclusion of theobromine in calcium chews, all codes with a serving size unit code for "chew" (DSD122U = 20) were selected. For all of these codes, the average daily consumption in g was based on the reported intake over the previous 30 days. The proposed use level for theobromine in calcium chews was then applied to derive an estimated daily intake of theobromine from these products. This intake was then added to the estimated intake of theobromine from all remaining proposed food uses for each identified consumer, which in this instance was limited to 16 individuals within the total U.S. population (Table A-7).

Table 3-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Theobromine in the U.S.				
Food Category	Proposed Food-Uses	Theobromine Level (mg/serving)	Serving Size (g or mL)	Use-Levels (%)
Baked Goods and Baking Mixes	Bread	15	25 ²	0.060
Breakfast Cereals	Instant and Regular Oatmeal	30	37 ²	0.13
	Ready-to-Eat Cereals	30	30 ²	0.10
Beverages and Beverage Bases	Sports, Isotonic, and Energy Drinks	60	488 ²	0.012
	Meal Replacement Beverages, Non Milk-Based	75	240	0.031
Bottled Water	Vitamin, Enhanced, and Bottled Waters	40	240	0.017
Chewing Gum	Chewing Gum	10	3	0.33
Coffee and Tea	Tea	40	488 ²	0.0082
Dairy Product Analogs	Soy Milk	40	250	0.016
Gelatins, Puddings, and Custard	Gelatin	40	85 ²	0.047
Hard Candy	Mints	5	2	0.25
Milk Products	Meal Replacement Beverages, Milk-Based	75	240	0.031
	Yogurt (fresh, not-chocolate)	50	170 ²	0.029
	Yogurt Drinks ³	25	28 ²	0.089

Table 3-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Theobromine in the U.S.

Food Category	Proposed Food-Uses	Theobromine Level (mg/serving)	Serving Size (g or mL)	Use-Levels (%)
Processed Fruits and Fruit Juices	Fruit Smoothies	50	366 ²	0.014
	Powdered Fruit-Flavored Drinks	50	8 ²	0.62
Vitamin and Mineral Supplements	Calcium Chews	60	4.5	0.013

¹ Unless otherwise indicated serving sizes were based on the Reference Amounts Customarily Consumed per Eating Occasion (RACC) (21 CFR §101.12 - CFR, 2009b). When a range of values is reported for a proposed food-use, particular foods within that food-use may differ with respect to their RACC.

² Serving size provided by The Tarka Group, Inc.

³ Food codes from yogurt drinks are not included in the NHANES survey data and therefore codes for dairy-based fruit smoothie drinks were employed as surrogate codes.

4.0 FOOD SURVEY RESULTS

Estimated for the background intake of theobromine based on the USDA nutrient data for the NHANES data, as described in Section 3.0, are presented in Section 4.1. Estimates for the total daily intakes of theobromine from all proposed food-uses alone are provided in Tables 4.2-1 and 4.2-2, while the combined intakes are presented in Tables 4.2-3 and 4.2-4. Estimates for the daily intake of theobromine from individual proposed food-uses in the U.S. are summarized in Tables A-1 to A-7 and B-1 to B-7 of Appendices A and B, respectively. Tables A-1 to A-7 provide estimates for the daily intake of theobromine per person (mg/day), whereas Tables B-1 to B-7 provide estimates for the daily intake of theobromine on a per kilogram body weight basis (mg/kg body weight/day).

4.1 Estimated Daily Background Intake of Theobromine

As described in Section 3.0, the USDA nutrient database was combined with the NHANES 2003-2004, 2005-2006 dietary intake data to estimate the background intake of theobromine. Approximately 65.1% of the total U.S. population was identified as potential consumers of theobromine on a regular basis. Consumption of a standard diet by the total U.S. population resulted in estimated daily mean all-person and all-user intakes of theobromine of 43 mg/person/day (0.8 mg/kg body weight/day) and 61 mg/person/day (1.1 mg/kg body weight/day), respectively. The estimated daily 90th percentile all-person and all-user intakes of theobromine within the total population were 123 mg/person/day (2.2 mg/kg body weight/day) and 147 mg/person/day (2.9 mg/kg body weight/day), respectively.

Table 4.1-1 Summary of the Estimated Daily Intake of Theobromine from All Background Levels in the U.S. by Population Group (2003-2004, 2005-2006 NHANES Data)

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption (mg)		All-User Consumption (mg)	
				Mean	90 th Percentile	Mean	90 th Percentile
Infants	0 to 2	37.9	705	18	57	41	111
Children	3 to 11	78.8	2,153	61	150	74	158
Female Teenagers	12 to 19	69.2	1,375	44	111	61	136
Male Teenagers	12 to 19	66.4	1,289	52	159	75	199
Female Adults	20 and Up	68.0	2,911	40	115	55	133
Male Adults	20 and Up	62.4	2,397	40	122	61	152
Total Population	All Ages	65.1	10,830	43	123	61	147

The intake of theobromine from the typical diet was most prevalent among children with 78.8% of this population group identified as consumers of foods containing theobromine. Within the individual population groups, the largest mean daily all-person intake of theobromine was identified as occurring in children with an intake of 61 mg/person/day. The largest mean daily all-user intake was observed to occur in male teenagers for whom the background daily intake of theobromine was equivalent to 75 mg/person/day. Infants displayed the lowest estimate for the mean daily all-person and all-user intakes of theobromine on an absolute basis with values of 18 and 41 mg/person/day, respectively. On a body weight basis, estimated mean daily all-person intake of theobromine was observed to be highest in children at 2.3 mg/kg body weight/day while the highest estimate for the mean daily all-user intake of theobromine was observed to occur in infants at 3.2 mg/kg body weight/day. The lowest all-person and all-user mean daily intakes of theobromine on a per kilogram body weight basis were observed to occur in male adults at 0.5 and 0.7 mg/kg body weight/day, respectively.

Table 4.1-2 Summary of the Estimated Daily per Kilogram Body Weight Intake of Theobromine from All Background Levels in the U.S. by Population Group (2003-2004, 2005-2006 NHANES Data)

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption (mg/kg)		All-User Consumption (mg/kg)	
				Mean	90 th Percentile	Mean	90 th Percentile
Infants	0 to 2	37.9	705	1.4	4.6	3.2	8.1
Children	3 to 11	78.8	2,153	2.3	5.6	2.8	6.2
Female Teenagers	12 to 19	69.2	1,375	0.8	2.0	1.1	2.3
Male Teenagers	12 to 19	66.4	1,289	0.9	2.6	1.2	3.2
Female Adults	20 and Up	68.0	2,911	0.6	1.6	0.8	1.9
Male Adults	20 and Up	62.4	2,397	0.5	1.4	0.7	1.8
Total Population	All Ages	65.1	10,830	0.8	2.2	1.1	2.9

When heavy consumers (90th percentile) were assessed, the largest daily all-person and all-user intakes of theobromine were determined to occur in male teenagers at 159 and 199 mg/person/day, respectively. The lowest 90th percentile all-person and all-user mean daily intakes of theobromine were identified in infants, with values of 57 and 111 mg/person/day, respectively, on an absolute basis. On a body weight basis, children and infants were determined to have the greatest all-person and all-user 90th percentile intakes of theobromine respectively, with values of 5.6 and 8.1 mg/kg body weight/day, respectively. The lowest all-person and all-user 90th percentile intakes of theobromine on a body weight basis were observed to occur in male adults with intakes of 1.4 and 1.8 mg/kg body weight/day, respectively.

4.2 Estimated Daily Intake of Theobromine from All Proposed Food-Uses

The estimated total intake of theobromine from all proposed food-uses in combination with the existing levels presented in foods in the U.S. by population group is summarized in Table 4.2-1. Table 4.2-2 presents these data on a per kilogram body weight basis.

Approximately 94.6% of the total U.S. population was identified as potential consumers of theobromine from either the proposed food-uses or naturally occurrence in foods (15,737 actual users identified). Consumption of all of these types of foods by the total U.S. population resulted in estimated mean all-person and all-user intakes of theobromine of 145 and 150 mg/person/day, respectively, equivalent to 2.6 and 2.7 mg/kg body weight/day, respectively, on a body weight basis. The 90th percentile all-person and all-user intakes of theobromine from all proposed food-uses and naturally occurring levels by the total population were 314 and 319 mg/person/day, respectively, or 5.7 and 5.8 mg/kg body weight/day, respectively.

Children represented the population group containing the largest percentage of theobromine consumers based on the background levels and proposed food uses with 99.3% of individuals within this groups identified as potential theobromine consumers. A high percentage of potential theobromine users were also identified in male and female adults and teenagers with more than 96% of these population groups identified as potential consumers of theobromine. On an individual population basis, the greatest mean all-person and all-user intakes of theobromine on an absolute basis were determined to occur in male teenagers, at 157 and 161 mg/person/day, respectively. Infants continue to be the population group with the lowest identified intake of theobromine with mean all-person and all-user intakes of theobromine of 63 and 77 mg/person/day, respectively. On a body weight basis, the mean all-person estimate for the intake of theobromine was highest in children at 5.6 mg/kg body weight/day. The mean all-user estimate for the intake of theobromine was highest in infant at 6.3 mg/kg body weight/day. The lowest all-person and all-user mean intakes of theobromine on a per kilogram body weight basis was observed to occur in male adults with a values of 1.8 and 1.9 mg/kg body weight/day, respectively.

Table 4.2-1 Summary of the Estimated Daily Intake of Theobromine from All Background Levels and Proposed Food Uses in the U.S. by Population Group (2003-2004, 2005-2006 NHANES Data)

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption (mg)		All-User Consumption (mg)	
				Mean	90 th Percentile	Mean	90 th Percentile
Infants	0 to 2	74.3	1,381	63	154	77	170
Children	3 to 11	99.3	2,713	148	285	149	285
Female Teenagers	12 to 19	97.2	1,931	133	275	137	280
Male Teenagers	12 to 19	96.8	1,877	157	329	161	335
Female Adults	20 and Up	96.7	4,142	144	318	148	321
Male Adults	20 and Up	96.1	3,693	155	339	160	341
Total Population	All Ages	94.6	15,737	145	314	150	319

When heavy consumers (90th percentile) were assessed, all-person and all-user intakes of theobromine from all proposed food-uses and background sources were determined to be greatest in male adults at 339 and 341 mg/person/day, respectively. The lowest 90th percentile all-person and all-user intake estimates were identified as occurring in infants, with values of 154 and 177 mg/person/day, respectively, on an absolute basis. On a body weight basis, infants were determined to have the greatest all-person and all-user 90th percentile intakes of theobromine with values of 12.7 and 14.1 mg/kg body weight/day, respectively. The lowest all-person and all-user 90th percentile intakes of theobromine on a body weight basis were observed in male adults with intake values of 4.0 and 4.1 mg/kg body weight/day, respectively.

Table 4.2-2 Summary of the Estimated Daily per Kilogram Body Weight Intake of Theobromine from All Background Levels and Proposed Food Uses in the U.S. by Population Group (2003-2004, 2005-2006 NHANES Data)

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption (mg/kg bw)		All-User Consumption (mg/kg bw)	
				Mean	90 th Percentile	Mean	90 th Percentile
Infants	0 to 2	74.3	1,381	5.1	12.7	6.3	14.1
Children	3 to 11	99.3	2,713	5.6	10.9	5.7	10.9
Female Teenagers	12 to 19	97.2	1,931	2.3	4.7	2.3	4.7
Male Teenagers	12 to 19	96.8	1,877	2.5	5.5	2.5	5.5
Female Adults	20 and Up	96.7	4,142	2.0	4.4	2.1	4.5
Male Adults	20 and Up	96.1	3,693	1.8	4.0	1.9	4.1
Total Population	All Ages	94.6	15,737	2.6	5.7	2.7	5.8

4.3 Estimated Daily Intake of Theobromine from Individual Proposed Food-Uses

4.3.1 All-Person Intakes

Estimates for the mean and 90th percentile daily intakes of theobromine from each individual proposed food-use are summarized in Tables A-1 to A-7 and B-1 to B-7 on a mg/day and mg/kg body weight/day basis, respectively. Tables A-7 and B-7 summarize the estimates for the mean all-person intakes of theobromine by the total population (all ages) from each of the individual proposed food-uses on a mg/person/day and mg/kg body weight/day basis, respectively. The total U.S. population was identified as being significant consumers of bread (67.6% users), ready-to-eat cereals (42.3% users), and vitamin, enhanced, and bottled waters (22.5% users).

Consumption of vitamin, enhanced, and bottled waters provided the largest mean and 90th percentile all-person intakes of theobromine at 28 and 105 mg/person/day, respectively, within the total U.S. population. The intakes were equivalent to 0.41 and 1.85 mg/kg body weight/day on a body weight basis. In addition, high mean and 90th percentile all-person intakes of theobromine resulted from the consumption of bread (20 and 28 mg/person/day, respectively), ready-to-eat cereals (15 and 36 mg/person/day, respectively), and powdered fruit-flavored drinks (12 mg/person/day for the mean, insufficient users identified to establish a 90th percentile intake). On a body weight basis, mean and 90th percentile all-person intakes for bread were 0.34 and 0.50 mg/kg body weight/day, for ready-to-eat cereals were 0.29 and 0.10 mg/kg body weight/day, and for powdered fruit-flavored drinks were 0.22 and 0.43 mg/kg body weight/day, respectively.

Within the individual population groups, the highest mean all-person intakes of theobromine resulting from consumption of any individual proposed food uses were determined to result from

the consumption of vitamin, enhanced, and bottled waters in male and female adults and teenagers. The consumption of ready-to-eat cereals produced the greatest mean all-person intakes of theobromine in children and infants (Tables A-1 to A-6 and Tables B-1 to B-6). For the 90th percentile intake of theobromine, the consumption of vitamin, enhanced, and bottled waters again produced the largest intake of theobromine in male and female adults and female teenagers, with the consumption of ready-to-eat cereals producing the largest intake of theobromine in male teenagers, children, and infants. The highest mean and 90th percentile all-person intakes of theobromine resulting from the consumption of any individual proposed food use for theobromine were observed to occur in reported in female adults consuming vitamin, enhanced, and bottled waters which produced an intake estimates of 35 and 138 mg/person/day, respectively. On a body weight basis, consumption of ready-to-eat cereals by children led to the highest estimates for the mean and 90th percentile all-person intake of theobromine at 0.82 and 1.96 mg/kg body weight/day, respectively.

4.3.2 All-User Intakes

Tables A-7 and B-7 also summarize the estimates for the mean all-user intakes of theobromine by the total population (all ages) from each of the individual food-uses on a mg/person/day and mg/kg body weight/day basis, respectively. For all-user intakes, the contribution of each food-use to the overall intake is a function of both the estimated intake of theobromine resulting from the consumption of the food, as well as the percentage of users identified as consumers of the food. For example, within the total population, the consumption of fruit smoothies resulted in an estimated mean all-user theobromine intake of 192 mg/person/day; however, only 164 users (1.0% of the total population) of fruit smoothies meal replacement drinks were identified and therefore, the contribution of this food-use to the mean all-user intake of theobromine was not as important as the contribution of powdered fruit-flavored drinks with an intake of 135 mg/person/day in 1,704 users (10.2% of the population).

The consumption of vitamin, enhanced, and bottled waters made the greatest contribution to the mean and 90th percentile all-user intakes of theobromine at 126 and 281 mg/person/day, respectively, equivalent to 1.85 and 3.91 3.83 mg/kg body weight/day, respectively. Of the other proposed food-uses, the consumption of bread, ready-to-eat cereals, and powdered fruit-flavored drinks also made significant contributions to the estimates for the mean (27, 36, and 135 mg/person/day, respectively) and 90th percentile (54, 69, and 291 mg/person/day, respectively) all-user intake of theobromine by the total population. On a body weight basis, these intakes were equivalent to 0.47, 0.72, and 2.53 mg/kg body weight/day at the mean and 0.94, 1.50 and 5.13 mg/kg body weight/day at the 90th percentile.

Within the individual population groups, the consumption of instant and regular oatmeal and ready-to-eat cereals made the most significant contribution to the estimates for the mean intake of theobromine in infants and children, respectively. At the 90th percentile, the consumption of instant and regular oatmeal and powdered fruit-flavored drinks made the most significant

contribution to the all-user intake in infants and children, respectively. The consumption of vitamin, enhanced, and bottled waters was observed to make the most significant contribution to the mean and 90th percentile all-user intake of theobromine in male and female teenagers and adults. Female adults consuming ready-to-drink coffee made the largest contribution to the estimates for the mean and 90th all-user intake of theobromine with values of 142 and 311 mg/person/day, respectively. On a per kilogram body weight basis, infants consuming instant and regular hot oatmeal experienced the highest statistically reliable mean and 90th percentile all-user intakes of theobromine at 7.14 and 14.06 mg/kg body weight/day, respectively.

The estimated intakes of theobromine were considered statistically unreliable if the CV was equal to or greater than 30% or the sample size was less than 30 individuals. Assessing the CV for all-user intake estimates found the intake for calcium chews to be statistically unreliable in all population groups. Soy milk, yogurt drinks, fruit smoothies, and meal replacement beverages (milk based and non-milk based) were food categories in which the intakes were statistically unreliable in the infant, children, and female and male teenager population groups. Assessing the sample size for all-user intake estimates found the intake for chewing gum to be statistically unreliable in infants. Gelatins also had a low number of users in children and male and female teenagers resulting in higher CV values.

5.0 CONCLUSIONS

Consumption data and information pertaining to the individual proposed food-uses of theobromine were used to estimate the all-person and all-user intakes of theobromine for specific demographic groups and for the total U.S. population. This type of intake methodology is generally considered to be “worst case” as a result of several conservative assumptions made in the consumption estimates. For example, it is often assumed that all food products within a food category contain the ingredient at the maximum specified level of use. In addition, it is well established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the typical 2- or 3-day dietary surveys, overestimate the consumption of food products that are consumed relatively infrequently.

In summary, on an all-user basis, the mean intake of theobromine by the total U.S. population from all proposed food-uses was estimated to be 150 mg/person/day or 2.7 mg/kg body weight/day. The heavy consumer (90th percentile) all-user intake of theobromine by the total U.S. population from all proposed food-uses was estimated to be 319 mg/person/day or 5.8 mg/kg body weight/day.

6.0 REFERENCES

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APPENDIX A

Estimated Daily Intake of Theobromine from Individual Proposed Food-Uses by Different Population Groups Within the United States

Table A-1 Estimated Daily Intake of Theobromine from Individual Proposed Food-Uses by Infants (Aged 0 to 2 Years) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg)		All-User Consumption (mg)	
			Mean	90 th Percentile	Mean	90 th Percentile
<u>Baked Goods and Baking Mixes</u>						
Bread	39.9	765	6	21	13	26
<u>Breakfast Cereals</u>						
Instant and Regular Oatmeal	9.2	176	8	na	83	144
Ready-to-Eat Cereals	42.0	803	8	23	15	30
<u>Beverages and Beverage Bases</u>						
Sports, Isotonic, and Energy Drinks	3.7	70	1	na	34	84
Meal Replacement Beverages, Non Milk-Based	0.3	5	<1*	na	44*	69*
<u>Bottled Water</u>						
Vitamin, Enhanced, and Bottled waters	18.7	358	5	15	33	75
<u>Chewing Gum</u>						
Chewing Gum	1.1	21	<0.1*	na	6*	14*
<u>Coffee and Tea</u>						
Tea	2.5	47	<0.1	na	7	19
<u>Dairy Product Analogs</u>						
Soy Milk	1.0	20	1*	na	55*	125*
<u>Gelatins, Puddings, and Custard</u>						
Gelatin	3.1	59	1	na	24	56
<u>Hard Candy</u>						
Mints	9.2	176	2	na	18	53
<u>Milk Products</u>						
Meal Replacement Beverages, Milk-Based	0.1	2	<1*	na	11*	13*
Yogurt (fresh)	14.7	281	5	18	23	41
Yogurt Drinks	0.6	12	<1*	na	18*	52*
<u>Processed Fruits and Fruit Juices</u>						

Table A-1 Estimated Daily Intake of Theobromine from Individual Proposed Food-Uses by Infants (Aged 0 to 2 Years) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg)		All-User Consumption (mg)	
			Mean	90 th Percentile	Mean	90 th Percentile
Powdered Fruit-Flavored Drinks	7.7	148	5	na	61	121
Fruit Smoothies	0.9	19	1*	na	136*	338*
<u>Vitamin and Mineral Supplements</u>						
Calcium Chews	0.1	1	<1*	na	<1*	<1*

na = not available

*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% (see Section 2.3).

Table A-2 Estimated Daily Intake of Theobromine from Individual Proposed Food-Uses by Children (Aged 3 to 11 Years) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg)		All-User Consumption (mg)	
			Mean	90 th Percentile	Mean	90 th Percentile
<u>Baked Goods and Baking Mixes</u>						
Bread	70.8	1,934	17	42	24	46
<u>Breakfast Cereals</u>						
Instant and Regular Oatmeal	6.8	186	7	na	103	172
Ready-to-Eat Cereals	67.8	1,851	21	50	31	58
<u>Beverages and Beverage Bases</u>						
Sports, Isotonic, and Energy Drinks	3.7	102	1	na	30	44
Meal Replacement Beverages, Non Milk-Based	0.3	7	<0.1*	na	33*	49*
<u>Bottled Water</u>						
Vitamin, Enhanced, and Bottled waters	21.8	598	11	39	54	116
<u>Chewing Gum</u>						
Chewing Gum	6.7	182	1	na	10	19
<u>Coffee and Tea</u>						
Tea	6.1	166	1	na	15	25
<u>Dairy Product Analogs</u>						
Soy Milk	1.0	26	1*	na	35*	78*
<u>Gelatins, Puddings, and Custard</u>						
Gelatin	4.6	126	1	na	32	56
<u>Hard Candy</u>						
Mints	17.6	479	5	18	27	58
<u>Milk Products</u>						
Meal Replacement Beverages, Milk-Based	0.4	10	<0.1*	na	35*	60*
Yogurt (fresh)	13.5	368	4	17	26	50
Yogurt Drinks	0.8	23	<0.1*	na	17*	28*
<u>Processed Fruits and Fruit Juices</u>						
Powdered Fruit-Flavored Drinks	17.7	482	16	49	103	239

Table A-2 Estimated Daily Intake of Theobromine from Individual Proposed Food-Uses by Children (Aged 3 to 11 Years) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg)		All-User Consumption (mg)	
			Mean	90 th Percentile	Mean	90 th Percentile
Fruit Smoothies	1.0	28	1*	na	100*	129*
<u>Vitamin and Mineral Supplements</u>						
Calcium Chews	0.1	4	<1*	na	2*	3*

na = not available

*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% (see Section 2.3).

Table A-3 Estimated Daily Intake of Theobromine from Individual Proposed Food-Uses by Female Teenagers (Aged 12 to 19 Years) Within the United States (2003-2004, 2005-2006 NHANES Data)						
Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg)		All-User Consumption (mg)	
			Mean	90 th Percentile	Mean	90 th Percentile
<u>Baked Goods and Baking Mixes</u>						
Bread	64.2	1,277	19	47	28	55
<u>Breakfast Cereals</u>						
Instant and Regular Oatmeal	2.5	50	3	na	109	219
Ready-to-Eat Cereals	42.8	849	16	48	36	64
<u>Beverages and Beverage Bases</u>						
Sports, Isotonic, and Energy Drinks	3.4	68	1	na	28	41
Meal Replacement Beverages, Non Milk-Based	0.3	5	<0.1*	na	19*	85*
<u>Bottled Water</u>						
Vitamin, Enhanced, and Bottled waters	26.4	526	26	99	105	222
<u>Chewing Gum</u>						
Chewing Gum	9.1	181	1	na	13	37
<u>Coffee and Tea</u>						
Tea	12.2	242	2	10	21	42
<u>Dairy Product Analogs</u>						
Soy Milk	0.7	14	<0.1*	na	53*	97*
<u>Gelatins, Puddings, and Custard</u>						
Gelatin	1.6	31	<0.1*	na	25*	51*
<u>Hard Candy</u>						
Mints	12.3	245	2	3	21	52
<u>Milk Products</u>						
Meal Replacement Beverages, Milk-Based	0.7	13	1*	na	63*	85*
Yogurt (fresh)	6.7	132	2	na	33	58
Yogurt Drinks	1.2	23	<0.1*	na	26*	50*

Table A-3 Estimated Daily Intake of Theobromine from Individual Proposed Food-Uses by Female Teenagers (Aged 12 to 19 Years) Within the United States (2003-2004, 2005-2006 NHANES Data)						
Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg)		All-User Consumption (mg)	
			Mean	90 th Percentile	Mean	90 th Percentile
<u>Processed Fruits and Fruit Juices</u>						
Powdered Fruit-Flavored Drinks	13.5	268	12	30	106	212
Fruit Smoothies	1.7	34	2*	na	185*	326*
<u>Vitamin and Mineral Supplements</u>						
Calcium Chews	0.2	3	<1*	na	1*	4*

na = not available

*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% (see Section 2.3).

Table A-4 Estimated Daily Intake of Theobromine from Individual Proposed Food-Uses by Male Teenagers (Aged 12 to 19 Years) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg)		All-User Consumption (mg)	
			Mean	90 th Percentile	Mean	90 th Percentile
<u>Baked Goods and Baking Mixes</u>						
Bread	68.7	1,332	21	49	31	56
<u>Breakfast Cereals</u>						
Instant and Regular Oatmeal	2.0	38	2	na	116	190
Ready-to-Eat Cereals	44.8	868	21	66	48	89
<u>Beverages and Beverage Bases</u>						
Sports, Isotonic, and Energy Drinks	7.1	137	5	na	48	90
Meal Replacement Beverages, Non Milk-Based	0.7	13	<0.1*	na	28*	44*
<u>Bottled Water</u>						
Vitamin, Enhanced, and Bottled waters	21.5	419	22	62	105	247
<u>Chewing Gum</u>						
Chewing Gum	7.2	139	1	na	12	28
<u>Coffee and Tea</u>						
Tea	12.2	236	4	15	29	50
<u>Dairy Product Analogs</u>						
Soy Milk	0.5	9	<0.1*	na	33*	50*
<u>Gelatins, Puddings, and Custard</u>						
Gelatin	1.4	28	1*	na	45*	90*
<u>Hard Candy</u>						
Mints	9.2	179	2	na	24	53
<u>Milk Products</u>						
Meal Replacement Beverages, Milk-Based	0.6	11	<0.1*	na	50*	70*
Yogurt (fresh)	3.6	70	2	na	40	74
Yogurt Drinks	0.7	14	<0.1*	na	32*	48*

Table A-4 Estimated Daily Intake of Theobromine from Individual Proposed Food-Uses by Male Teenagers (Aged 12 to 19 Years) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg)		All-User Consumption (mg)	
			Mean	90 th Percentile	Mean	90 th Percentile
<u>Processed Fruits and Fruit Juices</u>						
Powdered Fruit-Flavored Drinks	13.0	253	21	64	162	305
Fruit Smoothies	1.1	22	2*	na	215*	279*
<u>Vitamin and Mineral Supplements</u>						
Calcium Chews	0.1	2	<1*	na	<1*	1*

na = not available

*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% (see Section 2.3).

Table A-5 Estimated Daily Intake of Theobromine from Individual Proposed Food-Uses by Female Adults (Aged 20 Years and Over) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg)		All-User Consumption (mg)	
			Mean	90 th Percentile	Mean	90 th Percentile
<u>Baked Goods and Baking Mixes</u>						
Bread	72.9	3,123	18	42	24	46
<u>Breakfast Cereals</u>						
Instant and Regular Oatmeal	12.9	553	13	39	123	219
Ready-to-Eat Cereals	34.7	1,485	11	39	33	61
<u>Beverages and Beverage Bases</u>						
Sports, Isotonic, and Energy Drinks	1.1	49	1	na	45	111
Meal Replacement Beverages, Non Milk-Based	1.2	54	0	na	33	73
<u>Bottled Water</u>						
Vitamin, Enhanced, and Bottled waters	24.6	1,059	35	138	142	311
<u>Chewing Gum</u>						
Chewing Gum	4.1	175	1	na	17	28
<u>Coffee and Tea</u>						
Tea	13.0	557	4	15	31	64
<u>Dairy Product Analogs</u>						
Soy Milk	2.7	115	1	na	30	70
<u>Gelatins, Puddings, and Custard</u>						
Gelatin	3.6	155	1	na	31	66
<u>Hard Candy</u>						
Mints	6.9	292	2	na	22	45
<u>Milk Products</u>						
Meal Replacement Beverages, Milk-Based	2.1	93	2	na	64	107
Yogurt (fresh)	10.9	469	4	17	31	52
Yogurt Drinks	1.3	57	1	na	27	52

Table A-5 Estimated Daily Intake of Theobromine from Individual Proposed Food-Uses by Female Adults (Aged 20 Years and Over) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg)		All-User Consumption (mg)	
			Mean	90 th Percentile	Mean	90 th Percentile
<u>Processed Fruits and Fruit Juices</u>						
Powdered Fruit-Flavored Drinks	6.9	294	10	na	139	316
Fruit Smoothies	1.9	79	2	na	152	270
<u>Vitamin and Mineral Supplements</u>						
Calcium Chews	0.1	5	<1*	na	3*	5*

na = not available

*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% (see Section 2.3).

Table A-6 Estimated Daily Intake of Theobromine from Individual Proposed Food-Uses by Male Adults (Aged 20 Years and Over) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg)		All-User Consumption (mg)	
			Mean	90 th Percentile	Mean	90 th Percentile
<u>Baked Goods and Baking Mixes</u>						
Bread	74.6	2,868	26	59	34	63
<u>Breakfast Cereals</u>						
Instant and Regular Oatmeal	9.3	360	12	na	159	314
Ready-to-Eat Cereals	31.2	1,199	15	54	46	87
<u>Beverages and Beverage Bases</u>						
Sports, Isotonic, and Energy Drinks	4.2	160	2	na	45	99
Meal Replacement Beverages, Non Milk-Based	1.8	68	1	na	51	117
<u>Bottled Water</u>						
Vitamin, Enhanced, and Bottled waters	20.9	805	32	119	145	316
<u>Chewing Gum</u>						
Chewing Gum	3.3	126	<1	na	12	28
<u>Coffee and Tea</u>						
Tea	13.3	509	5	19	40	85
<u>Dairy Product Analogs</u>						
Soy Milk	1.6	61	1	na	33	72
<u>Gelatins, Puddings, and Custard</u>						
Gelatin	2.6	104	1	na	39	76
<u>Hard Candy</u>						
Mints	5.5	210	1	na	23	53
<u>Milk Products</u>						
Meal Replacement Beverages, Milk-Based	1.6	62	2	na	88	200
Yogurt (fresh)	5.4	206	2	na	32	59
Yogurt Drinks	0.9	35	1	na	39	93

Table A-6 Estimated Daily Intake of Theobromine from Individual Proposed Food-Uses by Male Adults (Aged 20 Years and Over) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg)		All-User Consumption (mg)	
			Mean	90 th Percentile	Mean	90 th Percentile
<u>Processed Fruits and Fruit Juices</u>						
Powdered Fruit-Flavored Drinks	6.8	259	11	na	172	370
Fruit Smoothies	1.4	54	3	na	280	630
<u>Vitamin and Mineral Supplements</u>						
Calcium Chews	0.1	1	<1*	na	<1*	<1*

na = not available

*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% (see Section 2.3).

Table A-7 Estimated Daily Intake of Theobromine from Individual Proposed Food-Uses by the Total Population (All Ages) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg)		All-User Consumption (mg)	
			Mean	90 th Percentile	Mean	90 th Percentile
<u>Baked Goods and Baking Mixes</u>						
Bread	67.6	11,299	20	47	28	54
<u>Breakfast Cereals</u>						
Instant and Regular Oatmeal	8.1	1,363	11	na	130	234
Ready-to-Eat Cereals	42.3	7,055	15	48	36	70
<u>Beverages and Beverage Bases</u>						
Sports, Isotonic, and Energy Drinks	3.5	586	1	na	41	88
Meal Replacement Beverages, Non Milk-Based	0.9	152	1	na	41	103
<u>Bottled Water</u>						
Vitamin, Enhanced, and Bottled waters	22.5	3,765	28	105	126	281
<u>Chewing Gum</u>						
Chewing Gum	5.0	824	1	na	14	27
<u>Coffee and Tea</u>						
Tea	10.5	1,757	4	13	33	67
<u>Dairy Product Analogs</u>						
Soy Milk	1.5	245	1	na	33	77
<u>Gelatins, Puddings, and Custard</u>						
Gelatin	3.0	503	1	na	34	64
<u>Hard Candy</u>						
Mints	9.5	1,581	2	na	24	53
<u>Milk Products</u>						
Meal Replacement Beverages, Milk-Based	1.1	191	1	na	72	113
Yogurt (fresh)	9.1	1,526	3	na	30	54
Yogurt Drinks	1.0	164	<1	na	30	52

Table A-7 Estimated Daily Intake of Theobromine from Individual Proposed Food-Uses by the Total Population (All Ages) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg)		All-User Consumption (mg)	
			Mean	90 th Percentile	Mean	90 th Percentile
<u>Processed Fruits and Fruit Juices</u>						
Powdered Fruit-Flavored Drinks	10.2	1,704	12	na	135	291
Fruit Smoothies	1.4	236	2	na	192	338
<u>Vitamin and Mineral Supplements</u>						
Calcium Chews	<0.1	16	<1*	na	2*	5*

na = not available

*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% (see Section 2.3).

APPENDIX B

Estimated Daily per Kilogram Body Weight Intake of Theobromine from Individual Proposed Food-Uses by Different Population Groups Within the United States

Table B-1 Estimated Daily per Kilogram Body Weight Intake of Theobromine from Individual Proposed Food-Uses by Infants (Aged 0 to 2 Years) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg/kg bw)		All-User Consumption (mg/kg bw)	
			Mean	90 th Percentile	Mean	90 th Percentile
<u>Baked Goods and Baking Mixes</u>						
Bread	39.9	765	0.53	1.57	1.03	2.12
<u>Breakfast Cereals</u>						
Instant and Regular Oatmeal	9.2	176	0.67	na	7.14	14.06
Ready-to-Eat Cereals	42.0	803	0.64	1.90	1.23	2.49
<u>Beverages and Beverage Bases</u>						
Sports, Isotonic, and Energy Drinks	3.7	70	0.11	na	3.10	8.16
Meal Replacement Beverages, Non Milk-Based	0.3	5	0.01*	na	3.23*	5.79*
<u>Bottled Water</u>						
Vitamin, Enhanced, and Bottled waters	18.7	358	0.44	1.26	2.77	5.81
<u>Chewing Gum</u>						
Chewing Gum	1.1	21	0.01*	na	0.47*	1.01*
<u>Coffee and Tea</u>						
Tea	2.5	47	0.02	na	0.61	1.51
<u>Dairy Product Analogs</u>						
Soy Milk	1.0	20	0.07*	na	4.65*	11.59*
<u>Gelatins, Puddings, and Custard</u>						
Gelatin	3.1	59	0.07	na	2.02	4.37
<u>Hard Candy</u>						
Mints	9.2	176	0.14	na	1.44	3.96
<u>Milk Products</u>						
Meal Replacement Beverages, Milk-Based	0.1	2	<0.01*	na	0.81*	1.07*
Yogurt (fresh)	14.7	281	0.40	1.59	1.94	3.36
Yogurt Drinks	0.6	12	0.02*	na	1.51*	4.71*

Table B-1 Estimated Daily per Kilogram Body Weight Intake of Theobromine from Individual Proposed Food-Uses by Infants (Aged 0 to 2 Years) Within the United States (2003-2004, 2005-2006 NHANES Data)						
Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg/kg bw)		All-User Consumption (mg/kg bw)	
			Mean	90 th Percentile	Mean	90 th Percentile
<u>Processed Fruits and Fruit Juices</u>						
Powdered Fruit-Flavored Drinks	7.7	148	0.42	na	4.98	12.55
Fruit Smoothies	0.9	19	0.09*	na	11.35*	30.71*
<u>Vitamin and Mineral Supplements</u>						
Calcium Chews	0.1	1	<0.01*	na	<0.01*	<0.01*

na = not available

*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% (see Section 2.3).

Table B-2 Estimated Daily per Kilogram Body Weight Intake of Theobromine from Individual Proposed Food-Uses by Children (Aged 3 to 11 Years) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg/kg bw)		All-User Consumption (mg/kg bw)	
			Mean	90 th Percentile	Mean	90 th Percentile
<u>Baked Goods and Baking Mixes</u>						
Bread	70.8	1,934	0.65	1.62	0.90	1.84
<u>Breakfast Cereals</u>						
Instant and Regular Oatmeal	6.8	186	0.29	na	4.34	7.91
Ready-to-Eat Cereals	67.8	1,851	0.82	1.96	1.20	2.28
<u>Beverages and Beverage Bases</u>						
Sports, Isotonic, and Energy Drinks	3.7	102	0.04	na	0.90	2.07
Meal Replacement Beverages, Non Milk-Based	0.3	7	<0.01*	na	2.33*	4.10*
<u>Bottled Water</u>						
Vitamin, Enhanced, and Bottled waters	21.8	598	0.40	1.39	2.03	4.25
<u>Chewing Gum</u>						
Chewing Gum	6.7	182	0.03	na	0.37	0.72
<u>Coffee and Tea</u>						
Tea	6.1	166	0.03	na	0.50	0.79
<u>Dairy Product Analogs</u>						
Soy Milk	1.0	26	0.02*	na	1.51*	3.16*
<u>Gelatins, Puddings, and Custard</u>						
Gelatin	4.6	126	0.06	na	1.36	2.71
<u>Hard Candy</u>						
Mints	17.6	479	0.19	0.64	1.02	2.33
<u>Milk Products</u>						
Meal Replacement Beverages, Milk-Based	0.4	10	<0.01*	na	1.17*	2.05*
Yogurt (fresh)	13.5	368	0.17	0.64	1.16	2.08
Yogurt Drinks	0.8	23	0.01*	na	0.64*	0.99*

Table B-2 Estimated Daily per Kilogram Body Weight Intake of Theobromine from Individual Proposed Food-Uses by Children (Aged 3 to 11 Years) Within the United States (2003-2004, 2005-2006 NHANES Data)						
Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg/kg bw)		All-User Consumption (mg/kg bw)	
			Mean	90 th Percentile	Mean	90 th Percentile
<u>Processed Fruits and Fruit Juices</u>						
Powdered Fruit-Flavored Drinks	17.7	482	0.62	1.84	3.98	7.44
Fruit Smoothies	1.0	28	0.03*	na	3.54*	4.63*
<u>Vitamin and Mineral Supplements</u>						
Calcium Chews	0.1	4	<0.01*	na	0.10*	0.12*

na = not available

*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% (see Section 2.3).

Table B-3 Estimated Daily per Kilogram Body Weight Intake of Theobromine from Individual Proposed Food-Uses by Female Teenagers (Aged 12 to 19 Years) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg/kg bw)		All-User Consumption (mg/kg bw)	
			Mean	90 th Percentile	Mean	90 th Percentile
<u>Baked Goods and Baking Mixes</u>						
Bread	64.2	1,277	0.32	0.81	0.48	0.95
<u>Breakfast Cereals</u>						
Instant and Regular Oatmeal	2.5	50	0.05	na	1.81	4.32
Ready-to-Eat Cereals	42.8	849	0.29	0.79	0.64	1.29
<u>Beverages and Beverage Bases</u>						
Sports, Isotonic, and Energy Drinks	3.4	68	0.02	na	0.51	0.84
Meal Replacement Beverages, Non Milk-Based	0.3	5	<0.01*	na	0.38*	1.71*
<u>Bottled Water</u>						
Vitamin, Enhanced, and Bottled waters	26.4	526	0.41	1.49	1.67	3.62
<u>Chewing Gum</u>						
Chewing Gum	9.1	181	0.02	na	0.21	0.54
<u>Coffee and Tea</u>						
Tea	12.2	242	0.04	0.14	0.35	0.68
<u>Dairy Product Analogs</u>						
Soy Milk	0.7	14	0.01*	na	0.93*	1.53*
<u>Gelatins, Puddings, and Custard</u>						
Gelatin	1.6	31	0.01*	na	0.45*	1.06*
<u>Hard Candy</u>						
Mints	12.3	245	0.04	0.05	0.37	0.93
<u>Milk Products</u>						
Meal Replacement Beverages, Milk-Based	0.7	13	0.01*	na	1.00*	1.34*
Yogurt (fresh)	6.7	132	0.04	na	0.56	1.08
Yogurt Drinks	1.2	23	0.01	na	0.45	0.68

Table B-3 Estimated Daily per Kilogram Body Weight Intake of Theobromine from Individual Proposed Food-Uses by Female Teenagers (Aged 12 to 19 Years) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg/kg bw)		All-User Consumption (mg/kg bw)	
			Mean	90 th Percentile	Mean	90 th Percentile
<u>Processed Fruits and Fruit Juices</u>						
Powdered Fruit-Flavored Drinks	13.5	268	0.20	0.53	1.71	3.63
Fruit Smoothies	1.7	34	0.03*	na	3.19*	5.41*
<u>Vitamin and Mineral Supplements</u>						
Calcium Chews	0.2	3	<0.01*	na	0.02*	0.10*

na = not available

*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% (see Section 2.3).

Table B-4 Estimated Daily per Kilogram Body Weight Intake of Theobromine from Individual Proposed Food-Uses by Male Teenagers (Aged 12 to 19 Years) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg/kg bw)		All-User Consumption (mg/kg bw)	
			Mean	90 th Percentile	Mean	90 th Percentile
<u>Baked Goods and Baking Mixes</u>						
Bread	68.7	1,332	0.33	0.81	0.47	0.92
<u>Breakfast Cereals</u>						
Instant and Regular Oatmeal	2.0	38	0.04	na	2.11	4.26
Ready-to-Eat Cereals	44.8	868	0.35	1.08	0.80	1.48
<u>Beverages and Beverage Bases</u>						
Sports, Isotonic, and Energy Drinks	7.1	137	0.07	na	0.76	1.59
Meal Replacement Beverages, Non Milk-Based	0.7	13	<0.01*	na	0.46*	0.72*
<u>Bottled Water</u>						
Vitamin, Enhanced, and Bottled waters	21.5	419	0.33	0.95	1.58	3.79
<u>Chewing Gum</u>						
Chewing Gum	7.2	139	0.01	na	0.20	0.47
<u>Coffee and Tea</u>						
Tea	12.2	236	0.05	0.23	0.43	0.77
<u>Dairy Product Analogs</u>						
Soy Milk	0.5	9	<0.01*	na	0.54*	0.83*
<u>Gelatins, Puddings, and Custard</u>						
Gelatin	1.4	28	0.01*	na	0.76*	1.40*
<u>Hard Candy</u>						
Mints	9.2	179	0.04	na	0.42	0.83
<u>Milk Products</u>						
Meal Replacement Beverages, Milk-Based	0.6	11	<0.01*	na	0.86*	1.54*
Yogurt (fresh)	3.6	70	0.04	na	0.72	1.26
Yogurt Drinks	1.1	22	0.01*	na	0.51*	0.99*

Table B-4 Estimated Daily per Kilogram Body Weight Intake of Theobromine from Individual Proposed Food-Uses by Male Teenagers (Aged 12 to 19 Years) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg/kg bw)		All-User Consumption (mg/kg bw)	
			Mean	90 th Percentile	Mean	90 th Percentile
<u>Processed Fruits and Fruit Juices</u>						
Powdered Fruit-Flavored Drinks	13.0	253	0.30	0.94	2.32	3.86
Fruit Smoothies	0.7	14	0.03*	na	3.52*	5.92*
<u>Vitamin and Mineral Supplements</u>						
Calcium Chews	0.1	2	<0.01*	na	0.01*	0.02*

na = not available

*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% (see Section 2.3).

Table B-5 Estimated Daily per Kilogram Body Weight Intake of Theobromine from Individual Proposed Food-Uses by Female Adults (Aged 20 Years and Over) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg/kg bw)		All-User Consumption (mg/kg bw)	
			Mean	90 th Percentile	Mean	90 th Percentile
<u>Baked Goods and Baking Mixes</u>						
Bread	72.9	3,123	0.25	0.59	0.34	0.65
<u>Breakfast Cereals</u>						
Instant and Regular Oatmeal	12.9	553	0.18	0.51	1.74	3.26
Ready-to-Eat Cereals	34.7	1,485	0.16	0.54	0.47	0.90
<u>Beverages and Beverage Bases</u>						
Sports, Isotonic, and Energy Drinks	1.1	49	0.01	na	0.67	1.34
Meal Replacement Beverages, Non Milk-Based	1.2	54	0.01	na	0.47	1.19
<u>Bottled Water</u>						
Vitamin, Enhanced, and Bottled waters	24.6	1,059	0.48	1.89	1.97	3.97
<u>Chewing Gum</u>						
Chewing Gum	4.1	175	0.01	na	0.27	0.40
<u>Coffee and Tea</u>						
Tea	13.0	557	0.06	0.21	0.42	0.80
<u>Dairy Product Analogs</u>						
Soy Milk	2.7	115	0.01	na	0.47	1.00
<u>Gelatins, Puddings, and Custard</u>						
Gelatin	3.6	155	0.01	na	0.44	0.96
<u>Hard Candy</u>						
Mints	6.9	292	0.02	na	0.33	0.69
<u>Milk Products</u>						
Meal Replacement Beverages, Milk-Based	2.1	93	0.02	na	0.90	2.00
Yogurt (fresh)	10.9	469	0.05	0.19	0.44	0.79
Yogurt Drinks	1.9	79	0.01	na	0.38	0.74

Table B-5 Estimated Daily per Kilogram Body Weight Intake of Theobromine from Individual Proposed Food-Uses by Female Adults (Aged 20 Years and Over) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg/kg bw)		All-User Consumption (mg/kg bw)	
			Mean	90 th Percentile	Mean	90 th Percentile
<u>Processed Fruits and Fruit Juices</u>						
Powdered Fruit-Flavored Drinks	6.9	294	0.15	na	1.93	4.53
Fruit Smoothies	1.3	57	0.03	na	2.22	4.35
<u>Vitamin and Mineral Supplements</u>						
Calcium Chews	0.1	5	<0.01*	na	0.04*	0.08*

na = not available

*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% (see Section 2.3).

Table B-6 Estimated Daily per Kilogram Body Weight Intake of Theobromine from Individual Proposed Food-Uses by Male Adults (Aged 20 Years and Over) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg/kg bw)		All-User Consumption (mg/kg bw)	
			Mean	90 th Percentile	Mean	90 th Percentile
<u>Baked Goods and Baking Mixes</u>						
Bread	74.6	2,868	0.31	0.69	0.41	0.78
<u>Breakfast Cereals</u>						
Instant and Regular Oatmeal	9.3	360	0.14	na	1.91	3.63
Ready-to-Eat Cereals	31.2	1,199	0.18	0.66	0.55	1.10
<u>Beverages and Beverage Bases</u>						
Sports, Isotonic, and Energy Drinks	4.2	160	0.02	na	0.54	1.16
Meal Replacement Beverages, Non Milk-Based	1.8	68	0.01	na	0.65	1.73
<u>Bottled Water</u>						
Vitamin, Enhanced, and Bottled waters	20.9	805	0.36	1.36	1.65	3.50
<u>Chewing Gum</u>						
Chewing Gum	3.3	126	0.01	na	0.14	0.31
<u>Coffee and Tea</u>						
Tea	13.3	509	0.06	0.21	0.44	0.92
<u>Dairy Product Analogs</u>						
Soy Milk	1.6	61	0.01	na	0.43	0.83
<u>Gelatins, Puddings, and Custard</u>						
Gelatin	2.6	104	0.01	na	0.45	0.86
<u>Hard Candy</u>						
Mints	5.5	210	0.01	na	0.28	0.61
<u>Milk Products</u>						
Meal Replacement Beverages, Milk-Based	1.6	62	0.02	na	1.10	1.49
Yogurt (fresh)	5.4	206	0.02	na	0.38	0.72
Yogurt Drinks	1.4	54	0.01	na	0.49	1.12

Table B-6 Estimated Daily per Kilogram Body Weight Intake of Theobromine from Individual Proposed Food-Uses by Male Adults (Aged 20 Years and Over) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg/kg bw)		All-User Consumption (mg/kg bw)	
			Mean	90 th Percentile	Mean	90 th Percentile
<u>Processed Fruits and Fruit Juices</u>						
Powdered Fruit-Flavored Drinks	6.8	259	0.12	na	1.94	3.85
Fruit Smoothies	0.9	35	0.04	na	3.55	8.43
<u>Vitamin and Mineral Supplements</u>						
Calcium Chews	0.1	1	<0.01*	na	<0.01*	<0.01*

na = not available

*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% (see Section 2.3).

Table B-7 Estimated Daily per Kilogram Body Weight Intake of Theobromine from Individual Proposed Food-Uses by the Total Population (All Ages) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg/kg bw)		All-User Consumption (mg/kg bw)	
			Mean	90 th Percentile	Mean	90 th Percentile
<u>Baked Goods and Baking Mixes</u>						
Bread	67.6	11,299	0.34	0.80	0.47	0.94
<u>Breakfast Cereals</u>						
Instant and Regular Oatmeal	8.1	1,363	0.19	na	2.34	4.52
Ready-to-Eat Cereals	42.3	7,055	0.29	0.89	0.72	1.50
<u>Beverages and Beverage Bases</u>						
Sports, Isotonic, and Energy Drinks	3.5	586	0.03	na	0.77	1.63
Meal Replacement Beverages, Non Milk-Based	0.9	152	0.01	na	0.61	1.42
<u>Bottled Water</u>						
Vitamin, Enhanced, and Bottled waters	22.5	3,765	0.41	1.57	1.85	3.91
<u>Chewing Gum</u>						
Chewing Gum	5.0	824	0.01	na	0.24	0.51
<u>Coffee and Tea</u>						
Tea	10.5	1,757	0.05	0.18	0.43	0.82
<u>Dairy Product Analogs</u>						
Soy Milk	1.5	245	0.01	na	0.69	1.26
<u>Gelatins, Puddings, and Custard</u>						
Gelatin	3.0	503	0.02	na	0.71	1.45
<u>Hard Candy</u>						
Mints	9.5	1,581	0.05	na	0.58	1.33
<u>Milk Products</u>						
Meal Replacement Beverages, Milk-Based	1.1	191	0.02	na	0.98	2.00
Yogurt (fresh)	9.1	1,526	0.07	na	0.71	1.41
Yogurt Drinks	1.4	236	0.01	na	0.48	0.95

Table B-7 Estimated Daily per Kilogram Body Weight Intake of Theobromine from Individual Proposed Food-Uses by the Total Population (All Ages) Within the United States (2003-2004, 2005-2006 NHANES Data)

Food-Use Category	% Users	Actual # of Total Users	All-Person Consumption (mg/kg bw)		All-User Consumption (mg/kg bw)	
			Mean	90 th Percentile	Mean	90 th Percentile
<u>Processed Fruits and Fruit Juices</u>						
Powdered Fruit-Flavored Drinks	10.2	1,704	0.22	na	2.53	5.13
Fruit Smoothies	1.0	164	0.04	na	3.13	5.77
<u>Vitamin and Mineral Supplements</u>						
Calcium Chews	<0.1	16	<0.01*	na	0.04*	0.12*

na = not available

*Indicates an intake estimate that may not be statistically reliable, as the CV of the mean is equal to or greater than 30% (see Section 2.3).

APPENDIX C

Representative NHANES 2003-2004, 2005-2006 Food Codes for All Proposed Food-Uses of Theobromine in the United States

**Representative NHANES 2003-2004, 2005-2006 Food Codes for All Proposed Food-Uses
of Theobromine in the United States**

Baked Goods and Baking Mixes

Bread

[Theobromine] = 0.060%

51000100	Bread, NS as to major flour
51000110	Toast, NS as to major flour
51101000	Bread, white
51101010	Bread, white, toasted
51102010	Bread, white with whole wheat swirl
51102020	Bread, white with whole wheat swirl, toasted
51105010	Bread, Cuban
51105040	Bread, Cuban, toasted
51106010	Bread, Native, Puerto Rican style (Pan Criollo)
51106020	Bread, Native, Puerto Rican style, toasted (Pan Criollo)
51106100	Bread, Native water, Puerto Rican style (Pan de agua)
51106200	Bread, lard, Puerto Rican style (Pan de manteca)
51106210	Bread, lard, Puerto Rican style, toasted (Pan de manteca)
51106300	Bread, caressed, Puerto Rican style (Pan sobao)
51106310	Bread, caressed, Puerto Rican style, toasted (Pan sobao)
51107010	Bread, French or Vienna
51107040	Bread, French or Vienna, toasted
51108010	Focaccia, Italian flatbread, plain
51108100	Naan, Indian flatbread
51109010	Bread, Italian, Grecian, Armenian
51109040	Bread, Italian, Grecian, Armenian, toasted
51109100	Bread, pita
51109110	Bread, pita, toasted
51109150	Bread, pita with fruit
51109200	Bread, pita with fruit, toasted
51110010	Bread, batter
51111010	Bread, cheese
51111040	Bread, cheese, toasted
51113010	Bread, cinnamon
51113100	Bread, cinnamon, toasted
51115010	Bread, cornmeal and molasses
51115020	Bread, cornmeal and molasses, toasted
51119010	Bread, egg, Challah
51119040	Bread, egg, Challah, toasted
51119100	Bread, lowfat, 98% fat free
51119110	Bread, lowfat, 98% fat free, toasted
51121010	Bread, garlic
51121040	Bread, garlic, toasted
51121110	Bread, onion
51122000	Bread, reduced calorie and/or high fiber, white or NFS
51122010	Bread, reduced calorie and/or high fiber, white or NFS, toasted
51122050	Bread, reduced calorie and/or high fiber, Italian
51122060	Bread, reduced calorie and/or high fiber, Italian, toasted
51122100	Bread, reduced calorie and/or high fiber, white or NFS, with fruit and/or nuts
51122110	Bread, reduced calorie and/or high fiber, white or NFS, with fruit and/or nuts, toasted
51122300	Bread, white, special formula, added fiber

51122310	Bread, white, special formula, added fiber, toasted
51123010	Bread, high protein
51123020	Bread, high protein, toasted
51126010	Bread, milk and honey
51126020	Bread, milk and honey, toasted
51127010	Bread, potato
51127020	Bread, potato, toasted
51129010	Bread, raisin
51129020	Bread, raisin, toasted
51130510	Bread, white, low sodium or no salt
51130520	Bread, white, low sodium or no salt, toasted
51133010	Bread, sour dough
51133020	Bread, sour dough, toasted
51134000	Bread, sweetpotato
51135000	Bread, vegetable
51135010	Bread, vegetable, toasted
51140100	Bread, dough, fried
51201010	Bread, whole wheat, 100%
51201020	Bread, whole wheat, 100%, toasted
51201110	Bread, whole wheat, 100%, with raisins
51201120	Bread, whole wheat, 100%, with raisins, toasted
51201150	Bread, pita, whole wheat, 100%
51201160	Bread, pita, whole wheat, 100%, toasted
51204010	Bread, wheat germ
51204020	Bread, wheat germ, toasted
51207010	Bread, sprouted wheat
51207020	Bread, sprouted wheat, toasted
51300110	Bread, whole wheat, other than 100% or NS as to 100%
51300120	Bread, whole wheat, other than 100% or NS as to 100%, toasted
51300180	Bread, puri or poori (Indian puffed bread), whole wheat, other than 100% or NS as to 100%, filled wi
51300210	Bread, whole wheat, NS as to 100%, with raisins
51300220	Bread, whole wheat, NS as to 100%, with raisins, toasted
51301010	Bread, wheat or cracked wheat
51301020	Bread, wheat or cracked wheat, toasted
51301120	Bread, wheat or cracked wheat, with raisins
51301130	Bread, wheat or cracked wheat, with raisins, toasted
51301510	Bread, wheat or cracked wheat, reduced calorie and/or high fiber
51301520	Bread, wheat or cracked wheat, reduced calorie and/or high fiber, toasted
51301600	Bread, pita, whole wheat, other than 100% or NS as to 100%
51301610	Bread, pita, whole wheat, other than 100% or NS as to 100%, toasted
51301620	Bread, pita, wheat or cracked wheat
51301630	Bread, pita, wheat or cracked wheat, toasted
51302010	Bread, wheat bran
51302020	Bread, wheat bran, toasted
51302050	Bread, wheat bran, with raisins
51302060	Bread, wheat bran, with raisins, toasted
51401010	Bread, rye
51401020	Bread, rye, toasted
51401030	Bread, marble rye and pumpernickel
51401040	Bread, marble rye and pumpernickel, toasted
51401060	Bread, rye, reduced calorie and/or high fiber
51401070	Bread, rye, reduced calorie and/or high fiber, toasted
51404010	Bread, pumpernickel
51404020	Bread, pumpernickel, toasted
51407010	Bread, black

51407020	Bread, black, toasted
51501010	Bread, oatmeal
51501020	Bread, oatmeal, toasted
51501040	Bread, oat bran
51501050	Bread, oat bran, toasted
51501060	Bread, oat bran, reduced calorie and/or high fiber
51501070	Bread, oat bran, reduced calorie and/or high fiber, toasted
51601010	Bread, multigrain, toasted
51601020	Bread, multigrain
51601210	Bread, multigrain, with raisins
51601220	Bread, multigrain, with raisins, toasted
51602010	Bread, multigrain, reduced calorie and/or high fiber
51602020	Bread, multigrain, reduced calorie and/or high fiber, toasted
51801010	Bread, barley
51802010	Bread, triticale
51802020	Bread, triticale, toasted
51803010	Bread, buckwheat
51803020	Bread, buckwheat, toasted
51804010	Bread, soy
51804020	Bread, soy, toasted
51805010	Bread, sunflower meal
51805020	Bread, sunflower meal, toasted
51806010	Bread, rice
51806020	Bread, rice, toasted
51807000	Injera (American-style Ethiopian bread)
51808000	Bread, low gluten
51808010	Bread, low gluten, toasted

Mixtures containing bread

(adjusted for a bread content of 46 to 68%)

[Theobromine] = 0.0026 to 0.014%

14640000	Cheese sandwich
14640100	Cheese sandwich, grilled
14640300	Cheese spread sandwich
27500050	Sandwich, NFS
27500100	Meat sandwich, NFS
27510210	Cheeseburger, plain, on bun
27510500	Hamburger, plain, on bun
27510590	Hamburger, with mayonnaise or salad dressing, on bun
27510600	Hamburger, 1 oz meat, plain, on miniature bun
27515150	Steak patty (breaded, fried) sandwich, with mayonnaise or salad dressing, lettuce, and tomato, on bu
27520110	Bacon sandwich, with spread
27520520	Pork sandwich
27540110	Chicken sandwich, with spread
27540170	Chicken patty sandwich, miniature, with spread
27560000	Luncheon meat sandwich, NFS, with spread
27560110	Bologna sandwich, with spread
27560120	Bologna and cheese sandwich, with spread
27560410	Puerto Rican sandwich (Sandwich criollo)
27560510	Salami sandwich, with spread
27560710	Sausage sandwich
27563010	Meat spread or potted meat sandwich
27570310	Hors d'oeuvres, with spread
32201000	Fried egg sandwich

32204010 Scrambled egg sandwich
42301010 Peanut butter sandwich
42302010 Peanut butter and jelly sandwich

Mixtures containing bread
(adjusted for a bread content of 35 to 45%)
[Theobromine] = 0.021 to 0.027%

14640200 Cheese sandwich, hoagie
27510000 Beef sandwich, NFS
27510130 Beef barbecue submarine sandwich, on bun
27510220 Cheeseburger, with mayonnaise or salad dressing, on bun
27510240 Cheeseburger, 1/4 lb meat, plain, on bun
27510270 Double cheeseburger (2 patties), plain, on bun
27510290 Double cheeseburger (2 patties), plain, on double-decker bun
27510310 Cheeseburger with tomato and/or catsup, on bun
27510311 Cheeseburger, 1 oz meat, plain, on miniature bun
27510420 Taco burger, on bun
27510510 Hamburger, with tomato and/or catsup, on bun
27510520 Hamburger, with mayonnaise or salad dressing and tomatoes, on bun
27510530 Hamburger, 1/4 lb meat, plain, on bun
27510610 Hamburger, 1 oz meat, with tomato and/or catsup, on miniature bun
27510650 Double hamburger (2 patties), plain, on bun
27510720 Pizzaburger (hamburger, cheese, sauce) on whole bun
27510910 Corned beef sandwich
27511010 Pastrami sandwich
27513010 Roast beef sandwich
27513060 Roast beef sandwich with bacon and cheese sauce
27520120 Bacon and cheese sandwich, with spread
27520130 Bacon, chicken, and tomato club sandwich, with lettuce and spread
27520300 Ham sandwich, with spread
27520310 Ham sandwich with lettuce and spread
27520330 Ham and egg sandwich
27520340 Ham salad sandwich
27520410 Cuban sandwich, (Sandwich cubano), with spread
27520420 Midnight sandwich, (Media noche), with spread
27540120 Chicken salad or chicken spread sandwich
27540130 Chicken barbecue sandwich
27540190 Chicken patty sandwich, with lettuce and spread
27540240 Chicken fillet, (broiled), sandwich, on whole wheat roll, with lettuce, tomato and spread
27540310 Turkey sandwich, with spread
27540320 Turkey salad or turkey spread sandwich
27550100 Fish sandwich, on bun, with cheese and spread
27560320 Frankfurter or hot dog, plain, on bun
27560340 Frankfurter or hot dog, with catsup and/or mustard, on bun
27560400 Chicken frankfurter or hot dog, plain, on bun
27560670 Sausage and cheese on English muffin
42303010 Peanut butter and banana sandwich
54304100 Cracker, cheese, reduced fat
58128220 Dressing with chicken or turkey and vegetables
58128250 Dressing with meat and vegetables
74701000 Tomato sandwich

Mixtures containing bread
(adjusted for a bread content of 25 to 25%)
[Theobromine] = 0.015 to 0.021%

13210150	Puerto Rican bread pudding made with evaporated milk and rum (Budin de pan)
21420100	Beef, sandwich steak (flaked, formed, thinly sliced)
27250450	Shrimp toast, fried
27510110	Beef barbecue or Sloppy Joe, on bun
27510230	Cheeseburger, with mayonnaise or salad dressing and tomatoes, on bun
27510250	Cheeseburger, 1/4 lb meat, with mayonnaise or salad dressing, on bun
27510260	Cheeseburger, 1/4 lb meat, with mushrooms in sauce, on bun
27510280	Double cheeseburger (2 patties), with mayonnaise or salad dressing, on bun
27510300	Double cheeseburger (2 patties), with mayonnaise or salad dressing, on double-decker bun
27510320	Cheeseburger, 1/4 lb meat, with tomato and/or catsup, on bun
27510330	Double cheeseburger (2 patties), with tomato and/or catsup, on bun
27510340	Double cheeseburger (2 patties), with mayonnaise or salad dressing and tomatoes, on bun
27510350	Cheeseburger, 1/4 lb meat, with mayonnaise or salad dressing and tomatoes, on bun
27510360	Cheeseburger with mayonnaise or salad dressing, tomato and bacon, on bun
27510400	Bacon cheeseburger, 1/4 lb meat, with tomato and/or catsup, on bun
27510410	Chiliburger, on bun
27510440	Bacon cheeseburger, 1/4 lb meat, with mayonnaise or salad dressing and tomatoes, on bun
27510450	Cheeseburger, 1/4 lb meat, with ham, on bun
27510480	Cheeseburger (hamburger with cheese sauce), 1/4 lb meat, with grilled onions, on rye bun
27510540	Double hamburger (2 patties), with tomato and/or catsup, on bun
27510550	Double hamburger (2 patties), with mayonnaise or salad dressing and tomatoes, on double-decker bun
27510560	Hamburger, 1/4 lb meat, with mayonnaise or salad dressing and tomatoes, on bun
27510570	Hamburger, 2-1/2 oz meat, with mayonnaise or salad dressing and tomatoes, on bun
27510620	Hamburger, 1/4 lb meat, with tomato and/or catsup, on bun
27510630	Hamburger, 1/4 lb meat, with mayonnaise or salad dressing, on bun
27510660	Double hamburger (2 patties), with mayonnaise or salad dressing, on bun
27510670	Double hamburger (2 patties), with mayonnaise or salad dressing and tomatoes, on bun
27510680	Double hamburger (2 patties, 1/4 lb meat each), with tomato and/or catsup, on bun
27510950	Reuben sandwich (corned beef sandwich with sauerkraut and cheese), with spread
27513040	Roast beef submarine sandwich, on roll, with lettuce, tomato and spread
27513050	Roast beef sandwich with cheese
27516010	Gyro sandwich (pita bread, beef, lamb, onion, condiments), with tomato and spread
27520140	Bacon and egg sandwich
27520150	Bacon, lettuce, and tomato sandwich with spread
27520160	Bacon, chicken, and tomato club sandwich, on multigrain roll with lettuce and spread
27520320	Ham and cheese sandwich, with lettuce and spread
27520350	Ham and cheese sandwich, with spread, grilled
27520360	Ham and cheese sandwich, on bun, with lettuce and spread
27520370	Hot ham and cheese sandwich, on bun
27520510	Pork barbecue or Sloppy Joe, on bun
27520530	Pork sandwich, with gravy
27520540	Ham and tomato club sandwich, with lettuce and spread
27540140	Chicken fillet (breaded, fried) sandwich
27540150	Chicken fillet (breaded, fried) sandwich with lettuce, tomato and spread
27540230	Chicken patty sandwich with cheese, on wheat bun, with lettuce, tomato and spread
27540250	Chicken fillet, broiled, sandwich with cheese, on whole wheat roll, with lettuce, tomato and non-may

27540260	Chicken fillet, broiled, sandwich, on oat bran bun, with lettuce, tomato, spread
27540270	Chicken fillet, broiled, sandwich, with lettuce, tomato, and non-mayonnaise type spread
27540280	Chicken fillet, broiled, sandwich with cheese, on bun, with lettuce, tomato and spread
27540350	Turkey submarine sandwich, on roll, with cheese, lettuce, tomato and spread
27550510	Sardine sandwich, with lettuce and spread
27550710	Tuna salad sandwich, with lettuce
27550720	Tuna salad sandwich
27550730	Tuna melt sandwich
27560330	Frankfurter or hot dog, with cheese, plain, on bun
27560360	Frankfurter or hot dog, with chili, on bun
27560370	Frankfurter or hot dog with chili and cheese, on bun
27560660	Sausage griddle cake sandwich
27560720	Sausage and spaghetti sauce sandwich
32202055	Egg, cheese, and sausage griddle cake sandwich
32202075	Egg, cheese, and bacon griddle cake sandwich
32203010	Egg salad sandwich
41901020	Soyburger, meatless, with cheese on bun
58128210	Dressing with oysters

Mixtures containing bread
(adjusted for a bread content of 4 to 23%)
[Theobromine] = 0.0026 to 0.014%

13210110	Pudding, bread
13210180	Pudding, Mexican bread (Capirotada)
13210190	Pudding, Mexican bread (Capirotada), lower fat
27214600	Creamed dried beef on toast
27510370	Double cheeseburger (2 patties, 1/4 lb meat each), with mayonnaise or salad dressing, on bun
27510380	Triple cheeseburger (3 patties, 1/4 lb meat each), with mayonnaise or salad dressing and tomatoes, o
27510390	Double bacon cheeseburger (2 patties, 1/4 lb meat each), on bun
27510430	Double bacon cheeseburger (2 patties, 1/4 lb meat each), with mayonnaise or salad dressing and tomat
27510690	Double hamburger (2 patties, 1/4 lb meat each), with mayonnaise or salad dressing and tomatoes and/o
27510710	Pizzaburger (hamburger, cheese, sauce) on 1/2 bun
27513020	Roast beef sandwich, with gravy
27513030	Roast beef sandwich dipped in egg, fried, with gravy and spread
27515050	Fajita-style beef sandwich with cheese, on pita bread, with lettuce and tomato
27520390	Ham and cheese submarine sandwich, on multigrain roll, with lettuce, tomato and spread
27540200	Fajita-style chicken sandwich with cheese, on pita bread, with lettuce and tomato
27540330	Turkey sandwich, with gravy
27550000	Fish sandwich, on bun, with spread
27560310	Corny dog, with chili, on bun
27560910	Submarine, cold cut sandwich, on bun, with lettuce
32105190	Egg casserole with bread, cheese, milk and meat
55310100	Bread fritters, Puerto Rican style (Torrejas, Galician fritters)

Breakfast Cereals

Instant and Regular Oatmeal

[Theobromine] = 0.13%

56202960	Oatmeal, cooked, NS as to regular, quick or instant; NS as to fat added in cooking
56202970	Oatmeal, cooked, quick (1 or 3 minutes), NS as to fat added in cooking

56202980	Oatmeal, cooked, regular, NS as to fat added in cooking
56203000	Oatmeal, cooked, NS as to regular, quick or instant, fat not added in cooking
56203010	Oatmeal, cooked, regular, fat not added in cooking
56203020	Oatmeal, cooked, quick (1 or 3 minutes), fat not added in cooking
56203030	Oatmeal, cooked, instant, fat not added in cooking
56203040	Oatmeal, cooked, NS as to regular, quick, or instant, fat added in cooking
56203050	Oatmeal, cooked, regular, fat added in cooking
56203060	Oatmeal, cooked, quick (1 or 3 minutes), fat added in cooking
56203070	Oatmeal, cooked, instant, fat added in cooking
56203080	Oatmeal, cooked, instant, NS as to fat added in cooking
56203110	Oatmeal with maple flavor, cooked
56203200	Oatmeal with fruit, cooked
56203210	Oatmeal, NS as to regular, quick, or instant, made with milk, fat not added in cooking
56203220	Oatmeal, NS as to regular, quick, or instant, made with milk, fat added in cooking
56203230	Oatmeal, NS as to regular, quick, or instant, made with milk, NS as to fat added in cooking
56203540	Oatmeal, made with evaporated milk and sugar, Puerto Rican style
56203600	Oatmeal, multigrain, cooked, NS as to fat added in cooking
56203610	Oatmeal, multigrain, cooked, fat not added in cooking
56203620	Oatmeal, multigrain, cooked, fat added in cooking

Ready-to-Eat Cereals

[Theobromine] = 0.10%

57000000	Cereal, NFS
57000050	Kashi cereal, NS as to ready to eat or cooked
57000100	Oat cereal, NFS
57100100	Cereal, ready-to-eat, NFS
57100400	Character cereals, TV or movie, General Mills
57100500	Character cereals, TV or movie, Kelloggs
57101000	All-Bran
57101020	All-Bran with Extra Fiber
57102000	Alpen
57103000	Alpha-Bits
57103020	Alpha-bits with marshmallows
57103050	Amaranth Flakes
57103100	Apple Cinnamon Cheerios
57103500	Apple Cinnamon Squares Mini-Wheats, Kellogg's (formerly Apple Cinnamon Squares)
57104000	Apple Jacks
57106050	Banana Nut Crunch Cereal (Post)
57106100	Basic 4
57106250	Berry Berry Kix
57106260	Berry Burst Cheerios
57106530	Blueberry Morning, Post
57107000	Booberry
57110000	All-Bran Bran Buds, Kellogg's (formerly Bran Buds)
57111000	Bran Chex
57117000	Cap'n Crunch
57117500	Cap'n Crunch's Christmas Crunch
57119000	Cap'n Crunch's Crunch Berries
57120000	Cap'n Crunch's Peanut Butter Crunch
57123000	Cheerios
57124000	Chex cereal, NFS
57124200	Chocolate flavored frosted puffed corn cereal
57124500	Cinnamon Grahams, General Mills
57125000	Cinnamon Toast Crunch

57125900	Honey Nut Clusters (formerly called Clusters)
57126000	Cocoa Krispies
57126500	Cocoa Blasts, Quaker
57127000	Cocoa Pebbles
57128000	Cocoa Puffs
57128880	Common Sense Oat Bran, plain
57130000	Cookie-Crisp
57131000	Crunchy Corn Bran, Quaker
57132000	Corn Chex
57134000	Corn flakes, NFS
57134090	Corn flakes, low sodium
57135000	Corn flakes, Kellogg
57137000	Corn Puffs
57138000	Total Corn Flakes
57139000	Count Chocula
57143000	Cracklin' Oat Bran
57143500	Cranberry Almond Crunch, Post
57144000	Crisp Crunch
57148000	Crispix
57148500	Crispy Brown Rice Cereal
57148600	Harmony cereal, General Mills
57151000	Crispy Rice
57152000	Crispy Wheats'n Raisins
57201800	Disney cereals, Kellogg's
57206000	Familia
57206700	Fiber One
57206800	Fiber 7 Flakes, Health Valley
57207000	Bran Flakes, NFS (formerly 40% Bran Flakes, NFS)
57208000	Complete Wheat Bran Flakes, Kellogg's (formerly 40% Bran Flakes)
57209000	Natural Bran Flakes, Post (formerly called 40% Bran Flakes, Post)
57211000	Frankenberry
57212100	French Toast Crunch, General Mills
57213000	Froot Loops
57213850	Frosted Cheerios
57213900	Frosted Chex
57214000	Frosted Mini-Wheats
57214100	Frosted Wheat Bites
57215000	Frosty O's
57216000	Frosted rice, NFS
57218000	Frosted Rice Krispies
57219000	Fruit & Fibre (fiber), NFS
57221000	Fruit & Fibre (fiber) with dates, raisins, and walnuts
57221650	Fruit Harvest cereal, Kellogg's
57221700	Fruit Rings, NFS
57221800	Fruit Whirls
57223000	Fruity Pebbles
57224000	Golden Grahams
57227000	Granola, NFS
57228000	Granola, homemade
57229000	Granola, lowfat, Kellogg's
57229500	Granola with Raisins, lowfat, Kellogg's
57230000	Grape-Nuts
57231000	Grape-Nut Flakes
57231200	Great Grains, Raisin, Date, and Pecan Whole Grain Cereal, Post
57231250	Great Grains Double Pecan Whole Grain Cereal, Post
57232100	Healthy Choice Almond Crunch with raisins, Kellogg's

57237100	Honey Bunches of Oats
57237300	Honey Bunches of Oats with Almonds, Post
57238000	Honeycomb, plain
57239000	Honeycomb, strawberry
57239100	Honey Crunch Corn Flakes, Kellogg's
57240100	Honey Nut Chex
57241000	Honey Nut Cheerios
57241200	Honey Nut Shredded Wheat, Post
57243000	Honey Smacks
57243870	Jenny O's
57244000	Just Right
57245000	Just Right Fruit and Nut (formerly Just Right with raisins, dates, and nuts)
57250000	Pokemon, Kellogg's
57301100	Kaboom
57301500	Kashi, Puffed
57301510	Kashi GoLean
57301511	Kashi GoLean Crunch
57301520	Kashi Good Friends
57301530	Kashi Heart to Heart
57302100	King Vitaman
57303100	Kix
57304100	Life (plain and cinnamon)
57305100	Lucky Charms
57305150	Frosted oat cereal with marshmallows
57305170	Malt-O-Meal Coco-Roos
57305180	Malt-O-Meal Corn Bursts
57305200	Malt-O-Meal Crispy Rice
57305210	Malt-O-Meal Frosted Flakes
57305500	Malt-O-Meal Honey and Nut Toasty O's
57305600	Malt-O-Meal Marshmallow Mateys
57306100	Malt-O-Meal Puffed Rice
57306120	Malt-O-Meal Puffed Wheat
57306500	Malt-O-Meal Golden Puffs (formerly Sugar Puffs)
57306700	Malt-O-Meal Toasted Oat Cereal
57306800	Malt-O-meal Tootie Fruities
57307010	Maple Pecan Crunch Cereal, Post
57307150	Marshmallow Safari, Quaker
57307500	Millet, puffed
57308150	Mueslix cereal, NFS
57308190	Muesli with raisins, dates, and almonds
57308300	Multi Bran Chex
57308400	Multi Grain Cheerios
57308900	Natural Muesli, Jenny's Cuisine
57309100	Nature Valley Granola, with fruit and nuts
57311700	Nu System Cuisine Toasted Grain Circles
57316200	Nutty Nuggets, Ralston Purina
57316300	Oat Bran Flakes, Health Valley
57316410	Apple Cinnamon Oatmeal Crisp (formerly Oatmeal Crisp with Apples)
57316450	Oatmeal Crisp with Almonds
57316500	Oatmeal Raisin Crisp
57316710	Oh's, Honey Graham
57316750	Oh's, Fruitangy, Quaker
57318000	100% Bran
57319000	100% Natural Cereal, plain, Quaker
57319500	Sun Country 100% Natural Granola, with Almonds
57320500	100 % Natural Cereal, with oats, honey and raisins, Quaker

57321500	100 % Natural Wholegrain Cereal with raisins, lowfat, Quaker
57321700	Optimum, Nature's Path
57321800	Optimum Slim, Nature's Path
57322500	Oreo O's cereal, Post
57323000	Sweet Crunch, Quaker (formerly called Popeye)
57323050	Sweet Puffs, Quaker
57324000	Peanut Butter Toast Crunch, General Mills
57325000	Product 19
57327450	Quaker Oat Bran Cereal
57327500	Quaker Oatmeal Squares (formerly Quaker Oat Squares)
57328000	Quisp
57329000	Raisin bran, NFS
57330000	Raisin Bran, Kellogg
57330010	Raisin Bran Crunch, Kellogg's
57331000	Raisin Bran, Post
57332050	Raisin Bran, Total
57332100	Raisin Nut Bran
57335500	Raisin Squares Mini-Wheats, Kellogg's (formerly Raisin Squares)
57335550	Reese's Peanut Butter Puffs cereal
57336000	Rice Chex
57337000	Rice Flakes, NFS
57339000	Rice Krispies
57339500	Rice Krispies Treats Cereal (Kellogg's)
57340000	Rice, puffed
57340700	Scooby Doo Cinnamon Marshmallow Cereal, Kellogg's
57341000	Shredded Wheat'N Bran
57341200	Smart Start, Kellogg's
57342010	Smorz, Kellogg's
57344000	Special K
57344010	Special K Red Berries
57344015	Special K Fruit & Yogurt
57344020	Special K Vanilla Almond
57346500	Toasted Oatmeal, Honey Nut (Quaker)
57347000	Corn Pops
57347500	Strawberry Squares Mini-Wheats, Kellogg's (formerly Strawberry Squares)
57348000	Frosted corn flakes, NFS
57349000	Frosted Flakes, Kellogg
57349020	Frosted Flakes 1/3 Less Sugar, Kellogg's
57355000	Golden Crisp (Formerly called Super Golden Crisp)
57401100	Toasted oat cereal
57403100	Toasties, Post
57404100	Malt-O-Meal Toasty O's
57404200	Malt-O-Meal Apple and Cinnamon Toasty O's
57406100	Total
57407100	Trix
57408100	Uncle Sam's Hi Fiber Cereal
57409100	Waffle Crisp, Post
57410000	Weetabix Whole Wheat Cereal
57411000	Wheat Chex
57412000	Wheat germ, plain
57413000	Wheat germ, with sugar and honey
57416000	Wheat, puffed, plain
57416010	Wheat, puffed, presweetened with sugar
57417000	Shredded Wheat, 100%
57418000	Wheaties
57419000	Yogurt Burst Cheerios

Beverages and Beverage Bases

Sports, Isotonic, and Energy Drinks

[Theobromine] = 0.012%

92553000	Fruit-flavored thirst quencher beverage, low calorie
92560000	Fruit-flavored thirst quencher beverage
92570100	Fluid replacement, electrolyte solution
92570500	Fluid replacement, 5% glucose in water
92650000	Red Bull Energy Drink
92651000	Energy drink

Powdered sports, isotonic, and energy drinks

(adjusted for reconstitution based on 16 g of powder needed to produce a 240 mL beverage)

[Theobromine] = 0.18%

92900300	Fruit-flavored thirst quencher beverage, dry concentrate, not reconstituted
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Meal Replacement Beverages, Non Milk-Based

[Theobromine] = 0.031%

41430000	Protein powder, NFS
41430200	Meal replacement or supplement, soy- and milk-base, powder, reconstituted with water
41440010	Meal replacement or supplement, liquid, soy-base, high protein
41440020	Ensure with fiber, liquid
41440050	Ensure Plus liquid nutrition
41440100	Meal replacement or supplement, liquid, soy-based

Powdered, non-milk based meal replacement beverages

(adjusted for reconstitution based on 50 g of powder needed to produce a 250 mL beverage)

[Theobromine] = 0.15%

41430310	Protein diet powder with soy and casein
41430010	Protein supplement, powdered
41440000	Textured vegetable protein, dry

Bottled Water

Vitamin, Enhanced, and Normal Bottled Waters

[Theobromine] = 0.017%

94100100	Water, bottled, unsweetened
94100200	Water, bottled, sweetened, with low or no calorie sweetener
94210100	Propel Fitness Water
94210200	Vitamin Water

Chewing Gum

Chewing Gum

[Theobromine] = 0.33%

91800100	Chewing gum, NFS
91801000	Chewing gum, sugared
91802000	Chewing gum, uncoated, sugarless

Coffee and Tea

Tea

[Theobromine] = 0.0082%

92301000	Tea, NS as to type, unsweetened
92301060	Tea, NS as to type, presweetened with sugar
92301080	Tea, NS as to type, presweetened with low calorie sweetener
92301100	Tea, NS as to type, decaffeinated, unsweetened
92301130	Tea, NS as to type, presweetened, NS as to sweetener
92301160	Tea, NS as to type, decaffeinated, presweetened with sugar
92301180	Tea, NS as to type, decaffeinated, presweetened with low calorie sweetener
92301190	Tea, NS as to type, decaffeinated, presweetened, NS as to sweetener
92304000	Tea, made from frozen concentrate, unsweetened
92304700	Tea, made from frozen concentrate, decaffeinated, presweetened with low calorie sweetener
92305000	Tea, made from powdered instant, presweetened, NS as to sweetener
92305010	Tea, made from powdered instant, unsweetened
92305040	Tea, made from powdered instant, presweetened with sugar
92305050	Tea, made from powdered instant, decaffeinated, presweetened with sugar
92305090	Tea, made from powdered instant, presweetened with low calorie sweetener
92305110	Tea, made from powdered instant, decaffeinated, presweetened with low calorie sweetener
92305180	Tea, made from powdered instant, decaffeinated, unsweetened
92305800	Tea, made from powdered instant, decaffeinated, presweetened, NS as to sweetener
92306020	Tea, herbal, presweetened with sugar
92306030	Tea, herbal, presweetened with low calorie sweetener
92306040	Tea, herbal, presweetened, NS as to sweetener

Powdered tea

[adjusted for reconstitution based on 70 g powder to produce a 488 mL beverage (value provided by The Tarka Group, Inc.)]

[Theobromine] = 0.057%

92307000	Tea, powdered instant, unsweetened, dry
92307400	Tea, powdered instant, sweetened, NS as to sweetener, dry

Dairy Product Analogs

Soy Milk

[Theobromine] = 0.016%

11320000	Milk, soy, ready-to-drink, not baby's
11321000	Milk, soy, ready-to-drink, not baby's, chocolate

Gelatins, Puddings, and Custard

Gelatin

[Theobromine] = 0.047%

91500200	Gelatin powder, sweetened, dry
91501010	Gelatin dessert
91501015	Gelatin snacks
91510100	Gelatin powder, dietetic, sweetened with low calorie sweetener, dry

91511010 Gelatin dessert, dietetic, sweetened with low calorie sweetener
91580000 Gelatin, frozen, whipped, on a stick

Mixtures containing gelatin
(adjusted for a gelatin content of 76 to 95%
[Theobromine] = 0.035 to 0.044%

91501020 Gelatin dessert with fruit
91501030 Gelatin dessert with whipped cream
91501040 Gelatin dessert with fruit and whipped cream
91501050 Gelatin dessert with cream cheese
91501060 Gelatin dessert with sour cream
91501070 Gelatin dessert with fruit and sour cream
91501080 Gelatin dessert with fruit and cream cheese
91501110 Gelatin dessert with fruit and whipped topping
91501120 Gelatin dessert with fruit and vegetables
91511030 Gelatin dessert, dietetic, with whipped topping, sweetened with low calorie sweetener
91511050 Gelatin dessert, dietetic, with cream cheese, sweetened with low calorie sweetener
91511060 Gelatin dessert, dietetic, with sour cream, sweetened with low calorie sweetener
91511100 Gelatin salad, dietetic, with vegetables, sweetened with low calorie sweetener

Mixtures containing gelatin
(adjusted for a gelatin content of 31 to 71%
[Theobromine] = 0.015 to 0.033%

14610200 Cheese, cottage cheese, with gelatin dessert
14610210 Cheese, cottage cheese, with gelatin dessert and fruit
91501090 Gelatin dessert with fruit, vegetable, and nuts
91511020 Gelatin dessert, dietetic, with fruit, sweetened with low calorie sweetener
91511070 Gelatin dessert, dietetic, with fruit and sour cream, sweetened with low calorie sweetener
91511080 Gelatin dessert, dietetic, with fruit and cream cheese, sweetened with low calorie sweetener
91511090 Gelatin dessert, dietetic, with fruit and vegetable(s), sweetened with low calorie sweetener
91511110 Gelatin dessert, dietetic, with fruit and whipped topping, sweetened with low calorie sweetener

Mixtures containing gelatin
(adjusted for a gelatin content of 2 to 24%
[Theobromine] = 0.0010 to 0.012%

11460190 Yogurt, frozen, NS as to flavor, nonfat milk
13250100 Mousse, not chocolate
14610250 Cheese, cottage cheese, with gelatin dessert and vegetables
53101250 Cake, angel food, with fruit and icing or filling
53104580 Cheesecake -type dessert, made with yogurt, with fruit
53207050 Cookie, chocolate, with chocolate filling or coating, fat free
53347100 Pie, raspberry cream
53370000 Pie, chiffon, not chocolate
53371000 Pie, chiffon, chocolate
53371100 Pie, chiffon, with liqueur
53373000 Pie, black bottom
63411010 Cranberry salad, congealed
74501010 Tomato aspic
75657000 Vegetable broth, bouillon

91501100 Gelatin salad with vegetables
91520100 Yookan (Yokan), a Japanese dessert made with bean paste and sugar

Hard Candy

Mints

[Theobromine] = 0.047%

91745020 Hard candy
91770020 Dietetic or low calorie hard candy
91770050 Dietetic or low calorie mints

Milk Products

Meal Replacement Beverages, Milk-Based

[Theobromine] = 0.030%

11611000 Instant breakfast, fluid, canned
11612000 Instant breakfast, powder, milk added
11613000 Instant breakfast, powder, sweetened with low calorie sweetener, milk added
11621000 Diet beverage, liquid, canned
11622000 Diet beverage, powder, milk added
11623000 Meal supplement or replacement, commercially prepared, ready-to-drink
11631000 High calorie beverage, canned or powdered, reconstituted
11641000 Meal supplement or replacement, milk-based, high protein, liquid
11651010 Meal replacement formula, Cambridge diet, reconstituted, all flavors

Powdered milk-based meal replacement beverages

(adjusted for reconstitution based on 50 g of powder needed to produce a 250 mL beverage)

[Theobromine] = 0.15%

11830800 Instant breakfast, powder, not reconstituted
11830810 Instant breakfast, powder, sweetened with low calorie sweetener, not reconstituted
11830850 High calorie milk beverage, powder, not reconstituted
11830900 Protein supplement, milk-based, powdered, not reconstituted
11830940 Meal replacement, high protein, milk based, fruit juice mixable formula, powdered, not reconstituted
11830970 Meal replacement, protein type, milk-based, powdered, not reconstituted
11830990 Nutrient supplement, milk-based, powdered, not reconstituted
11831500 Nutrient supplement, milk-based, high protein, powdered, not reconstituted
11832000 Meal replacement, protein type, milk- and soy-based, powdered, not reconstituted
11835000 Meal replacement or nutritional supplement, Cambridge diet formula, powdered, nonfat milk solids base
11835100 Meal replacement, Amway's Nutrilite brand Positrim Drink Mix, powdered nonfat dry milk-based, dry, n
11835150 Dynatrim, meal replacement, powder

Yogurt (fresh, not-chocolate)

[Theobromine] = 0.029%

11410000 Yogurt, NS as to type of milk or flavor
11411010 Yogurt, plain, NS as to type of milk
11411100 Yogurt, plain, whole milk
11411200 Yogurt, plain, lowfat milk
11411300 Yogurt, plain, nonfat milk

11420000	Yogurt, vanilla, lemon, or coffee flavor, NS as to type of milk
11421000	Yogurt, vanilla, lemon, or coffee flavor, whole milk
11422000	Yogurt, vanilla, lemon, maple, or coffee flavor, lowfat milk
11423000	Yogurt, vanilla, lemon, maple, or coffee flavor, nonfat milk
11424000	Yogurt, vanilla, lemon, maple, or coffee flavor, nonfat milk, sweetened with low calorie sweetener
11430000	Yogurt, fruit variety, NS as to type of milk
11431000	Yogurt, fruit variety, whole milk
11432000	Yogurt, fruit variety, lowfat milk
11432500	Yogurt, fruit variety, lowfat milk, sweetened with low-calorie sweetener
11433000	Yogurt, fruit variety, nonfat milk
11433500	Yogurt, fruit variety, nonfat milk, sweetened with low-calorie sweetener
11444000	Yogurt, fruit and nuts, NS as to type of milk
11445000	Yogurt, fruit and nuts, lowfat milk

Yogurt Drinks

[Theobromine] = 0.089%

11553000	Fruit smoothie drink, made with fruit or fruit juice and dairy products
11553100	Fruit smoothie drink, NFS

Processed Fruits and Fruit Juices

Fruit Smoothies

[Theobromine] = 0.084%

11553000	Fruit smoothie drink, made with fruit or fruit juice and dairy products
11553100	Fruit smoothie drink, NFS
64134000	Fruit smoothie drink, made with fruit or fruit juice only (no dairy products)

Powdered Fruit-Flavored Drinks

Dry fruit flavored drink powders

[Theobromine] = 0.62%

92900100	Tang, dry concentrate
92900110	Fruit-flavored concentrate, dry powder, with sugar and vitamin C added
92900200	Fruit-flavored beverage, dry concentrate, low calorie, not reconstituted

Reconstituted powdered fruit flavored drinks

(adjusted for reconstitution based on 8 g powder (value provided by The Tarka Group, Inc.) needed to produce a 240 mL beverage)

[Theobromine] = 0.021%

92531210	Strawberry-flavored drink with vitamin C added
92541010	Fruit-flavored drink, made from sweetened powdered mix (fortified with vitamin C)
92541020	Lemonade-flavored drink, made from powdered mix, with sugar and vitamin C added
92541040	Lemonade-flavored drink, made from powdered mix, low calorie, with vitamin C added
92541100	Apple cider-flavored drink, made from powdered mix, with sugar and vitamin C added
92541120	Apple cider-flavored drink, made from powdered mix, low calorie, with vitamin C added
92542000	Fruit-flavored drink, made from powdered mix, mainly sugar, with high vitamin C added
92544000	Fruit-flavored drink, made from unsweetened powdered mix (fortified with vitamin C), with sugar added in preparation
92552000	Fruit-flavored drink, made from powdered mix with high vitamin C added, low calorie
92552010	Fruit flavored drink, made from powdered mix, low calorie
92582000	Fruit-flavored drink, low calorie, calcium fortified

92731000 Fruit-flavored drink, non-carbonated, made from powdered mix, with sugar
92741000 Fruit-flavored drink, non-carbonated, made from low calorie powdered mix

Vitamin and Mineral Supplements

Calcium Chews

(codes selected from NHANES Dietary Supplement Survey)

[Theobromine] = 0.013%

Supplement ID

1000060000
1000060000
1000248700
1000248700
1000292800
1000292800
1000418200
1000436300
1000455400
1000482200
1000492900
1000492900
1000532000
1000532000
1000564000
1000572800
1000590200
1000592800
1000604800
1000630800
1000635300
1000643700
1000665300
1000673600
1888060002
1888292801
1888292801

Original article

Biotransformation of caffeine, paraxanthine, theobromine and theophylline by cDNA-expressed human CYP1A2 and CYP2E1

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Six human cytochrome P450s expressed in HepG2 cells using vaccinia virus cDNA-directed expression, were used to study the biotransformation of caffeine and its metabolites. CYP1A2 alone was responsible for caffeine 3-demethylation and paraxanthine 7-demethylation; in addition, 1A2 catalysed virtually all reactions related to caffeine and its metabolites. The metabolic profile of caffeine biotransformation by CYP1A2 averaged 81.5% for paraxanthine, 10.8% for theobromine and 5.4% for theophylline formation. It remained quite uniform when caffeine concentrations were varied. The most striking finding was that CYP2E1 (the ethanol-inducible form) had major influences upon caffeine metabolism: in particular, it catalysed the formation of theophylline and theobromine from caffeine. Thus, the *in vivo* metabolite profiling of caffeine may reveal CYP2E1 activities in addition to the previously documented activities of CYP1A2, polymorphic N-acetyltransferase and xanthine oxidase.

Introduction

Caffeine (137X, see Fig. 1) is almost completely metabolized in the body (Somani & Gupta, 1988). The demethylated xanthines and ring hydroxylated urates together with the acetylated uracil account for the major portion of caffeine metabolism in humans (Arnaud & Welsch, 1980; Tang *et al.*, 1983; Tang-Liu *et al.*, 1983). Hydroxylation of caffeine to give 1,3,7-trimethyluric acid (137U) is only important for *in vitro* studies (Fig. 1). It has been established by Butler *et al.* (1989) that the major route of caffeine 3-demethylation in humans is catalysed by CYP1A2. Berthou *et al.* (1991) confirmed this observation and suggested that the formation of other demethylated metabolites (e.g. theobromine, 37X, and theophylline, 13X) is mediated, at least partly, by other P450 enzymes.

Since the urinary metabolite ratio which was derived from the molar fraction of 7-demethylation of paraxanthine (17X) after ingestion of caffeine correlated well with caffeine 3-demethylation, it was concluded that 7-demethylation of paraxanthine was

catalysed mostly or completely by the same caffeine metabolizing enzyme (Campbell *et al.*, 1987; Kalow & Tang, 1991). Subsequently, the urinary metabolite ratio was proposed as the CYP1A2 index (Kalow & Tang, 1991). The objectives of this study are to investigate the role of CYP1A2 in the pathways of caffeine metabolism and to explore whether any of the six available P450s are involved in the biotransformation of caffeine and its metabolites; the human P450s were produced in human hepatoma-derived HepG2 cells through vaccinia virus cDNA expression.

Materials and methods

Chemicals

Caffeine, paraxanthine, theobromine and theophylline were obtained from Sigma Chemical Co. (St Louis, USA). The purity of the chemicals was checked by HPLC and they were found to be free of interfering substances except for paraxanthine, which was received approximately 95% pure and was further purified by HPLC before use to reach a purity of higher than 99.99%.

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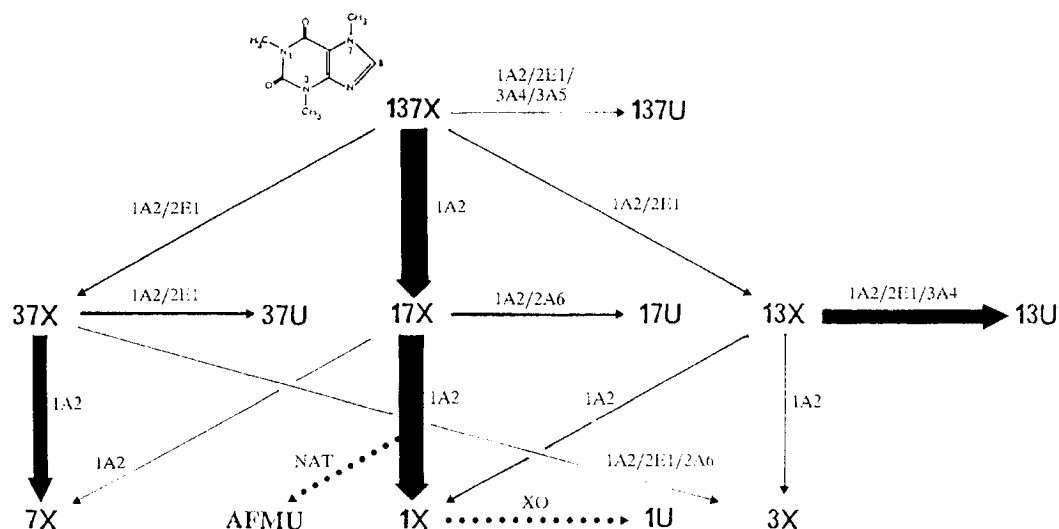


Fig. 1. Involvement of human cytochrome P450 isozymes from HepG2 cells through vaccinia virus cDNA expression in the biotransformation of caffeine and metabolites. Heavy arrows indicate the relative proportions of the pathway via CYP1A2. 137X: caffeine; 137U: 1,3,7-trimethyluric acid; 17X: paraxanthine; 37X: theobromine; 13X: theophylline. 2A3 is now known as 2A6.

Human cytochrome P450 isozymes

Six human vaccinia virus-expressed P450s were used in this study. They were CYP1A2, 2A6, 2B6, 2E1, 3A4 and 3A5. The preparation of these P450s has been described previously (Gonzalez *et al.*, 1991). Briefly, corresponding human P450 cDNAs were inserted into vaccinia viruses and the recombinant viruses were used to infect HepG2 cells. Twenty-four hours after infection, the intact cells were collected and stored as a cell pellet at -70°C . Prior to use, the cell pellets were thawed on ice, sonicated briefly and diluted with phosphate buffer solution (0.2 M, $\text{pH} = 7.4$) so that the protein concentration was 10 mg/ml as estimated by the bicinchoninic acid protein assay kit (Pierce Chemical Co., Rockford, IL).

Caffeine, paraxanthine, theobromine and theophylline assays

Methylxanthine assays were based on the methods of Grant *et al.* (1983) and Campbell *et al.* (1987). The incubate mixture of 0.4 ml contained 0.1 ml of caffeine or dimethylxanthine as substrate, 0.1 ml of phosphate buffer solution (0.2 M, $\text{pH} = 7.4$), 0.1 ml of 1.15% KCl, 0.1 ml of 0.95% MgCl_2 and 0.5 mg NADPH. The mixture was pre-incubated for 2 min at 37°C and the reaction was started by the addition of 1 mg cell protein which had been kept at 4°C . After incubation at 37°C for 60 min, the reaction was terminated by the addition of 0.05 ml of 1.5 M HCl.

Then 0.02 ml of *N*-acetyl-4-aminophenol (1 mg $100\text{ ml}^{-1}\text{ H}_2\text{O}$) as internal standard and 150 mg of ammonium sulfate was added. The mixture was extracted with 8 ml of chloroform:isopropanol (85:15, v:v), vortexed for 30 s and centrifuged for 5 min at 2500 rpm. The organic phase was then dried under nitrogen. The residue was redissolved in 0.15 ml of the mobile phase (11% methanol in 0.05% acetic acid, v:v) and 0.1 ml was injected onto the Ultrasphere ODS column (Beckman, $5\text{ }\mu\text{m}$, $25\text{ cm} \times 4.6\text{ mm}$ I.D.). The xanthines and urates were monitored by ultraviolet spectrophotometer at 280 nm.

Various concentrations of caffeine and paraxanthine, ranging from 0.125 to 2.0 mM, were used with CYP1A2 as catalyst for the estimation of Michaelis-Menten parameters using the program ENZFITTER (Elsevier-Biosoft Co., 1987).

Results

Caffeine

CYP1A2 catalysed both 1-, 3- and 7-demethylations and ring hydroxylation of caffeine (Fig. 1). The paraxanthine (17X) formation represented about 79% of total biotransformation via 1A2 (Table 1). Theobromine (37X), theophylline (13X) and 1,3,7-trimethylurate (137U) constituted 10.9%, 6.2% and 3.7%, respectively. The overall rate of caffeine biotransformation was about three times higher

Table 1. Biotransformation of caffeine, paraxanthine theobromine and theophylline by cDNA expressed human cytochrome P450 isozymes*

P450	1A2	2A6	2B6	2E1	3A4	3A5
137X						
13X	1.8 (6.2%)	—	—	2.1 (33.1%)	—	—
17X	22.6 (79.2%)	—	—	—	—	—
37X	3.1 (10.9%)	—	—	0.9 (13.8%)	—	—
137U	1.1 (3.7%)	—	—	3.4 (53.1%)	3.5	1.1
Sum	28.6 (100%)			6.4 (100%)		
17X						
1X	7.0 (75.8%)	—	—	—	—	—
7X	—	—	—	—	—	—
17U	2.2 (24.2%)	2.8	—	—	—	—
Sum	9.2 (100%)					
37X						
3X	0.7 (6.7%)	2.1	—	0.6 (19.1%)	—	—
7X	6.3 (61.4%)	—	—	—	—	—
37U	3.3 (31.8%)	—	—	2.6 (80.9%)	—	—
Sum	10.2 (100%)			3.2 (100%)		
13X						
1X	2.4 (23.0%)	—	—	—	—	—
3X	2.4 (23.0%)	—	—	—	—	—
13U	5.5 (54.1%)	—	—	4.6 (100%)	1.4	—
Sum	10.2 (100%)					

*All substrate concentrations were 1 mM, incubated with 1 mg cell protein for 60 min.

Values were averaged velocity of metabolite formation from two measurements ($\text{pmol min}^{-1} \text{mg}^{-1}$ protein with normalized rate % in brackets).

— indicated below level of detection (detection limit = 20 nM).

than the biotransformation rate of any of the dimethylxanthines.

Interestingly, CYP2E1 (the ethanol-inducible form of cytochrome P450) was the only other P450 that showed *N*-demethylation activity with distinct metabolite profiles. While there was no detectable amount of paraxanthine, theophylline (33%) and theobromine (13.8%) represented the major *N*-demethylation products of caffeine via 2E1 (Table 1, Fig. 2).

In addition, 8-hydroxylation of caffeine was manifest after incubation with both 2E1 and 3A4, which showed higher activity than 3A5. CYP2B6 did not metabolize caffeine under the present experimental conditions.

Table 2 shows the biotransformation of caffeine and paraxanthine via 1A2. The K_m for caffeine metabolites ranged from 0.93 to 2.44 mM which is not significantly different from that of 1-methylxanthine formation from paraxanthine (2.5 mM).

It should be noted that the conversion percentage of each metabolite of caffeine is virtually constant when the concentration of caffeine is varied from 0.125 to 2 mM, with an average of 81.5% for paraxanthine, 10.8% for theobromine and 5.4% for theophylline (Table 2, Fig. 2).

Paraxanthine

It was demonstrated that 1A2 could catalyse the 7-demethylation of paraxanthine (Table 1) to give 1-methylxanthine (75.9%). Furthermore, 1A2 was

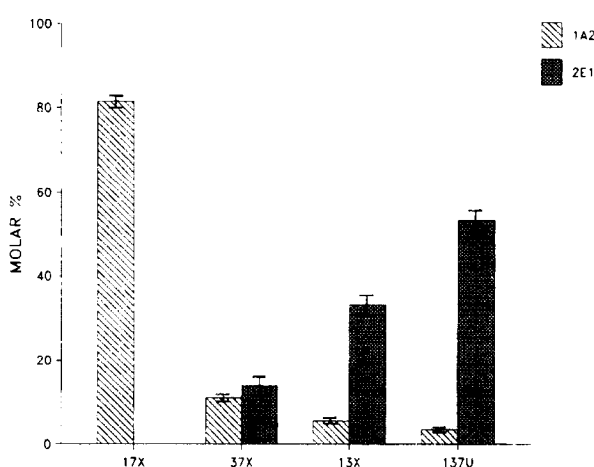


Fig. 2. Distinct metabolite profiles of caffeine catalysed by CYP1A2 and CYP2E1. Standard deviations are marked at the top of each bar for CYP1A2 and ranges for CYP2E1.

Table 2. Biotransformation of various concentrations of caffeine and paraxanthine by CYP1A2 to form products as indicated

Caffeine (mM)	pmol min ⁻¹ mg ⁻¹ (normalized rate %)				Para- xanthine (mM)	pmol min ⁻¹ mg ⁻¹ (normalized rate %)		
	17X	37X	13X	137U		1X	7X	17U
0.125	4.8 (85.8)	0.6 (11.2)	—	0.2 (2.9)	0.125	—	—	—
0.25	10.3 (82.4)	1.3 (10.3)	0.5 (4.2)	0.4 (3.1)	0.25	1.7 (80.2)	—	0.4 (19.8)
0.5	15.7 (80.3)	2.3 (11.6)	1.0 (4.9)	0.6 (3.3)	0.5	3.5 (77.5)	—	1.0 (22.5)
1	22.6 (79.2)	3.1 (10.9)	1.8 (6.2)	1.1 (3.7)	1	7.0 (75.8)	—	2.2 (24.2)
2	32.4 (79.6)	4.2 (10.3)	2.6 (6.4)	1.5 (3.7)	2	9.9 (80.6)	0.4 (3.0)	2.0 (16.4)
Km*	1.08 ± 0.15	0.93 ± 0.10	2.44 ± 0.45	1.52 ± 0.15	Km	2.5 ± 0.9	—	—
Kcat	49.2 ± 3.47	6.1 ± 0.30	5.9 ± 0.68	2.6 ± 0.14	Kcat	22.7 ± 5.4	—	—

*Km (mM) and Kcat (pmol min⁻¹ mg⁻¹) were estimated from the program ENZFITTER.

the only P450 which catalysed the demethylation of paraxanthine (2E1 could not metabolize paraxanthine). 7-methylxanthine was detected only at a concentration higher than 1 mM of paraxanthine (Table 2). However, 8-hydroxylation of paraxanthine could be mediated by both 1A2 and 2A6.

Theobromine

7-methylxanthine (61.4%), the major metabolite of theobromine, was formed only by 1A2. The second major metabolite, 3,7-dimethylurate (31.8%) could be formed by P450s 1A2 and 2E1 (Table 1).

The metabolite profile of theobromine was quite different when 2E1 was used as a catalyst for incubation. The major metabolite was 37U (80.9%) and there was no 7X formation.

Theophylline

Theophylline was N-demethylated and hydroxylated by 1A2. In contrast, 1,3-dimethylurate (13U) was the only product detected after incubation with 2E1.

Discussion

Results from cDNA-expressed CYP1A2 gave direct evidence that 1A2 is the primary P450 responsible for caffeine 3-demethylation and paraxanthine 7-demethylation. This study confirms our use of the

urinary caffeine ratio, which is based on the molar fraction of 7-demethylation of paraxanthine after ingestion of caffeine, as the index of CYP1A2 activity (Campbell *et al.*, 1987; Kalow & Tang, 1991); indeed, CYP1A2 catalysed virtually all reactions related to caffeine and metabolites, although other P450 enzymes also contribute to the biotransformation of caffeine (Fig. 1 and Table 1).

The relative rates of paraxanthine, theobromine and theophylline formation via CYP1A2 remained unchanged as caffeine concentrations were varied. Equivalent measurements in preparations of human liver microsomes showed more formation of theophylline and of theobromine than produced by CYP1A2 (Table 3). This is as expected since microsomes must contain CYP2E1. Differences of CYP2E1 content in the microsomes from different livers may account for the larger standard deviations in the studies with microsomes compared to those with CYP1A2.

CYP2E1, the ethanol-inducible P450 form, is known to metabolically-activate small molecular weight nitrosamines and many small solvent molecules (Koop & Tierney, 1990; Lieber, 1990; Yang *et al.*, 1990; Guengerich *et al.*, 1991). The present results suggest that the contribution of 2E1 to the 7- and 1-demethylation of caffeine can be estimated because of the distinct patterns of dimethylxanthines

Table 3. Mean (±SD) of relative rate ratios (% of sum) for the formations of three dimethylxanthines from caffeine

	[137X]	17X	37X	13X
CYP1A2*	0.125–2 mM	84.3 (2.3)	11.2 (0.5)	5.6 (1.0)
Microsomes**	0.012 mM	70.0 (7.5)	17.4 (5.4)	12.6 (2.9)

*Average of normalized rates from Table 2 at various concentrations of caffeine. Data from this study.

**Average of normalized rates of 5 human microsome preparations from Table 4 of Berthou *et al.* (1989).

formation via 2E1 and 1A2. Since 3-demethylation of caffeine was catalysed only by CYP1A2, paraxanthine formation may serve as the basis for the *in vivo* estimation of the expected contribution of 1A2 to the theophylline and theobromine pathways. Subtracting these expected contribution from the observed rates should yield the contributions of 2E1 to the 7- and 1-demethylation pathways. However, the exact involvement of 2E1 in caffeine metabolism and estimation of 2E1 activity through the shifting of the 3-demethylation to the 1- and 7-demethylation pathways need to be validated. Thus caffeine, which can be used as an index of activity of three enzymes, the carcinogen-activating CYP1A2, the polymorphic N-acetyltransferase, and xanthine oxidase (Tang *et al.*, 1991; Kalow & Tang, 1991), may also be useful in estimating the activity of CYP2E1, the ethanol-inducible cytochrome P450.

1,3,7-trimethylurate formation represents a minor metabolic pathway via 1A2 and in the *in vivo* situation. In contrast, it is a major pathway in *in vitro* metabolism by human microsomes (Campbell *et al.*, 1987; Berthou *et al.*, 1989). This discrepancy has yet to be explained.

Since in our previous studies, 8-hydroxylation activity correlated poorly with the demethylation activity in microsomes, it was suggested that hydroxylation and demethylation functions are mediated by different enzymes (Kalow & Campbell, 1988). This is confirmed by the evidence of this study that the formation of all four urates was catalysed by more than a single cytochrome: CYP2E1 exhibited high activity for the formation of all urates except for 17U formation (Table 1, Fig. 1). Since the CYP3A P450s, which are among the most abundantly expressed in humans they may contribute to *in vivo* production of 1,3,7-trimethylurate.

In summary, six human cDNA-expressed P450s were tested in a study of the biotransformation of caffeine and three dimethylxanthines. It was demonstrated that CYP1A2 participated in all the demethylations and ring hydroxylations of caffeine and of the dimethylxanthine metabolites. The second major P450 involved in caffeine demethylation was CYP2E1 which exhibited major influences in theophylline and theobromine formation. Moreover, CYP2E1 demonstrated high activity for the 8-hydroxylations of caffeine, theobromine and theophylline. Of the other six cytochrome P450s only 2A6 showed demethylation activity in the 7-demethylation of theobromine. CYP2A6 was also involved in the 8-hydroxylation of paraxanthine. While CYP3A4 could catalyse the 8-hydroxylation of both caffeine and theophylline,

CYP3A5 could only catalyse caffeine 8-hydroxylation. CYP2B6 did not show any activity in the biotransformation of caffeine or its metabolites.

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MATERNAL SERUM PARAXANTHINE, A CAFFEINE METABOLITE, AND THE RISK OF SPONTANEOUS ABORTION

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ABSTRACT

Background Whether the consumption of caffeine during pregnancy increases the risk of spontaneous abortion is controversial. Prior studies have determined caffeine consumption by questionnaire. We used a biologic marker, serum paraxanthine, a metabolite of caffeine, to measure the dose of caffeine.

Methods In a nested case-control study, we measured serum paraxanthine in 591 women who had spontaneous abortions at less than 140 days' gestation and in 2558 matched women from the same clinic who gave birth to live infants at 28 weeks' gestation or later and who had serum drawn on the same day of gestation as the women who had abortions. The women were enrolled in the Collaborative Perinatal Project during the period from 1959 to 1966, and serum paraxanthine was measured over 30 years later.

Results A total of 487 women who had spontaneous abortions (82 percent) and 2087 controls (82 percent) had quantifiable serum paraxanthine concentrations. However, the mean serum paraxanthine concentration was higher in the women who had spontaneous abortions than in the controls (752 vs. 583 ng per milliliter, $P < 0.001$). The odds ratio for spontaneous abortion was not significantly elevated in the women who had serum paraxanthine concentrations of 1845 ng per milliliter or lower, corresponding to the 95th percentile of the matched women. However, the adjusted odds ratio for spontaneous abortion among women with serum paraxanthine concentrations higher than 1845 ng per milliliter, as compared with women who had concentrations below 50 ng per milliliter, was 1.9 (95 percent confidence interval, 1.2 to 2.8).

Conclusions Only extremely high serum paraxanthine concentrations are associated with spontaneous abortion. This suggests that moderate consumption of caffeine is unlikely to increase the risk of spontaneous abortion. (N Engl J Med 1999;341:1639-44.)

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WHETHER consumption of caffeine during pregnancy increases the risk of spontaneous abortion is controversial. Several studies have indicated that even moderate caffeine consumption is associated with a risk of fetal loss that is more than double the risk in women who do not consume caffeine.^{1,2} Others have reported that the risk is elevated only for women who consume three or more cups of coffee per day³ or those who consume large amounts of caffeine and

who are nauseated during pregnancy.⁴ Still other studies have found no increase in risk even among women who consume large amounts of caffeine.⁵⁻⁸

Possible reasons for these discrepant results include small samples, particularly with respect to the groups of women who consumed large amounts of caffeine; retrospective or prospective ascertainment of caffeine consumption, spontaneous abortion, or both; changes in caffeine consumption during pregnancy; differences in the adequacy of statistical control for nausea during pregnancy; and problems of assessing caffeine intake on the basis of responses to a questionnaire.⁹ Wide variations in individual rates of caffeine metabolism make it difficult to translate even an accurately reported intake into serum concentrations of caffeine and its metabolites.¹⁰ We measured serum paraxanthine, the primary metabolite of caffeine, to determine whether the consumption of caffeine is associated with spontaneous abortion.

METHODS

Study Subjects

Our sample consisted of women enrolled in the Collaborative Perinatal Project, a prospective study of pregnancy, labor, and child development conducted at 12 sites in the United States from 1959 to 1966. The women in that study were enrolled when they presented for prenatal care and were followed for the remainder of their pregnancy. There were approximately 55,000 births to 42,000 women.¹¹ Although no information was collected on the consumption of coffee, tea, or soft drinks, serum was obtained approximately every two months during pregnancy, at delivery, and six weeks after delivery. Information about vomiting was obtained at enrollment and at each prenatal visit, and gestational age was estimated on the basis of the reported first day of the last menstrual period.

A total of 830 women had early fetal losses (less than 140 days after the first day of the last menstrual period); serum was obtained during the pregnancy from 704 of these women. The relatively small number of women with early fetal losses was due to the late gestational age at which many women were enrolled in the study.¹¹ The women were stratified according to the clinical center and the day of gestation on which the earliest serum sample was obtained. For the women with early fetal losses in each stratum, we selected four times the number of women at the same center who gave birth to live infants after at least 28 weeks of gestation and who had serum drawn on the same day of gestation (con-

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trols). Serum was obtained at an unknown time of day during clinic visits and during hospitalization for delivery.

Serum caffeine and paraxanthine were assayed. In a pilot study,¹² serum caffeine and paraxanthine concentrations were positively correlated with the caffeine consumption reported by pregnant women, but the serum paraxanthine concentration was more closely correlated with caffeine consumption than was the serum caffeine concentration, particularly among smokers. The serum concentration of paraxanthine is less sensitive than that of caffeine to very recent caffeine intake.¹⁰ Accordingly, our primary objective was to test the hypothesis that the mean serum paraxanthine concentration was higher in women who had spontaneous abortions than in women who delivered live infants. An additional objective was to determine whether there was a threshold above which the serum paraxanthine concentration was associated with spontaneous abortion.

Biochemical Assays

Serum caffeine and paraxanthine were measured with the use of high-performance liquid chromatography.¹² The limit of quantitation was established at 50 ng per milliliter for caffeine and paraxanthine; the limit of detection was 25 ng per milliliter. The intraassay and interassay coefficients of variation were less than 6.9 percent at 200, 800, and 2000 ng per milliliter. The laboratory personnel who performed the assays were unaware of the outcome of each pregnancy. Serum samples from the women who had spontaneous abortions and from the matched controls were analyzed in the same batch; the order of the samples varied from batch to batch. Since this analysis involved previously collected specimens from which identifying information had been removed, the Office of Human Subjects Research found it to be exempt from the requirement for approval by an institutional review board.

Statistical Analysis

Continuous variables were compared with use of Student's *t*-test or analysis of variance, and categorical variables were compared with use of the chi-square test. The standard deviation for the serum paraxanthine concentration was proportional to the mean, violating the assumptions of the *t*-test and analysis of variance. Log transformation of the serum paraxanthine values solved this problem. Since the results with the use of log-transformed data did not differ substantially from the results with the use of untransformed data, only the latter are reported here. The association between the serum paraxanthine concentration and spontaneous abortion was analyzed by conditional logistic regression.¹³

RESULTS

There were 704 women who had early fetal losses and 2816 controls. Since the Collaborative Perinatal Project had only one code for all early fetal losses, the original study records were reviewed to identify the women who had spontaneous abortions. Forty-six of the women with early fetal losses had induced abortions, ectopic pregnancies, or iatrogenic termination of pregnancy or died during pregnancy. For the group of 658 women in whom fetal loss was due to spontaneous abortion, it was not possible to determine from a review of the records whether serum drawn on the day of spontaneous abortion was obtained before or after the event, so the 57 women in whom the serum sample had been obtained on the day of abortion were excluded from the analysis. In an additional 10 women who had spontaneous abortions, insufficient serum was available for analysis. The exclusion of these 113 women required the exclusion of 208 matched controls, and in 50 additional controls, in-

sufficient serum was available for analysis. The final study group thus comprised 591 women who had spontaneous abortions and 2558 matched controls.

We compared the group of 591 women with spontaneous abortions whose serum samples were available for analysis with the group of 193 women with spontaneous abortions for whom serum samples were not available. The median date of enrollment was January 1963 for the former group and December 1960 for the latter ($P < 0.001$), suggesting that study procedures improved over time, and the two groups of women were enrolled on day 76 and day 80 of gestation, respectively ($P = 0.02$). On average, 24 days elapsed from enrollment to the spontaneous abortion for women for whom serum was available, as compared with 12 days for women for whom serum was not available ($P < 0.001$). The proportion of women from whom serum was obtained varied significantly among the study sites, ranging from 70 to 100 percent.

The characteristics of the women who had spontaneous abortions and the controls are shown in Table 1. Serum was drawn on the same day of gestation in the two groups. The mean duration of pregnancy was slightly more than 14 weeks among the women who had spontaneous abortions and was 39 weeks among the controls. The median interval from the collection of serum to abortion was 17 days. The women who had spontaneous abortions were significantly older than the controls ($P < 0.001$), more likely to smoke ($P < 0.001$), and less likely to have vomited ($P < 0.001$) or to have taken medications containing caffeine ($P = 0.02$) during pregnancy.

The serum paraxanthine concentrations are shown in Table 2 according to the outcome of pregnancy and maternal characteristics. In both the group of women who had spontaneous abortions and the control group, higher serum paraxanthine concentrations were associated with increasing age, white race, smoking, and the absence of vomiting during pregnancy. The serum paraxanthine concentration was positively associated with the level of education only in the control group. In almost every category of each of these characteristics, the serum paraxanthine concentration was higher in the women who had spontaneous abortions than in the controls (Table 2).

A total of 487 women who had spontaneous abortions (82 percent) and 2087 controls (82 percent) had quantifiable serum paraxanthine concentrations ($P = 0.64$). However, the mean serum paraxanthine concentration was significantly higher in the abortion group than in the control group (752 vs. 583 ng per milliliter, $P < 0.001$). The odds ratios for spontaneous abortion according to the serum paraxanthine concentration, with the women who had unquantifiable serum paraxanthine concentrations (< 50 ng per milliliter) used as the reference group and with adjustment for smoking status, age, and race or ethnic group, are

TABLE 1. CHARACTERISTICS OF THE WOMEN WHO HAD SPONTANEOUS ABORTIONS AND THOSE WHO GAVE BIRTH TO LIVE INFANTS (CONTROLS).

CHARACTERISTIC	WOMEN WITH SPONTANEOUS ABORTIONS (N=591)		CONTROLS (N=2558)	
Length of gestation when blood sample obtained (days)				
Mean	78		78	
Median	76		76	
Length of gestation at time of abortion or delivery (days)				
Mean	100		274	
Median	99		278	
Interval between blood sample and abortion (days)				
Mean	22		—	
Median	17			
Mean age at enrollment (yr)	27		25	
Smoker (%)*	46		39	
Vomiting since last menstrual period (%)†	38		56	
Education (%)‡				
<12 yr	50		40	
12 yr	31		37	
≥13 yr	19		23	
Race or ethnic group (%)				
White	61		65	
Black	33		29	
Other or unknown§	6		6	
Diagnosis of diabetes mellitus before pregnancy (%)	3		2	
Use of medications containing caffeine (%)				
During month serum sample was obtained	6		9	
During month before serum sample was obtained	4		6	

*Data were available for 591 women who had spontaneous abortions and 2542 controls.

†Data were available for 515 women who had spontaneous abortions and 2544 controls.

‡Data were available for 447 women who had spontaneous abortions and 2518 controls.

§Eleven percent of the women in this category were Asian, 85 percent were Puerto Rican, and 4 percent were unclassified with respect to race or ethnic group.

shown in Figure 1. Data on vomiting during pregnancy and educational level were missing for a substantial number of women. However, adjustment for these factors did not substantially change the odds ratios (data not shown). The increased risk of spontaneous abortion was almost entirely restricted to women with serum paraxanthine concentrations higher than 1845 ng per milliliter, corresponding to the 5 percent of controls with the highest concentrations (adjusted odds ratio, 1.9; 95 percent confidence interval, 1.2 to 2.8). For the remainder of the analyses, the women were grouped according to their serum paraxanthine concentrations (<50 ng per milliliter, 50 to 1845 ng per milliliter, and >1845 ng per milliliter, corresponding roughly to <20th percentile of serum paraxanthine values in the controls, 20th to 95th percentile, and >95th percentile).

The association between serum paraxanthine con-

TABLE 2. MEAN SERUM CONCENTRATIONS OF PARAXANTHINE ACCORDING TO THE OUTCOME OF PREGNANCY AND MATERNAL CHARACTERISTICS.

CHARACTERISTIC	WOMEN WITH SPONTANEOUS ABORTIONS		CONTROLS	
	SERUM PARAXANTHINE	P VALUE	SERUM PARAXANTHINE	P VALUE
	ng/ml		ng/ml	
Age		<0.001*		<0.001*
<20 yr	447		359	
20–24 yr	512		498	
25–29 yr	835		681	
30–34 yr	1068		713	
≥35 yr	1024		870	
Education		0.74*		0.01*
<12 yr	714		515	
12 yr	748		593	
≥13 yr	680		612	
Smoker		<0.001		<0.001
Yes	899		762	
No	626		474	
Vomiting		<0.001		<0.001
Yes	585		536	
No	849		646	
Race or ethnic group		<0.001		<0.001
White	931		679	
Black	435		357	
Other or unknown	652		659	

*The P value is for trend.

centrations and spontaneous abortion according to other factors is shown in Table 3. All odds ratios were adjusted for maternal age, smoking status, and race or ethnic group. For women with very high serum paraxanthine concentrations, the odds ratio for spontaneous abortion did not differ significantly according to whether the abortion occurred at 100 or more days of gestation or earlier (100 days was the median interval), whether the serum sample had been obtained 17 or fewer days before spontaneous abortion or more than 17 days earlier (17 days was the median interval), or whether the woman had or had not vomited since her last menstrual period.

To determine whether differences in desiccation over time affected the results, we measured serum sodium in 3057 samples, using direct potentiometry with ion-selective electrodes. The mean (\pm SD) serum sodium concentration was 137 ± 27 mmol per liter. After standardization of the serum paraxanthine concentration to a serum sodium concentration of 135 mmol per liter, the results shown in Figure 1 were largely unchanged.

DISCUSSION

Our results indicate that the serum concentration of paraxanthine, the primary metabolite of caffeine, is higher in women who have spontaneous abortions than in women who give birth to live infants. However, the risk of spontaneous abortion is not in-

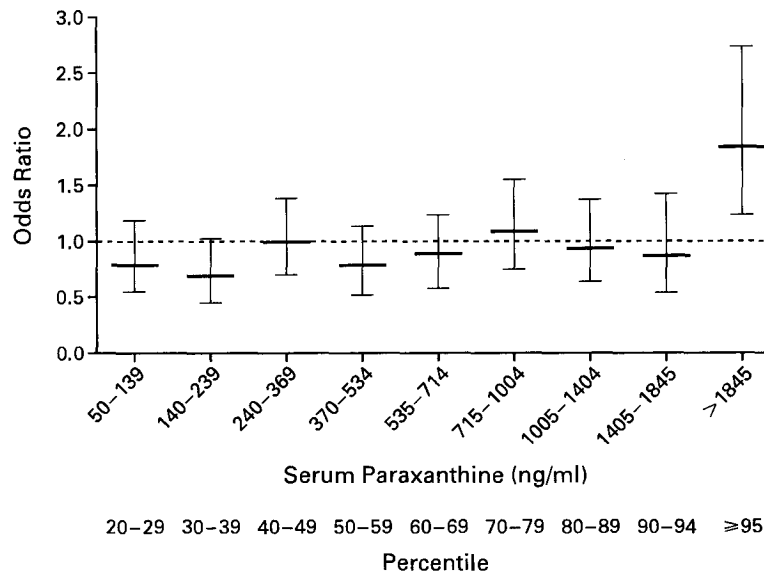


Figure 1. Adjusted Odds Ratios and 95 Percent Confidence Intervals for Spontaneous Abortion According to the Serum Concentration of Paraxanthine. The reference category is values of less than 50 ng per milliliter. The odds ratios have been adjusted for smoking status, age, and race or ethnic group. The percentiles are for the serum paraxanthine values in the controls.

creased until extremely high serum paraxanthine concentrations are reached. Our results support previous studies showing that the consumption of large amounts of caffeine is associated with an increased risk of spontaneous abortion¹⁻⁴ but that moderate consumption does not increase the risk.³⁻⁸

Since there is no precise way to equate a serum paraxanthine concentration with an amount of caffeine intake, our results cannot directly answer the question of how much caffeine is safe during pregnancy. However, there may be indirect ways to answer this question. Our pilot study¹² involved women who had participated in the Birmingham, Alabama, study of infant growth in the mid-1980s.¹⁴ The highest caffeine intake in that cohort was 1530 mg per day (equivalent to approximately 15 cups of coffee). The highest measured serum paraxanthine concentration was 1165 ng per milliliter, which was substantially lower than the value at the 95th percentile in this study (1845 ng per milliliter). Even with allowance for volume loss during storage, the 95th percentile of serum paraxanthine in this study is higher than the highest value in the Birmingham study. Extrapolating from our pilot data, a 60-kg woman who did not smoke and who consumed 600 mg of caffeine (about 6 cups of coffee) per day or a 60-kg woman who smoked and who consumed 1100 mg of caffeine (about 11 cups of coffee) per day would have an estimated serum paraxanthine concentration of 1845 ng per milliliter.

Additional information to equate serum paraxanthine concentrations with caffeine intake comes from the California Child Health and Development Studies,¹⁵ involving a prospective cohort of pregnant women in the 1960s. In that study, women were asked about their intake of coffee and tea. Assuming that a cup of tea contains half the caffeine of a cup of coffee, the 95th percentile of caffeine intake was equivalent to 8.5 cups of coffee per day, which is consistent with the extrapolated data from our pilot study and conservatively suggests that the 95th percentile of caffeine intake in the current study was the equivalent of more than 5 cups of coffee per day.

Several caveats should be noted. First, the women in the Collaborative Perinatal Project were enrolled relatively late in gestation, and the majority of abortions occurred in the second trimester. Furthermore, karyotype analyses were not performed for any of the aborted fetuses. Fetuses aborted early in gestation are more likely to have chromosomal abnormalities than are fetuses aborted later.¹⁶ However, abortion of chromosomally normal fetuses is a more sensitive indicator of exogenous risk factors.¹⁶ The association between caffeine intake and spontaneous abortion has been reported to be similar for chromosomally normal and abnormal fetuses,⁸ suggesting either that caffeine increases the risk of loss for both types of fetuses or that the association is not causal.

Second, the Collaborative Perinatal Project recorded data on vomiting during pregnancy but not on

TABLE 3. ADJUSTED ODDS RATIOS FOR SPONTANEOUS ABORTION ACCORDING TO THE SERUM PARAXANTHINE CONCENTRATION AND ADDITIONAL FACTORS.

FACTOR	WOMEN WITH SPONTANEOUS ABORTIONS	CONTROLS*	TOTAL	ADJUSTED ODDS RATIO (95% CI)†
	number			
>17 Days between collection of serum and spontaneous abortion‡				
Serum paraxanthine, <50 ng/ml	50	240	290	1.0
Serum paraxanthine, 50–1845 ng/ml	206	879	1085	1.0 (0.6–1.5)
Serum paraxanthine, >1845 ng/ml	33	62	95	2.0 (1.2–3.6)
≤17 Days between collection of serum and spontaneous abortion‡				
Serum paraxanthine, <50 ng/ml	54	231	285	1.0
Serum paraxanthine, 50–1845 ng/ml	212	1078	1290	0.8 (0.5–1.1)
Serum paraxanthine, >1845 ng/ml	36	68	104	1.8 (1.0–3.1)
Spontaneous abortion at ≥100 days' gestation‡				
Serum paraxanthine, <50 ng/ml	51	255	306	1.0
Serum paraxanthine, 50–1845 ng/ml	205	1042	1247	1.0 (0.7–1.4)
Serum paraxanthine, >1845 ng/ml	33	79	112	1.7 (1.0–3.0)
Spontaneous abortion at <100 days' gestation‡				
Serum paraxanthine, <50 ng/ml	53	216	269	1.0
Serum paraxanthine, 50–1845 ng/ml	213	915	1128	0.8 (0.6–1.1)
Serum paraxanthine, >1845 ng/ml	36	51	87	2.1 (1.2–3.6)
Vomiting since last menstrual period§				
Serum paraxanthine, <50 ng/ml	49	190	239	1.0
Serum paraxanthine, 50–1845 ng/ml	221	860	1081	1.1 (0.7–1.6)
Serum paraxanthine, >1845 ng/ml	47	73	120	2.2 (1.2–4.0)
No vomiting since last menstrual period§				
Serum paraxanthine, <50 ng/ml	49	277	326	1.0
Serum paraxanthine, 50–1845 ng/ml	133	1087	1220	0.7 (0.5–1.0)
Serum paraxanthine, >1845 ng/ml	16	57	73	1.8 (0.8–3.8)

*Controls were women who gave birth to live infants after at least 28 weeks of gestation, who were at the same clinic as the women who had spontaneous abortions, and who had serum drawn on the same day of gestation as the women who had spontaneous abortions.

†Odds ratios have been adjusted for maternal age, smoking status, and race or ethnic group. The reference group is women in both study groups who had serum paraxanthine concentrations of less than 50 ng per milliliter. CI denotes confidence interval.

‡Controls were randomly matched with women who had spontaneous abortions in this stratum.

§Data on vomiting were available for 515 women who had spontaneous abortions and 2544 controls.

nausea. Nausea is thought to be a marker for a healthy pregnancy, and nausea and food aversions may cause women to reduce their consumption of coffee and other foods with strong aromas.¹⁷ If so, then even the elevated risk of spontaneous abortion among women with extremely high serum paraxanthine concentrations may simply reflect the fact that a viable pregnancy causes a woman to reduce her intake of caffeine. Since nauseated women consume less caffeine than women without nausea and also have a reduced risk of spontaneous abortion, the likely effect of incomplete data on nausea and vomiting would be to overestimate the level of risk associated with high levels of caffeine consumption.

Third, although unlike the serum caffeine concentration, the serum paraxanthine concentration does not fluctuate greatly during the day, the serum half-

lives of the two substances are similar: approximately 5 hours during the first trimester and 10 hours during the second trimester.^{18,19} Therefore, serum paraxanthine is a marker only of short-term caffeine intake. Although we are unaware of any data that confirm this observation, the likelihood that caffeine intake is relatively constant from day to day provides support for the use of serum paraxanthine as a biologic marker of caffeine intake.

Fourth, the serum samples we used had been stored for over 30 years. The stability of paraxanthine during long-term storage at -20°C is unknown. In our pilot study,¹² we found that the paraxanthine concentration in serum samples stored for eight years at -70°C was closely correlated with the reported caffeine intake, suggesting that paraxanthine remains stable under these conditions. We quantified paraxan-

thine in 82 percent of the serum samples from the Collaborative Perinatal Project and detected it below the limit of quantitation in an additional 4 percent. In the California Child Health and Development Studies, 13 percent of the women reported that they consumed neither coffee nor tea. This finding is consistent with our 85 percent detection rate and suggests that marked deterioration of paraxanthine was unlikely to have occurred.

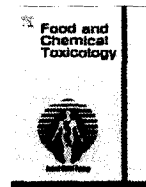
Our study has several strengths. The serum samples were collected in the 1960s, when few pregnant women were advised to reduce their intake of caffeine. Per capita coffee consumption in the United States peaked in 1962 and then declined, particularly among people less than 40 years old.²⁰ The Collaborative Perinatal Project is therefore likely to have enrolled many women who consumed large amounts of caffeine. Most investigators have found it difficult to enroll sufficient numbers of women who consumed large quantities of caffeine.^{1-4,6,7}

In conclusion, if caffeine causes spontaneous abortion, it does so only at serum paraxanthine concentrations, and presumably levels of caffeine intake, that were uncommonly high in the 1960s, and these high levels are probably even less common now.

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Review

A review of the epidemiologic evidence concerning the reproductive health effects of caffeine consumption: A 2000–2009 update

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ABSTRACT

This review of human studies of caffeine and reproductive health published between January 2000 and December 2009 serves to update the comprehensive review published by Leviton and Cowan (2002). The adverse reproductive outcomes addressed in this review include: (1) measures of subfecundity; (2) spontaneous abortion; (3) fetal death; (4) preterm birth; (5) congenital malformations; and (6) fetal growth restriction. Methodologic challenges and considerations relevant to investigations of each reproductive endpoint are summarized, followed by a brief critical review of each study. The evidence for an effect of caffeine on reproductive health and fetal development is limited by the inability to rule out plausible alternative explanations for the observed associations, namely confounding by pregnancy symptoms and smoking, and by exposure measurement error. Because of these limitations, the weight of evidence does not support a positive relationship between caffeine consumption and adverse reproductive or perinatal outcomes.

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Abbreviations: ART, assisted reproductive technology; BMI, body mass index; CgA, chromogranin A; CYP1A2, cytochrome P450 1A2; CYP1B1, cytochrome P450 1B1; CYP2E1, cytochrome P450 2E1; GIFT, gamete intra-fallopian transfer; GST, glutathione S-transferase; HR, hazard ratio; IVF, *In vitro* fertilization; IUGR, intrauterine growth restriction; LMP, last menstrual period; OR, odds ratio; NAT2, N-acetyltransferase 2; RR, relative risk; SGA, small for gestational age.

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1. Introduction

This review of the literature on consumption of caffeine-containing products and reproductive health is an update of the comprehensive report previously published by Leviton and Cowan (2002). As such, this review is restricted to human studies of caffeine and reproductive health published in English between January 2000 and December 2009. The search strategy consisted of a Pubmed search using the keywords caffeine, coffee or paraxanthine in combination with the following terms: pregnancy, reproduction, fetal development, miscarriage, spontaneous abortion, pregnancy loss, fetal death, stillbirth, congenital malformations, birth defects, fetal growth, growth restriction, growth retardation, small for gestational age, low birthweight, low birth weight, IUGR, preterm, fertility, fecundity, time to pregnancy, sperm, semen, twins, twinning, multiple gestation, or multiple pregnancy. The references cited in all original studies and review papers identified were also examined to ensure completeness.

The reproductive outcomes addressed in this review are organized into six categories: (1) measures of subfecundity; (2) spontaneous abortion; (3) fetal death; (4) preterm birth; (5) congenital malformations; and (6) fetal growth. For each topic, we begin by summarizing study design considerations relevant to investigations of the specific reproductive endpoint. In keeping with the un-

ique format of the previous summary, we then provide a detailed critical review of each study, identifying strengths as well as methodological limitations that may influence results and restrict inferences that can be drawn from individual studies. Individual studies are discussed by topic in chronological order. Table 1 lists the publications reviewed in this report by reproductive outcome evaluated. Two post-1999 publications (Cnattingius et al., 2000; Grosso et al., 2001) were reviewed previously in Leviton and Cowan (2002) and, thus, are not summarized in this review.

We begin with a discussion of general methodological concerns that should be considered when reviewing studies of consumption of caffeine-containing products and reproductive health.

1.1. Caffeine exposure assessment

Total caffeine consumption is difficult to measure accurately. Caffeine exposure can occur from various sources including beverages (coffee, tea, soft drinks), chocolate, and some medications. Furthermore, the caffeine content of individual beverage servings varies considerably by method of preparation, product brand, and cup size (Bracken et al., 2002). The most common justification for assessing caffeine exposure from coffee alone or from coffee and tea is that coffee is the predominant source of caffeine exposure and fewer women report high doses from other beverages, foods

Table 1
Chronological list of 2000–2009 publications by reproductive outcome evaluated.

Year	First author	Subfecundity/ infertility	Spontaneous abortion	Fetal death	Gestational age/preterm birth	Congenital malformations	Fetal growth
2000	Natsume					X	
2000	Torfs					X	
2000	Zusterzeel		X				
2001	Grosso						X
2001	Signorello		X				
2001	Wen		X				
2002	Claussion				X		X
2002	Klebanoff				X		X
2002	Klonoff-Cohen	X	X		X		
2003	Balat						X
2003	Bracken				X		X
2003	Giannelli		X				
2003	Horak	X					
2003	Orskou						X
2003	Rasch		X				
2003	Tolstrup		X				
2003	Tough				X		
2003	Vik						X
2003	Wisborg			X			
2004	Hassan	X					
2004	Khoury		X		X	X	
2005	Bech		X	X			
2005	Parazzini						X
2005	Santos				X		X
2005	Sata		X				
2005	Sobreiro	X					
2006	Bech			X			
2006	Chiaffarino				X		
2006	Cole	X					
2006	George		X				
2006	Grosso						X
2006	Karypidis		X				
2006	Matijasevich			X			
2006	Tsubouchi						X
2007	Bech				X		X
2007	Bille					X	
2007	Browne					X	
2007	Diego						X
2007	Infante-Rivard						X
2007	Maconochie		X				
2007	Schmid	X					
2008	CARE Study Group						X
2008	Haugen				X		
2008	Mikkelsen				X		
2008	Mongraw-Chaffin					X	
2008	Ramlau-Hansen	X					
2008	Savitz		X				
2008	Slickers					X	
2008	Weng		X				
2008	Xue						X
2009	Chavarro	X					
2009	Collier					X	
2009	Johansen					X	
2009	Killick	X					
2009	Miller					X	
2009	Schmidt					X	

or pharmaceuticals. Regardless of the population proportions consuming large quantities from other sources, consumption from all sources is important for accurate classification of total caffeine intake for individuals, since low to moderate intake from multiple sources can result in high cumulative caffeine exposure. Furthermore, because coffee also contains many chemicals other than caffeine, it is difficult to disentangle the potential effects of caffeine from those that may be attributed to other compounds.

Relying on coffee intake alone would likely result in underestimations of total caffeine exposure. The influence of this measurement error on observed associations would depend on whether use of caffeine from other sources was more, less or equally common among women experiencing the outcome under investigation. Exposure measurement error is commonly assumed to be

similar among those with and without each reproductive disorder, resulting in an underestimation of any coffee/caffeine relationship with the reproduction adversity. This underestimation (bias toward the null) tends to be predictable only when the exposure is dichotomous and the association is independent of other errors (Greenland and Gustafson, 2006). Much of the caffeine literature assesses more than two categories of exposure and thus, misclassification of caffeine intake could produce a bias either toward or away from the null, depending on the nature of the errors.

Other critical aspects of caffeine exposure assessment include the importance of measuring exposures during the relevant exposure time window and the need to capture changing intake patterns throughout pregnancy. Caffeine consumption tends to decrease during the early weeks of pregnancy, coinciding with

increasing pregnancy symptoms and aversions (Gadsby et al., 1993; Cnattingius et al., 2000; Lawson et al., 2004). Retrospective reports of caffeine intake collected at a single time point as the average number of daily servings across a large time span such as the first trimester or entire pregnancy (typically converted to caffeine in mg/day) will not accurately characterize true exposure fluctuations. This type of measurement error is especially relevant when the critical window of exposure for selected outcomes occurs during the time interval when consumption patterns are changing in early gestation.

Although a few studies designed for the purpose of investigating caffeine exposure have implemented detailed improvements in exposure assessment, variations in caffeine exposure by source, portion size, brewing method, metabolism and fluctuations over the course of pregnancy continue to result in exposure misclassification. Furthermore, the comparison of findings across studies is difficult due to the use of different categories of caffeine intake and different reference groups.

Self-reported caffeine exposure is not only imprecise, it also fails to account for variability in rates of degradation. The measurement of caffeine metabolites in biologic fluids provides better information about biologic dose in part because it reflects individual differences in caffeine metabolism (Klebanoff et al., 1998c), but such methods are not without limitations. As the major metabolite of caffeine, serum paraxanthine has a short half-life, ranging from 2 to 5 h in early pregnancy to 10 h in late pregnancy (Aldridge et al., 1981). Thus, serum paraxanthine concentrations reflect recent exposures within the day immediately preceding specimen collection. Moreover, studies that incorporate caffeine biomarkers are typically limited to specimens collected at a single time point. Therefore, biomarker concentrations would accurately represent previous caffeine intake patterns only when consumption remains relatively constant over time. The problem with this assumption is that caffeine consumption is known to decrease throughout the first months of pregnancy (Lawson et al., 2004). Thus, biomarker concentrations may also be susceptible to exposure misclassification when a single measurement is intended to represent long-term or usual patterns of exposure during pregnancy.

The rate at which caffeine is cleared from the body may influence biologic dose and exposure interval. Caffeine metabolites might be more important than caffeine in producing a biologic effect. Caffeine clearance rates differ between individuals and are affected by pregnancy and genetics as well as environmental factors such as cigarette smoking, drugs and diet (Aldridge et al., 1981; Kalow and Tange, 1991; Carrillo and Benitez, 2000; Lampe et al., 2000). Variations in caffeine metabolism between individuals are mostly attributed to differences in cytochrome P450 1A2 (CYP1A2) enzyme activity (Arnaud, 1994). Assessments of CYP1A2 phenotypes and genotypes have been used to characterize study populations according to high or low metabolic activity. A comprehensive review of the metabolic considerations for caffeine exposure assessment during pregnancy was published by Grosso and Bracken (2005).

1.2. Pregnancy signal phenomenon

Pregnancy symptoms, including aversions to taste and smells, nausea, and vomiting are more common in healthy pregnancies that result in live births and occur less frequently among women whose pregnancies end in spontaneous abortions (Weigel and Weigel, 1989; Weigel et al., 2006). This relationship is attributed to a stronger pregnancy signal produced by higher concentrations of pregnancy hormones in viable pregnancies (Stein and Susser, 1991; Lawson et al., 2002). Caffeine consumption has been shown to decrease with increasing pregnancy signal symptoms during the early weeks of pregnancy (Lawson et al., 2004; Cnattingius et al., 2000). For example, Lawson et al. (2004) reported that mean onset

of nausea, vomiting and appetite loss occurred between 5 and 6 weeks from the last menstrual period, accompanied by a 59% decrease in caffeine intake from coffee between weeks 4 and 6. Thus, women experiencing viable pregnancies are more likely to reduce their caffeine intake in response to the pregnancy signal than women who go on to have a spontaneous abortion. As a result, reduced caffeine consumption may be a consequence of pregnancy viability rather than increased consumption causing any reproductive adversity. "Reverse causation" is the term used to describe such errors in causal inference.

Control for confounding by pregnancy signal symptoms is critical for caffeine studies of spontaneous abortion and fetal death, and may have importance for studies of other adverse pregnancy outcomes. This task, however, is complicated by the difficulty of measuring pregnancy signal symptoms. Studies that include only dichotomous indicators for nausea and vomiting fail to capture the severity, frequency and duration of the symptoms. Furthermore, aversions to specific foods or beverages, which may be equally or more relevant to decreased caffeine consumption, are rarely assessed. Studies have revealed that women who decrease their coffee consumption during early pregnancy commonly attributed these changes to an acknowledged aversion to the taste, smell or thought of coffee (Lawson et al., 2004); whereas, nausea reported as number of hours per week was not statistically associated with reduced coffee consumption (Lawson et al., 2002). Thus, separate assessment of coffee aversion has been recommended for studies of caffeine and pregnancy outcomes (Lawson et al., 2002; Lawson and LeMasters, 2006). Efforts to disentangle this complex relationship will require improved, prospective measurement of all relevant dimensions of the pregnancy signal.

1.3. Residual confounding by smoking

Most investigators recognize the importance of controlling for confounding by smoking when evaluating the reproductive effects of caffeine. Smoking and caffeine use are strongly associated, as heavier smokers tend to consume more caffeine than others (Schreiber et al., 1988; Zavela et al., 1990). Furthermore, smoking is considered a risk factor for many adverse reproductive outcomes such as infertility, spontaneous abortion, fetal growth restriction, stillbirth, and preterm birth (Cnattingius, 2004). Controlling for self-reported smoking status, however, may not provide adequate control for confounding when smoking is measured inaccurately. The stigma of smoking during pregnancy may lead to inaccurate reporting of smoking status or under-reporting of the amount smoked per day (Ford et al., 1997; Klebanoff et al., 1998b; Lindqvist et al., 2002). Morrison (1984) described the potential for residual confounding to occur when the more socially acceptable behavior of caffeine consumption is more accurately reported than the less acceptable behavior of smoking tobacco. Because the two behaviors are highly correlated, the more accurately reported caffeine consumption conveys information about tobacco consumption. Furthermore, many investigators control only for smoking status (yes/no) without consideration of amount smoked. Thus, incomplete control for the effects of smoking may explain observed associations commonly attributed to caffeine use. Some authors have attempted to improve upon these measurements by incorporating cotinine measurements as biomarkers of nicotine exposure. It is important, however, to acknowledge that these biochemical markers reflect recent tobacco exposures and may or may not accurately control for actual smoking patterns during the relevant window of exposure.

1.4. Precision of results

Measures of association (e.g., odds ratios and relative risks) and confidence intervals are frequently reported to two decimal places

when, in fact, study precision is rarely sufficient for this degree of detail to be meaningful. Thus all results discussed in this review are rounded to one decimal place.

2. Subfecundity

Studies assessing the impact of caffeine on fertility potential have evaluated a variety of outcomes including time to pregnancy, infertility, semen quality and selected endpoints of assisted reproductive technologies.

2.1. Time to pregnancy studies

Time to pregnancy studies identify determinants of fecundability, defined as the probability of conception within a non-contracepting menstrual cycle (Baird et al., 1986). Each of the three studies that evaluated the relationship between caffeine/coffee exposure and time to conception, none of which were specifically designed to evaluate caffeine exposures, used a retrospective time to pregnancy design within a population of pregnant women (Hassan and Killick, 2004; Cole et al., 2006). The most prominent limitation of this pregnancy-based approach is that participation is restricted to those who have achieved a pregnancy (Tingen et al., 2004). Thus, sub-fecund couples are underrepresented, and sterile couples are excluded entirely. If exposure is associated with longer wait times or sterility, more highly exposed sub-fecund women would be excluded and associations would be underestimated (Spira, 1998; Joffe et al., 2005). Given the retrospective nature of the data collection, these studies are also vulnerable to recall errors for reports of exposure as well as recollected time to conception. Although recall of time to pregnancy has been demonstrated to be relatively accurate at the group level over several years (Joffe et al., 1993), women with longer time to pregnancies would have to recall their past exposures over a longer time period compared to women with shorter time to pregnancies. Thus, exposure reports may be influenced by the outcome of delayed conception. The retrospective time to pregnancy study design is also criticized for lack of ability to account for timing of intercourse, which may interfere with the ability to accurately identify cycles at risk of conception. Prospective time to pregnancy studies that collect data on ovulation and timing of intercourse as couples attempt to conceive are considered the best available, although not without some limitations, such as pregnancy planning bias, low participation rates and concerns regarding the generalizability of results from volunteer populations (Tingen et al., 2004).

2.2. Fecundability vs. Infertility

Infertility is defined as failure to conceive within 12 months. Few exposures would be anticipated to lead to all or nothing effects on fertility. Thus, a continuous measure of fecundability is more informative than a dichotomous indicator of infertility because it allows for detection of diminished fecundity (Savitz et al., 2002).

2.3. Semen quality and timing of exposure assessment

Semen characteristics, including volume, sperm concentration, motility, morphology, and markers of DNA damage in sperm, have been studied as indicators of male fertility potential. Caffeine studies evaluating semen quality have been mostly cross-sectional; thus, the timing of caffeine assessment does not coincide with the relevant window for spermatogenesis, which occurs over 74 days (Nussey and Whitehead, 2001). However, coffee consumption appears to be relatively stable in some populations (Barone

and Roberts, 1996). Thus, caffeine consumption assessed at the time of semen collection might generally reflect exposure throughout spermatogenesis. Patterns of exposure preceding the window for spermatogenesis would be relevant if delayed or permanent effects on the spermatogenic cycle were suspected via the mechanism of stem cell disruption (Jensen et al., 2006).

2.4. Selection bias in studies of semen quality

Participation rates in studies of semen quality are typically low, leading to concerns of selection bias when study volunteers differ from those who refuse (Cohn et al., 2002). If men with fertility problems are more motivated to participate in semen studies and more likely to modify their caffeine intake or other behaviors in response to their concerns, the association between caffeine and semen quality would be distorted toward the null value due to overrepresentation of affected individuals with low exposure.

2.5. Contribution of male exposures

Two studies recognized the importance of including caffeine exposures of male partners when assessing fecundity endpoints beyond semen characteristics (Klonoff-Cohen et al., 2002; Cole et al., 2006). These studies evaluated male and female caffeine consumption separately or the combined monthly caffeine intake for the couple, but the joint effects of caffeine use in both partners has not been fully explored.

2.6. Review of individual studies of subfecundity

2.6.1. Assisted reproductive technology (ART) outcomes

Klonoff-Cohen, H., Bleha, J., Lam-Kruglick, P., 2002. A prospective study of the effects of female and male caffeine consumption on the reproductive endpoints of IVF and gamete intra-fallopian transfer. *Hum. Reprod.* 17, 1746–1754. [Discussed also in 3. Spontaneous Abortion and 5. Gestational Age and Preterm Birth]

This study evaluated the possible contribution of caffeine in both partners. This prospective cohort study was conducted among 221 couples undergoing *in vitro* fertilization (IVF) and gamete intra-fallopian transfer (GIFT) in Southern California between 1993 and 1998. Consumption of caffeine in caffeinated or decaffeinated coffee, tea, soft drinks, cocoa drinks and chocolate was assessed in relation to sperm characteristics, number of oocytes retrieved and fertilized, number of embryos transferred, achieving a pregnancy, live birth deliveries, miscarriage, multiple gestations, and gestational age at birth.

For women, caffeine consumption was not associated with number of oocytes retrieved, number of oocytes fertilized, number of embryos transferred or achieving a clinical pregnancy when undergoing IVF or GIFT (measures of association not presented).

According to the definition provided by the authors, “not having a live birth” resulted from either not becoming pregnant or experiencing a miscarriage”. Among females receiving IVF and GIFT, associations between not achieving a live birth and caffeine intake of >50 mg/day compared to 0–2 mg/day during the week before [OR:3.8 (0.9–15.8)] and the week of the procedure [OR:4.0 (0.5–31.1)] were imprecise and not statistically significant. Statistically significant associations were identified for caffeine intake reported for other time periods further removed from the procedure, including usual lifetime intake and intake during the week of the initial clinical visit, (OR:3.9 (1.3–11.6) for >50 mg/day over lifetime; OR:3.8 (1.4–10.7) for >50 mg/day during the week of the initial clinic visit).

Male caffeine consumption was not associated with sperm count, motility or morphology or the occurrence of pregnancy (data not shown by authors). On the other hand, caffeine intake

among males was associated with an increased probability of multiple gestations (presumably mostly twins) in couples who achieved a pregnancy using IVF and GIFT (for increase of 100 mg/day OR = 2.2 (1.1–4.4) for usual caffeine intake; OR = 3.0 (1.2–7.4) for week of initial clinical visit; OR = 2.2 (0.9–5.0) for week before sperm collection). Only 57 of 71 pregnant couples had complete information on male caffeine intake (usual and week of initial clinical visit) and confounders and only 45 had complete data for the assessment during the week before sperm collection.

Strengths of this study include being the first to focus on end-points occurring within couples receiving assisted reproductive technology (ART) treatment and the assessment of caffeine intake across time.

The authors acknowledge the major limitation of the study was the small sample size, which resulted in limited statistical power and imprecise measures of association. This study population reported an unusually low level of caffeine exposure, presumably due to fertility concerns. Thus, the study's capacity to assess the effects of moderate caffeine intake was limited.

2.6.2. Time to pregnancy studies

Hassan, M.A., Killick, S.R., 2004. Negative lifestyle is associated with a significant reduction in fecundity. *Fertil. Steril.* 81, 384–392.

This retrospective study enrolled 2112 pregnant women from prenatal clinics in the United Kingdom. Several lifestyle factors, including coffee and tea consumption, were evaluated in relation to subfecundity measured as time to pregnancy >12 months (typically referred to as “infertility”) and mean time to pregnancy following cessation of birth control. The study strengths include a high response rate (99%) and large sample size.

No details regarding the measurement of “coffee and/or tea intake” were disclosed other than being reported retrospectively in cups per day (combined). Because a cup of coffee contains substantially more caffeine than a cup of tea and information on soda and energy drinks was lacking, the combined count of servings would misclassify total caffeine exposure. Women who consumed the most coffee and/or tea (≥ 7 cups/day) had a marginally longer average time to pregnancy (10.4 months, 95% CI 8.1–12.8) compared to “mild” (≤ 6 cups/day) users (8.4 months; 95% CI 7.7–9.1) ($p = 0.1$) and a greater odds of subfecundity [OR:1.7 (1.1–2.7)].

When mean time to pregnancy was compared graphically across five categories of coffee/tea consumption (0, ≤ 5 , 6–10, 11–15, and 16–20 cups/day), those with the most extreme intake (16–20 cups/day) appeared to have a longer time to pregnancy compared to non-consumers (19.7 vs. 9.6 months). The point estimate for the heaviest intake, however, was imprecise (number of women consuming 16–20 cups/day not reported).

The study limitations, which include potential recall bias and exposure misclassification, hinder interpretation.

Cole, D.C., Wainman, B., Sanin, L.H., Weber, J.P., Muggah, H., Ibrahim, S., 2006. Environmental contaminant levels and fecundability among non-smoking couples. *Reprod. Toxicol.* 22, 13–19.

This study recruited 41 couples receiving prenatal care during the third trimester of pregnancy with the goal of evaluating effects of persistent organic pollutants on fecundability. Participants were restricted to couples who were not smoking at the time of conception and in which the female partner was \leq age 35. According to the authors' description, similar numbers of couples were selected who conceived early (first month) and late (>5 months) as well as “a few” who conceived in between. Thus, subject selection was based on the time to conception outcome. As a result, the cohort design approach to data analysis is questionable and comparisons of the probability of pregnancy in any given month in the exposed and unexposed groups are likely to be invalid. For valid comparisons, the subjects in the cohort would need to be selected independently of their outcome status. The lack of details defining caffeine

exposure also raised concerns about the general quality of the retrospective exposure assessment and relevance of the exposure time frame. Given the methodological concerns, this null study offers no useful information regarding caffeine and fecundability.

Killick, S., Trussell, J., Cleland, K., Moreau, C., 2009. Factors associated with subfertility among women attending an antenatal clinic in Hull. *Hum. Fertil.* 12, 191–197.

The retrospective study of subfertility conducted among 2317 pregnant women was not designed to address risk associated with caffeine intake, but rather aimed to identify the most parsimonious models predicting time to pregnancy greater than 12 months and time to pregnancy greater than 24 months. Caffeine intake, defined as coffee and tea intake of 1–6 cups/week and ≥ 7 cups/week was included among the lifestyle variables assessed in this study. However, caffeine was not retained in the final models which were limited to statistically significant predictors of subfertility. No measures of association for caffeine were reported.

2.6.3. Ovulatory infertility

Chavarro, J.E., Rich-Edwards, J.W., Rosner, B.A., Willett, W.C., 2009. Caffeinated and alcoholic beverage intake in relation to ovulatory disorder infertility. *Epidemiology* 20, 374–381.

Women without a history of infertility in the Nurses Health Study II cohort ($n = 18,555$) who became pregnant or tried to become pregnant during the 1991–1999 follow-up period were selected for this analysis. Caffeine intake was collected from food frequency questionnaires administered in 1991 and 1995 to assess food and beverage intake during the previous year. Ovulatory disorder was reported as a reason for infertility in questionnaires administered every 2 years. Neither total caffeine intake from all sources nor frequency of coffee, decaffeinated coffee or tea intake were associated with infertility due to ovulatory disorder. Two or more soft drinks per day, however, were positively associated with risk of ovulatory infertility [OR:1.6 (1.2–2.2), independent of caffeine intake and total energy intake. Non-caffeinated, sugared and diet soft drinks showed similar associations with ovulatory disorder infertility, suggesting the association may be due to chance, components of soft drinks other than caffeine or sugar, or dietary patterns in which soft drinks are preferentially consumed to the exclusion of nutritious alternatives.

The results offer no evidence to indicate a role for caffeine in the development of ovulatory infertility. The findings, however, are limited by the potential for exposure misclassification. Although the exposure data were collected prospectively, measurement error was possible given the exposure assessment was applied to pregnancies or pregnancy attempts occurring up to 4 years after the food frequency questionnaire was administered.

2.6.4. Sperm quality

Horak, S., Polanska, J., Widlak, P., 2003. Bulky DNA adducts in human sperm: relationship with fertility, semen quality, smoking, and environmental factors. *Mutat. Res.* 537, 53–65.

This cross-sectional study evaluated correlations between coffee consumption and DNA damage in the semen of 179 men, approximately half of whom had impaired fertility. Men who drank >250 ml of coffee per day did not have higher levels of DNA adducts than “non-drinking or sporadically drinking subjects”. Furthermore, no correlations were observed between amount of coffee consumed and concentrations of DNA adducts or semen parameters.

This study has a number of limitations, including lack of detail about exposure assessment and lack of attention to possible confounding by age or other selection factors. Furthermore, reports of current coffee consumption may not reflect relevant exposures during the period of spermatogenesis.

The crude results presented in this study provide no suggestive evidence of a link between coffee intake and DNA damage in sperm.

Sobreiro, B.P., Lucon, A.M., Pasqualotto, F.F., Hallak, J., Athayde, K.S., Arap, S., 2005. Semen analysis in fertile patients undergoing vasectomy: reference values and variations according to age, length of sexual abstinence, seasonality, smoking habits and caffeine intake. *Sao Paulo Med. J.* 123, 161–166.

The purpose of this study was to establish reference standards for semen characteristics in Brazil and to examine effects of exposures such as caffeine consumption on semen measurements. Although described as a prospective study, these cross-sectional data were collected from a population of 500 healthy, fertile men age 24–63 scheduled for vasectomies. Semen samples were collected in the hospital before the vasectomy. Coffee use was categorized as 0, 1–3, 4–6 and >6 cups per day, but no details regarding the data collection procedures were provided. Mean values for semen volume, sperm concentration and sperm morphology did not vary meaningfully across the four categories of coffee use. Mean sperm motility, however, was observed to increase slightly with increasing coffee consumption, from 57.1% (sd 16.2) for non-users to 62.4% (sd 16.0) for men consuming >6 cups/day ($p = 0.037$). Thus, no adverse effects of coffee drinking on sperm quality were observed.

Despite the large study size, the lack of study design details and the absence of control for confounding limit this study's contribution to resolving the question of what effects coffee and/or caffeine may have on reproduction. By restricting their sample to men seeking a vasectomy (i.e., men who have reason to consider themselves fertile), the authors have eliminated men who might have limited fertility. Thereby, the authors have reduced their ability to identify a coffee effect.

Schmid, T.E., Eskenazi, B., Baumgartner, A., Marchetti, F., Young, S., Weldon, R., Anderson, D., Wyrobek, A.J., 2007. The effects of male age on sperm DNA damage in healthy non-smokers. *Hum. Reprod.* 22, 180–187.

A total of 80 non-smoking men participated in this cross-sectional study evaluating factors associated with DNA damage in sperm. DNA damage was measured using single-cell electrophoresis (sperm Comet) and reported as the average percentage of DNA staining outside the area of the sperm nucleus, referred to as % tail DNA.

Alkaline % tail DNA (indicating single-strand DNA breaks) was not related to caffeine consumption. Under neutral conditions representing double-stranded DNA breaks, men with the highest caffeine consumption (>308 mg/day) had 19% higher mean % tail DNA compared to non-users (39.1 (sd 10.5) vs. 32.8 (sd 6.7), $p = 0.005$). Neutral % tail DNA did not correlate with semen quality as measured by sperm concentration, total sperm count, motility, and progressive motility.

The results suggest that DNA damage in sperm may be slightly increased in healthy men consuming large quantities of caffeine, but bias due to confounding and exposure misclassification cannot be ruled out. Although the paper's focus was on the effects of male age, age and factors other than total kilocalorie intake and the history of urinary tract infections were not controlled in sub-analyses of caffeine use.

Ramlau-Hansen, C.H., Thulstrup, A.M., Bonde, J.P., Olsen, J., Bech, B.H., 2008. Semen quality according to prenatal coffee and present caffeine exposure: two decades of follow-up of a pregnancy cohort. *Hum. Reprod.* 23, 2799–2805.

Young-adult sons ($n = 343$) of women who were members of a Danish pregnancy cohort were assessed for semen quality and serum hormone concentrations in relation to intrauterine coffee exposure and current caffeine consumption patterns. Average maternal coffee exposure during pregnancy was obtained from

questionnaires completed during the 36th week of gestation, which collected categorical responses as 0–3, 4–7 and ≥ 8 cups/day. At the time of semen and serum collection, the male participants reported their current daily coffee and cola intake. Categorical responses for coffee and cola were each assigned an estimated caffeine concentration (e.g., 1–4 cups coffee/day = 250 mg; half to one liter of cola per day = 75 mg), which were combined to create categories of low (0–25 mg), medium (50–125 mg) and high (175–1075 mg) caffeine exposure. After adjustment for confounders, including mother's and son's smoking, a non-significant trend toward decreasing mean testosterone (19.2, 17.7 and 17.8 nmol/l for 0–3, 4–7, and ≥ 8 cups/day, $p = 0.06$) and inhibin B levels (181, 173, and 162 ng/ml; $p = 0.09$) was observed with increasing prenatal coffee exposure. Current caffeine consumption was associated with increased adjusted mean testosterone levels (17.9, 19.0, and 20.4 nmol/l for low, medium, and high caffeine; $p = 0.007$). No effects on sperm concentration, total sperm count, semen volume, morphology or motility were observed in adjusted analyses of either prenatal coffee or current caffeine exposures.

Among mothers with high coffee consumption during pregnancy, a higher proportion of sons drank coffee on a daily basis (25%) compared to sons of mothers with lower intake (16%). Results of sub-analyses for semen volume, sperm concentration, and testosterone levels are reported to be unchanged when also adjusted for the effects of maternal/son's caffeine consumptions.

The authors acknowledge the third trimester assessment of average exposure during pregnancy may not accurately reflect caffeine intake during the relevant window of testicular development. Furthermore, exposure misclassification was likely given the assignment of a mid-range caffeine concentration to all individuals within a broad category of current coffee or cola intake. Like most studies of semen quality, the participation rate was low (48.5%) introducing the possibility of selection bias. Although this report improves upon previous studies of semen quality, which had limited adjustment for confounders, the dichotomous measurement of current and maternal smoking may have failed to fully control for amount smoked. Overall, the study provides no indication that prenatal coffee consumption or current caffeine intake adversely affects semen quality in young adult males.

3. Spontaneous abortion

A good review of studies that have evaluated the relationship between caffeine consumption and spontaneous abortion has been published by Signorello and McLaughlin (2004).

3.1. Missing early losses

Some women conceive and then miscarry without any recognition of these events other than missing one period (Weinberg et al., 1992; Wilcox et al., 1999). By all definitions, they had a spontaneous abortion, and yet they did not seek medical care and thus are not included in studies of spontaneous abortion. Studies are biased to the extent that they miss these early spontaneous abortions. The best studies seek to improve outcome ascertainment by recruiting women when they first become pregnant or even earlier when trying to conceive, utilizing biomedical advances in early pregnancy detection. An alternative approach is to exclude all early losses before some specified gestational stage, e.g., 8 weeks, in an effort to create a homogeneous population for study. Because the causes of early losses appear to be distinct from those of later miscarriages, the results of such studies may not be readily extrapolated to populations at risk for early pregnancy loss.

Studies of caffeine and spontaneous abortion vary by the range of gestational ages included for study (unspecified (Wen et al., 2001; Giannelli et al., 2003); 6–12 weeks (Signorello et al., 2001), <13 weeks (Maconochie et al., 2007); 6–16 weeks (Rasch, 2003); ≤20 weeks (Khoury et al., 2004; Weng et al., 2008; Savitz et al., 2008b); <28 weeks (Tolstrup et al., 2003). Consequently, comparability is compromised.

3.2. Role of abnormal karyotypes

By and large, the earlier the miscarriage, the higher the proportion with chromosomal abnormalities (Yusuf and Naeem, 2004). Until evidence is presented to the contrary, it seems highly desirable to evaluate the antecedents of karyotypically normal miscarriages separately from those of karyotypically abnormal miscarriages. Only one study published since 2000 considered fetal karyotype (Signorello et al., 2001).

3.3. Pregnancy signal

The potential for confounding introduced by the pregnancy signal, which was discussed earlier, is relevant to studies of caffeine exposure and risk of spontaneous abortion. In this regard, smokers are less likely to experience nausea and vomiting during pregnancy than non-smokers (Weigel and Weigel, 1989; Louik et al., 2006). Thus, the effects of confounding due to nausea may differ by smoking status, with greater impact on non-smokers whose reports of increased caffeine intake may be more likely to reflect reduced nausea that accompanies nonviable pregnancies.

3.4. Proper control selection in case-control studies

Most case-control studies of caffeine-miscarriage relationships selected controls from among pregnant women seeking prenatal care. This inclusion criterion is often not applied to the case group, which includes women who had not sought routine prenatal care before presenting with vaginal bleeding (Giannelli et al., 2003; Rasch, 2003; Signorello et al., 2001; Karypidis et al., 2006; George et al., 2006). If some controls receive advice from their prenatal care provider to reduce caffeine intake, then selection bias will result. Some of this bias can be reduced if controls are interviewed about their caffeine intake at the first prenatal care visit before clinical advice would have an opportunity to influence intake patterns (Giannelli et al., 2003; Rasch, 2003).

3.5. The importance of pregnancy duration (gestational age)

Unless cases and controls are of comparable gestational age, the exposure opportunity of one group will be larger than that of the other. For example, the longer the duration of the pregnancy before caffeine/coffee assessment, the greater the likelihood of a pregnancy signal and the opportunity to decrease consumption. Some studies have selected controls from women who delivered a full term live birth (Sata et al., 2005) or from women who have not yet delivered (Giannelli et al., 2003), without consideration for duration of pregnancy. The timing of control selection also has implications for the quality of the retrospectively reported exposure data when the recall period is not similar for cases and controls. These errors can severely limit the value of a study's findings.

3.6. Late recognition of fetal demise

Some fetuses die *in utero* weeks before being expelled (Simpson, 1990). Exposures during the time between fetal demise and recognition of the loss are irrelevant. To the extent that this time interval is unrecognized, bias can result. Studies of caffeine and pregnancy

loss that attempt to exclude exposures occurring during the time period immediately preceding recognition of the loss (Cnattingius et al., 2000; Bech et al., 2005) may improve upon the approximation of the etiologically relevant time period.

3.7. Review of individual studies of spontaneous abortion

3.7.1. Cohort studies

Wen, W., Xiao, U.S., Jacobs, D.R., Brown, J.E., 2001. The associations of maternal caffeine consumption and nausea with spontaneous abortion. *Epidemiology* 12, 38–42.

In this prospective study, women who consumed ≥100 mg/day of caffeine during the first trimester were at elevated risk of spontaneous abortion [100–299 mg/day RR:2.0 (1.0–4.1); ≥300 mg/day RR:2.5 (1.0–6.4)] compared to those consuming <20 mg/day ($n = 584$). These increased risks were not apparent when the focus was consumption prior to the onset of nausea ($n = 206$) or in women who never experienced nausea ($n = 73$), but were limited to caffeine intake after the onset of nausea ($n = 498$) [RR:1.8 (0.8–3.9), RR:2.4 (0.9–6.2) and RR:5.4 (2.0–14.6) for intakes of 20–99, 100–299 and ≥300 mg/day, respectively]. These findings could be explained by the pregnancy signal.

Frequent assessments of caffeine intake throughout pregnancy and evaluation of the interaction between caffeine and nausea are strengths of this study. Limitations include the small number of events in the stratified analyses which resulted in imprecise measures of effect. In addition, control for potential confounders may not have been sufficient, since they were ascertained only at enrollment and were analyzed in broad categories (e.g., smoking at enrollment, yes/no), creating the opportunity for residual confounding. Furthermore, assumptions were made about the temporal association of caffeine consumption and spontaneous abortion, although the authors acknowledge that the exact timing of fetal demise was unknown. Duration of nausea was explored by restricting an analysis to women reporting nausea in at least two monthly questionnaires, but results were imprecise and non-significant.

Klonoff-Cohen, H., Bleha, J., Lam-Kruglick, P., 2002. A prospective study of the effects of female and male caffeine consumption on the reproductive endpoints of IVF and gamete intra-fallopian transfer. *Hum. Reprod.* 17, 1746–1754. [Discussed also in 2. Subfecundity and 5. Gestational Age and Preterm Birth]

Miscarriage occurred in 21 of 62 confirmed pregnancies following *in vitro* fertilization (IVF) and gamete intra-fallopian transfer (GIFT). Unstable but elevated point estimates were reported for caffeine intake [OR:6.2 (0.9–40.8) for >50 mg/day] during the week of the initial clinic visit. No associations with first trimester caffeine use were observed. The authors stated, "Because the sample size was small, the CIs were very wide and the magnitude of association for caffeine and miscarriage may be unreliable". The study controlled for several potential confounders, but certain known confounders such as smoking and pregnancy signal symptoms were not controlled in the analysis. Thus, the results of this study do not provide convincing evidence of a link between caffeine and spontaneous abortion among women receiving assisted reproductive technology (ART) treatment.

Khouri, J.C., Miodovnik, M., Buncher, C.R., Kalkwarf, H., McElvy, S., Khouri, P.R., Sibai, B., 2004. Consequences of smoking and caffeine consumption during pregnancy in women with type 1 diabetes. *J. Matern. Fetal Neonatal Med.* 15, 44–50. [Discussed also in 5. Gestational Age and Preterm Birth and 6. Congenital Malformations]

This study of adverse pregnancy outcomes was conducted among 191 pregnancies (23 spontaneous abortions ≤20 weeks) identified within a cohort of women with type 1 diabetes who were either pregnant or planning a pregnancy. Compared to no caffeine intake, the observed odds ratios for spontaneous abortions

≤20 weeks were 3.8 (0.8–16.9) for first trimester consumption of 1–2 cups of caffeinated beverages per day and 5.5 (1.2–22.0) for ≥3 cups per day.

The major strength of this study is the prospective collection of first trimester caffeine consumption, which avoids reporting bias that can result from post-outcome assessments. The major limitations are lack of control for pregnancy symptoms, imprecise point estimates due to small sample size and exposure misclassification resulting from the less than optimal definition of caffeine consumption (monthly average number of 8 oz equivalent cups with equal weighting for coffee, tea and soft drinks).

Bech, B.H., Nohr, E.A., Vaeth, M., Henriksen, T.B., Olsen, J., 2005. Coffee and fetal death: a cohort study with prospective data. *Am. J. Epidemiol.* 162, 983–990. [Discussed also in 4. Fetal Death]

This prospective cohort study of spontaneous abortion (<28 weeks) was conducted among 88,482 pregnancies within the Danish National Birth Cohort. Women were enrolled following their first prenatal visit and interviewed at 16 weeks of gestation on average (inter-quartile range 13–19 weeks). The authors observed that heavy coffee drinkers (≥8 cups per day) compared to non-coffee drinkers had a higher risk of spontaneous abortion before 20 weeks of gestation [HR:1.5 (1.0–2.2)]. Despite the advantage of such large numbers, the limitations include missing early spontaneous abortions, a lack of information about coffee consumption during the early weeks of pregnancy, and the absence of attention to the implications of early pregnancy symptoms.

Weng, X., Odouli, R., Li, D.K., 2008. Maternal caffeine consumption during pregnancy and the risk of miscarriage: a prospective cohort study. *Am. J. Obstet. Gynecol.* 198, 279–286.

This cohort study enrolled 1063 health plan members after confirmation of pregnancy (median gestational age at interview was 10 weeks). Women reported average daily intake of caffeine-containing beverages since their last menstrual period and whether their consumption patterns had changed since becoming pregnant. Because 59% of miscarriages occurred prior to contact for the study, caffeine consumption was reported retrospectively for a substantial number of participants.

Compared to those with no caffeine exposure, the adjusted hazard ratio (HR) for miscarriage associated with caffeine intake ≥200 mg/day was 2.2 (1.3–3.7). No significant interactions between consumption of caffeine ≥200 mg/day vs. <200 mg/day and presence or absence of nausea or vomiting since LMP or smoking status were observed. Among women who reduced caffeine intake and were therefore most likely to have experienced strong pregnancy signals, consumption of ≥200 mg/day was no longer associated with miscarriage [HR:1.5 (0.9–2.5)]. Thus, the association with caffeine appears to be explained by the pregnancy signal.

Incomplete control for confounding by amount smoked or severity or duration of nausea and vomiting was likely since these factors were operationalized as dichotomous indicators (yes/no). Caffeine intake measured through the end of pregnancy may also have inadvertently captured consumption patterns that increased following unrecognized fetal demise. Despite concerns about potential recall bias, results were similar when analyses were restricted to women interviewed before the miscarriage occurred (aHR = 2.8; 95% CI 1.1–7.0) (Li et al., 2008). However, confounding by the pregnancy signal is reasonably supported.

Savitz, D.A., Chan, R.L., Herring, A.H., Howards, P.P., Hartmann, K.E., 2008. Caffeine and miscarriage risk. *Epidemiology* 19, 55–62.

This study evaluated 258 spontaneous abortions (≤20 weeks of gestation) occurring among a cohort of 2407 women with clinically recognized pregnancies. Caffeine assessment considered daily consumption in a 'typical week' during three time periods – pre-pregnancy, four weeks after last menstrual period (LMP), and at the time of interview, referred to as 'current consumption' (i.e., before 16 completed weeks or when still pregnant for those with losses).

Overall, the study reported no association between coffee consumption or total caffeine intake before or during pregnancy and risk of spontaneous abortion up to 20 weeks of gestation.

Among women interviewed after the miscarriage, current caffeine consumption ≥144.3 mg/day was associated with an increased risk of spontaneous abortion [OR:1.9 (1.1–3.5)] compared to non-consumers. In contrast, among women interviewed before their loss, no association with current caffeine intake was observed [OR:1.1 (0.6–1.8)]. These disparate results could reflect recall bias, increased consumption patterns following fetal demise, true differences in consumption due to absence of a strong pregnancy signal, a relationship between caffeine consumption and later miscarriages only, or chance.

This study has several strengths including a large sample, a prospective design, use of an analytic approach that estimates the probability of having a loss in a given week, conditional on still being pregnant (i.e., at risk of spontaneous abortion) at the beginning of that week, and use of median and 75th percentile cut-points for defining exposure categories, which increase precision when biologically meaningful cut-points are unknown.

The results of this study do not support an association between spontaneous abortion and caffeine intake before or during pregnancy at the levels assessed. The most likely explanation of the associations produced by the sub-analysis of exposure data collected after the event of a loss is recall bias.

3.7.2. Nested case-control studies

Tolstrup, J.S., Kjaer, S.K., Munk, C., Madsen, L.B., Ottesen, B., Berg-holt, T., Grønbaek, M., 2003. Does caffeine and alcohol intake before pregnancy predict the occurrence of spontaneous abortion? *Hum. Reprod.* 18, 2704–2710.

This study used an atypical definition of spontaneous abortion as pregnancy loss within the first 28 weeks of pregnancy, thus complicating comparisons with other studies which apply the clinical definition, loss before 20 weeks. This nested case-control study was conducted within a cohort of 11,088 non-pregnant women randomly sampled from the general population of 20–29 year olds living in Copenhagen, Denmark. A baseline interview was conducted between May 1991 and January 1993. A follow-up interview was administered 2 years later to ascertain all pregnancies ($n = 1381$) and related outcomes ($n = 303$ spontaneous abortions) occurring during the follow-up interval. The Danish Hospital Discharge Registry was also used to confirm interview reports and to ascertain pregnancy outcomes in women who did not complete the follow-up questionnaire.

The adjusted odds ratio of miscarriage before 28 weeks gestation was significantly increased only for women consuming >900 mg caffeine per day from coffee or tea [OR:1.7 (1.0–3.0)], after adjustment for maternal age, marital status, cigarette smoking and alcohol intake. At consumption levels similar to those examined by others (e.g., 300 mg/d), average daily pre-pregnancy intake of caffeine was not associated with miscarriage.

This study had a number of methodological features that limit confidence in the results. Because of the time lag between ascertainment of caffeine exposure from the first interview and conception (mean 9.3 ± 6.5 months), caffeine data obtained at baseline may misclassify patterns of consumption more proximal to conception. Pregnancy symptoms were also not assessed and thus were not controlled in the analysis. Furthermore, while it is possible that the 20% of self-reported miscarriages that were not registry-confirmed represented very early fetal losses among women who did not seek medical care, it is also possible that they were not miscarriages. Test results to confirm pregnancies were not available.

3.7.3. Case-control studies

Giannelli, M., Doyle, P., Roman, E., Pelerin, M., Hermon, C., 2003. The effect of caffeine consumption and nausea on the risk of miscarriage. *Paediatr. Perinat. Epidemiol.* 17, 316–323.

This case-control study was conducted among nulliparous women seeking care for spontaneous abortion ($n = 160$ cases) or pregnancy ($n = 314$ controls). Cases were interviewed about 3 weeks after their pregnancy loss on average, whereas controls were interviewed at the first prenatal care visit which typically occurred at a more advanced gestational age.

When compared to women reporting <151 mg/day, women consuming more than 300 mg/day during pregnancy were at increased odds for spontaneous abortion [OR:1.9 (1.0–3.6) for 301–500 mg/day and OR:2.2 (1.1–4.4) for ≥ 500 mg/day]. No associations were observed for pre-pregnancy caffeine use.

The burden of recalling caffeine exposure was not equivalent for cases and controls. The authors report collecting data on the timing and explanation for variations in typical caffeine consumption patterns before and during pregnancy (but not actual change in amount consumed), although it does not appear that such changes were incorporated into the exposure estimates.

A strength of this study is its attempt to control for severity of nausea. As anticipated, significantly fewer cases than controls reported nausea in this study population (45% of case and 81% of controls interviewed in first trimester) and spontaneous abortion was strongly associated with less severe nausea. Despite these strong associations, controlling for severity of nausea (none, mild, moderate, and severe) had little impact on the magnitude of the reported point estimates and no interaction between nausea and caffeine intake was observed.

The limitations of this study include potential recall bias, incomplete control for smoking (excluded as a potential confounder when measured as yes/no) and inclusion of caffeine intake following fetal demise. The 2-fold increased odds observed in this study are similar in magnitude to the association produced in Savitz et al. (2008b) when exposure data were retrospectively reported by cases as compared to prospective reporting. The study also did not consider dietary aversions as an indicator of the pregnancy signal phenomenon, as sensitivity to odors and/or food/beverage aversions may be more closely associated with decreased caffeine consumption than nausea severity.

Rasch, V., 2003. Cigarette, alcohol, and caffeine consumption: risk factors for spontaneous abortion. *Acta Obstet. Gynecol. Scand.* 82, 182–188.

This case-control study included 303 women with clinically recognized spontaneous abortions between 6 and 16 weeks of gestation and 1168 control women pregnant with a live fetus in the same range of gestational age. Women reporting heavy caffeine use since becoming pregnant (≥ 375 mg/day) had a 2.2 (1.5–3.2) times greater odds of spontaneous abortion compared to women consuming 0–199 mg/day.

The major strengths of this study include the use of control pregnancies of similar gestational ages, which appropriately represents the source population for the cases, the relatively large sample size, and the high proportion of heavy caffeine users in the study population (46.6% of cases and 28.9% of controls). Among the study limitations considered by the author are no data to control for pregnancy symptoms, the possibility of including exposure following fetal demise and the possibility of overestimation due to recall bias given cases reported exposures while being hospitalized for evacuation of the pregnancy. The study author reasons recall bias is an unlikely explanation because concern about caffeine use during pregnancy was not widespread in Denmark during the mid-1990's study period.

A substantial proportion of the study population was excluded due to missing data on gestational age (23.8% of case respondents

and 21.4% of controls). In addition, it is not possible to disentangle the evidence to determine whether the observed associations reflect something other than a diminished or absent pregnancy signal in pregnancies destined to fail.

Maconochie, N., Doyle, P., Prior, S., Simmons, R., 2007. Risk factors for first trimester miscarriage – results from a UK-population-based case-control study. *Br. J. Obstet. Gynaecol.* 114, 170–186.

Data for this case-control study were derived from the National Women's Health Study of adult women living in the United Kingdom in 2001. Women with a pregnancy history identified in a two-stage population-based survey were selected as cases if their last pregnancy ended in a first trimester miscarriage (<13 completed weeks) or if they had a miscarriage in any pregnancy conceived since 1995 ($n = 603$). This latter group represented about 40% of all cases. Controls were women whose last pregnancy progressed to 13 weeks gestation or beyond ($n = 6116$). Eighty-three percent of cases were conceived since 1995, compared to 49% of controls, thus there was potential for differential recall between cases and controls.

Average caffeine intake from beverages >300 mg/day during the first 12 weeks of pregnancy was independently associated with an increased odds of miscarriage compared to no consumption [OR:1.5 (1.1–2.2) for 301–500 mg/d; OR:1.7 (1.2–2.4) for >500 mg/d]. After further adjustment for nausea severity, however, caffeine intake was no longer related to the odds of miscarriage [OR:1.0 (0.7–1.5) for 301–500 mg/d and OR:1.1 (0.8–1.7) for >500 mg/d].

The limitations of this study include the potential misclassification of exposure and outcome due to lengthy and differential time frames for exposure recall, self-reported outcomes, and confining caffeine intake to beverages. These data do not support an association between caffeine consumption from beverages and odds of first trimester miscarriage after adjusting for the confounding effect of nausea severity.

3.7.4. Caffeine metabolism

Signorello, L.B., Nordmark, A., Granath, F., Blot, W.J., McLaughlin, J.K., Annerén, G., Lundgren, S., Ekblom, A., Rane, A., Cnattingius, S., 2001. Caffeine metabolism and the risk of spontaneous abortion of normal karyotype fetuses. *Obstet. Gynecol.* 98, 1059–1066.

This study is one of three papers (Signorello et al., 2001; Karypidis et al., 2006; George et al., 2006) published using data collected as part of a case-control study of caffeine and miscarriage in Uppsala, Sweden (Cnattingius et al., 2000). The original publication by Cnattingius et al., was previously reviewed by Leviton and Cowan (2002). In the original study, 562 confirmed cases of spontaneous abortions between 6 and 12 weeks of gestation were identified from a university hospital, the sole source of care for women experiencing pregnancy loss. Controls ($n = 953$) were identified from women seeking prenatal care in Uppsala and were frequency matched to cases by completed weeks of gestation and area of residence. This study was well-designed in that controls represented the source population of the cases, i.e., women in their first trimester of pregnancy. A major strength of this study was karyotyping of cases when fetal tissue could be obtained, although this was available on fewer than half of the cases. Another positive quality was the measurement of caffeine consumption, which although ascertained retrospectively, incorporated multiple sources of exposure, method of preparation, serving size, and captured reports of caffeine use week by week. The potential drawback of the exposure assessment was the likely inclusion of caffeine intake following unrecognized fetal demise.

The 101 chromosomally normal spontaneous abortions were compared to the 953 controls. With the goal of evaluating variability in caffeine metabolism as a risk factor for spontaneous abortions, the authors estimated the activity levels of two enzymes,

cytochrome P4501A2 (CYP1A2) and *N*-acetyltransferase 2 (NAT2), given both are involved in the metabolism or detoxification of caffeine (Campbell et al., 1987a; Nebert et al., 1996; Arnaud, 1994). Using genomic DNA from whole blood, polymorphisms of the NAT2 gene were determined by polymerase chain reaction (PCR) amplification to identify slow (homozygous mutated alleles) and fast acetylator genotypes (heterozygous or homozygous wild type alleles). CYP1A2 phenotypes were determined using the index described by Campbell et al. (1987b), which calculates a ratio of four urinary metabolites of caffeine as an indicator of caffeine clearance. Low and high CYP1A2 activity were defined as log-transformed CYP1A2 index values below and above the median value identified among controls (median index value = 0.73).

When stratified by CYP1A2 activity, caffeine intake above 100 mg/day was associated with increased odds of spontaneous abortion among women with high CYP1A2 activity [OR:2.4 (1.0–5.8) for 100–299 mg/day and OR:3.2 (1.2–8.2) for ≥ 300 mg/day compared to 0–99 mg/day], but not among those with low activity. Independent of caffeine intake, smoking status, nausea score, maternal age and week of gestation, high CYP1A2 activity was associated with an elevated odds of spontaneous abortion [OR:2.9 (1.7–5.0)]. NAT2 genotype was not associated with spontaneous abortion. The authors suggest that prolonged exposure to caffeine metabolites might explain their unexpected findings.

A limitation of using spot urine samples in population-based studies to phenotype CYP1A2 activity (as opposed to the less feasible method of administering a standardized test dose of caffeine) is that recent caffeine consumption is required in order to detect the caffeine metabolites in urine (Nordmark et al., 1999). Thus, women consuming little or no caffeine (approximately 1/3 of cases and controls) were excluded from the analyses. Furthermore, the exclusions resulted in small sample sizes and imprecise estimates, particularly within the stratified analyses.

Because CYP1A2 is involved in the metabolism of numerous drugs in addition to caffeine, the authors recognize that the results may indicate potential links between spontaneous abortion and other unmeasured factors. Cigarette smoke is one compound that has been shown to induce high CYP1A2 activity (Campbell et al., 1987b). Classifying women as either smokers or non-smokers, instead of actual quantity smoked (or concentration of plasma cotinine) might have contributed to residual confounding by smoking. This may also explain why high CYP1A2 activity was associated with spontaneous abortion contrary to expectations that risk would increase with slower caffeine clearance. Thus, caution is advised when drawing inferences from these findings.

Karypidis, A.H., Söderström, T., Nordmark, A., Granath, F., Cnattingius, S., Rane, A., 2006. Association of cytochrome P450 1B1 polymorphism with first-trimester miscarriage. *Fertil. Steril.* 86, 1498–1503.

The Swedish case-control study was utilized again by Karypidis et al. (2006) to evaluate the odds of spontaneous abortion associated with CYP1B1 polymorphisms and a possible interaction with caffeine consumption. CYP1B1 is an enzyme that influences the metabolism of steroid hormones, such as testosterone, progesterone, and estradiol, and of caffeine, among other agents. CYP1B1 activity, like CYP1A2, is also induced by smoking (Piipari et al., 2000). Women who were homozygous for the Val allele had an adjusted odds of miscarriage 1.5 times higher than women homozygous for the Leu allele (95% CI = 1.0–2.1), and the association was consistent among non-smokers [OR:1.6 (1.1–2.4)]. The risk increased with increasing caffeine consumption, but almost only among Val/Val. This indicates a significant interaction between homozygosity for Val and caffeine intake.

Since the Leu variant contributes to inactivation of testosterone (Shimada et al., 1999) and higher levels of testosterone have been associated with miscarriage (Okon et al., 1998), Leu/Leu homozygotes may be protected from spontaneous abortion. The authors

acknowledge that CYP1B1 is involved in the metabolism of many compounds including steroid hormones and procarcinogens as well as caffeine; thus, the observed associations may not be directly attributed to caffeine metabolism.

3.7.5. Recurrent pregnancy loss

George, L., Granath, F., Johansson, A.L.V., Olander, B., Cnattingius, S., 2006. Risks of repeated miscarriage. *Paediat. Perinat. Epidemiol.* 20, 119–126.

This case-control study of repeated miscarriage was conducted within the Swedish study of spontaneous abortions reported by Cnattingius et al. (2000). Cases included 108 women from the original case group who had two or more consecutive miscarriages. Controls were women with at least two pregnancies ($n = 583$), of which the last was a normal intrauterine pregnancy, confirmed by vaginal ultrasound.

After adjustment for potential confounders, the odds of repeated miscarriage was not significantly increased in heavy caffeine users [OR:1.8 (0.8–3.9) for ≥ 300 mg/day]. Caffeine consumption ≥ 300 mg/day was related to repeated miscarriage in non-smokers [OR:2.7 (1.1–6.2)] but not smokers [OR:0.4 (0.05–4.1)]. The test for interaction was not statistically significant.

As with the original study, the increased odds of repeated miscarriage associated with high caffeine intake only in non-smokers may be a result of lower prevalence of other risk factors for miscarriage in non-smokers, making a relation with caffeine more apparent. It may also be due to the fact that smoking, as the authors report, increases the rate of elimination of caffeine. Lack of control for pregnancy symptoms could provide an alternative explanation for the association between caffeine consumption and repeated spontaneous abortion in non-smokers, as described in Section 3.3.

3.7.6. Recurrent pregnancy loss and caffeine metabolism

Zusterzeel, P.L., Nelen, W.L., Roelofs, H.M., Peters, W.H., Blom, H.J., Steegers, E.A., 2000. Polymorphisms in biotransformation enzymes and the risk for recurrent early pregnancy loss. *Mol. Hum. Reprod.* 6, 6474–6478.

This case-control study of recurrent early pregnancy loss evaluated associations with polymorphisms in glutathione S-transferase (GST) and cytochrome P450 genes. The authors reasoned that polymorphisms in these genes may reflect impaired drug metabolism and detoxification, which could increase susceptibility to adverse outcomes resulting from chemical exposures. The study included 187 case women who had at least two unexplained consecutive spontaneous abortions occurring <17 weeks of gestation. The cases were selected from a referral hospital and 109 controls were selected from unrelated acquaintances who had at least one uncomplicated pregnancy and no spontaneous abortions (and were, thus, considered matched by age, socioeconomic status and district). The ratio of less than one control per case is not explained, but suggests differential response rates for cases and controls. Details regarding coffee exposure assessment are also omitted.

Odds ratios for the effects of caffeine consumption were not reported, but our calculations show no observed associations between daily coffee intake and recurrent pregnancy loss [crude OR:0.8 (0.4–1.5) for 1–5 cups and crude OR:1.4 (0.7–2.9) for $>$ (and equal to ?) five cups compared to non-coffee drinkers]. The non-mutually exclusive categories of coffee consumption are those reported by the authors.

GSTP1b-1b genotype (representing lower enzyme activity) was associated with recurrent pregnancy loss (OR:2.9 (1.0–10.1), especially among coffee drinkers (OR:4.1 (1.2–13.3)). The increased OR among coffee drinkers could be attributable to poorer precision in the smaller subgroup, which is based on only three controls homozygous for the GSTP1b allele. It could also be explained by uncontrolled confounding by smoking.

Although the GSTP1b–1b polymorphism appeared to be more common among women with recurrent early pregnancy loss, the limited data presented in this paper offer little evidence to implicate a specific role for coffee intake via direct effects or interactive effects with GST polymorphisms.

Sata, F., Yamada, H., Suzuki, K., Saijo, Y., Kato, E.H., Morikawa, M., Minakami, H., Kishi, R., 2005. Caffeine intake, CYP1A2 polymorphism and the risk of recurrent pregnancy loss. *Mol. Hum. Reprod.* 11, 357–360.

The authors of this small case-control study (58 cases and 147 controls) reported no overall association between caffeine intake ≥ 300 mg/day and recurrent pregnancy loss [OR:1.8 (0.7–4.6)] when compared to intake of 0–99 mg/day. Among women homozygous for CYP1A2 *1F (A/A) alleles, considered to have heightened caffeine metabolism, caffeine intake ≥ 300 mg/day was associated with recurrent pregnancy loss [OR:5.2 (1.1–25.9)] when compared to limited caffeine use (0–99 mg/day). Effect modification by CYP1A2 genotype was not observed among women with other CYP1A2 genotypes.

The limitations of this study include the small sample size, poor response rates (56.6% for cases and 58.1% for controls), lack of information on the timing of recruitment in relation to the previous pregnancy and implications for accurate exposure recall, no assessment of pregnancy symptoms, and the possibility of residual confounding by smoking which was measured as never, quitter and continuous smoker. Smokers homozygous for the CYP1A2 *1F (A/A) allele have higher CYP1A2 activity compared to other CYP1A2 genotypes (A/C and C/C) (Sachse et al., 1999). Thus, associations within the CYP1A2 *1F strata may be explained by smoking, particularly if reported caffeine use reflects actual smoking patterns (as described by Morrison, 1984).

The methodological limitations do not allow unambiguous conclusions to be drawn about interactive effects of heavy caffeine use and CYP1A2 genotype on recurrent pregnancy loss.

4. Fetal death

Clinical convention distinguishes fetal death, defined as fetal demise after 20 weeks of gestation, from spontaneous abortion defined as pregnancy loss ≤ 20 weeks of gestation. The distinction is typically drawn at the mid-point of pregnancy because it approximates the point of fetal viability, which is generally regarded as occurring close to 23–24 weeks of gestation. Because both outcomes address fetal loss, but at different points along the continuum of pregnancy, many of the methodological considerations identified for studies of spontaneous abortions are also applicable to studies of fetal death.

4.1. Pregnancy signal

Studies of caffeine and fetal death tend to focus on mid-pregnancy exposures and thus may be particularly subject to confounding by the pregnancy signal. Most women decrease their coffee consumption during the early part of pregnancy and maintain their lower than pre-pregnancy levels of consumption throughout the remainder of their pregnancy (Boylan et al., 2008). Thus, women who are heavy coffee consumers in the last half of pregnancy may not have experienced a strong pregnancy signal early in pregnancy. Only one study of fetal death (Matijasevich et al., 2006) considered nausea and vomiting, but measurement was limited and food/drink aversions were not considered.

4.2. Heterogeneity

Efforts have been made to group fetal deaths into more etiologically homogeneous outcomes according to the time of fetal de-

mise (antepartum vs. intrapartum; early fetal deaths <28 weeks vs. stillbirths ≥ 28 weeks) and cause of death. The evaluation of fetal deaths by likely causes (e.g., congenital infection, malformations, placental abruption) is hindered by the difficulty of attributing deaths to a single cause, the challenge of systematic identification of all contributing causes, and the need for large sample sizes (Leviton, 1987). While a worthwhile goal, the quality of analyses which classify outcomes by cause of fetal death remain questionable.

4.3. Selection of controls

Controls selected from live, healthy births (Matijasevich et al., 2006; Bech et al., 2006) may introduce selection bias. As with studies of spontaneous abortion, controls selected from on-going pregnancies matched by gestational age would provide the most accurate representation of the exposure distribution among the population that generated the cases of fetal deaths.

4.4. Late recognition of fetal demise

The exact timing of death is rarely observed, but can occur weeks before the fetal loss is recognized. Like studies of spontaneous abortion, the inclusion of caffeine intake up to the point of pregnancy termination may inflate associations with fetal deaths if the diminished pregnancy signal following fetal demise leads to increased caffeine consumption (Stein and Susser, 1991).

4.5. Review of individual studies of fetal deaths

Wisborg, K., Kesmodel, U., Bech, B.H., Hedegaard, M., Henriksen, T.B., 2003. Maternal consumption of coffee during pregnancy and stillbirth and infant death in first year of life: prospective study. *BMJ* 326, 420–423.

This prospective cohort study of 18,478 singleton pregnancies in Denmark reported an increased odds of stillbirth (≥ 28 completed weeks of gestation) among pregnant women drinking ≥ 8 cups of coffee per day compared to non-coffee drinkers [OR:2.2 (1.0–4.7)]. The strength of this study was its prospective design, which avoided opportunities for recall bias. The measurement of caffeine/coffee intake, however, was limited to current coffee consumption at 16 weeks of gestation, without reference to a standard cup size. Although other sources of caffeine intake were collected, the authors ignored contributions from tea, cola or chocolate since few women reported high intakes from these sources. Because 3922 women contributed more than one pregnancy to the cohort, sub-analyses addressing the lack of independence between observations were conducted. Results were not presented, but were reported as “comparable”. If women with less viable pregnancies consume more caffeine because they have fewer pregnancy symptoms and aversions, then higher caffeine consumption would be a consequence of an unhealthy pregnancy rather than a cause. Wisborg et al. (2003) did not consider pregnancy signal symptoms in their analyses.

Bech, B.H., Nohr, E.A., Vaeth, M., Henriksen, T.B., Olsen, J., 2005. Coffee and fetal death: a cohort study with prospective data. *Am. J. Epidemiol.* 162, 983–990. [Discussed also in 3. Spontaneous Abortion]

In this prospective study of 88,482 pregnancies within the Danish National Birth Cohort, the fetuses of women who consumed 8 or more cups of coffee per day were at increased risk of death between 20 and 27 weeks of gestation [HR:2.3 (1.3–3.9)], but were not at increased risk of death after 27 weeks [HR:1.3 (0.7–2.4)]. Risk of stillbirth due to placental dysfunction was 2.3 times greater among women consuming ≥ 4 cups of coffee per day compared to women who consumed no coffee [HR:2.3 (1.2–4.3)]. No associations were observed for other stillbirth subgroups such as

unexplained intrauterine deaths, umbilical cord complications, congenital malformations, “other” conditions such as infection and maternal disease, or intrapartum deaths.

To minimize bias that can occur when caffeine consumption is increased as a result of undetected fetal demise, the authors repeated analyses after excluding losses occurring over a range of 2–28 days following the interview. The hazard ratios became attenuated with increasing lag time and non-significant in all exposure categories, suggesting the results could be explained by the pregnancy signal. Although the data are not shown, the authors note the decline in hazard ratios to be particularly strong among fetal deaths <20 weeks, but less so for fetal deaths ≥20 weeks. Associations with later fetal losses may be less susceptible to the influence of undetected fetal demise since exposure assessment (between 13 and 19 weeks of gestation) preceded the loss by a longer time period. However, reverse causation remains a possibility if higher coffee intake is a marker of placental dysfunction and thus a consequence of the reduced or absent symptoms that accompany less viable pregnancies, regardless of the timing of fetal demise. The authors acknowledge their inability to assess the impact of pregnancy symptoms on the observed associations.

Bech, B.H., Autrup, H., Nohr, E.A., Henriksen, T.B., Olsen, J., 2006. Stillbirth and slow metabolizers of caffeine: comparison by genotypes. *Int. J. Epidemiol.* 35, 948–953.

In this paper, the same research group as above conducted a nested case-control study within the Danish National Birth Cohort to evaluate the interrelationship between caffeine, caffeine metabolism and stillbirth. Cases ($n = 142$) were defined as stillbirths occurring ≥28 weeks of gestation, excluding intrapartum deaths. Controls ($n = 157$) were selected from live births, frequency matched by parity. The authors evaluated genotypes either known [cytochrome P4501A2 (CYP1A2) and N-acetyltransferase 2 (NAT2)], or suspected [glutathione S-transferase $\alpha 1$ (GSTA1)] to be active in caffeine metabolism. CYP1A2 genotypes were grouped into fast (A/A) and slow (A/C and C/C) oxidizers. NAT2 genotypes were grouped into fast (Fast/Fast and Fast/Slow) and slow (Slow/Slow) acetylators. GSTA1 genotypes were classified according to high activity (a/a) and reduced activity (a/b and b/b). Coffee was the only source of caffeine considered in this analysis. The methods used to ascertain coffee intake are not provided, but the limitations are presumed to be the same as described for Bech et al. (2005) (see Section 3.7.1).

When caffeine use was assessed without consideration for metabolic genotype, drinking ≥4 cups of coffee per day was not associated with stillbirth [OR:1.0 (0.5–2.3)]. Women with stillbirths had a 1.9-fold increased odds of having a slow caffeine metabolism as characterized by all three genotypes (slow CYP1A2, slow NAT2 and low GSTA1) compared to controls [OR:1.9 (1.0–3.4)]. However, when genotypes were assessed separately or in paired combinations, no associations with stillbirth were observed. The lack of interaction between genotype and caffeine consumption suggests the associations with these genotypes may not reflect causal pathways specific to caffeine metabolism.

Matijasevich, A., Barros, F.C., Santos, I.S., Yemini, A., 2006. Maternal caffeine consumption and fetal death: a case-control study in Uruguay. *Paediatr. Perinat. Epidemiol.* 20, 100–109.

Cases ($n = 382$) included all antepartum fetal deaths (≥20 weeks of gestational age or weighing >350 g) occurring in the 16 maternity hospitals within the capital city of Uruguay. Controls ($n = 792$) were healthy, full term, live births without growth restriction, frequency matched by hospital. Participants were interviewed within 24 h following delivery for caffeine intake from maté (herbal tea) and coffee during each trimester. The justification for limiting assessment to coffee and maté was that these are the primary sources of caffeine in South America. However, according to their own report of caffeine consumption within the

South American region (Santos et al., 1998), up to 48% of pregnant women reported consumption of other sources such as soft drinks, chocolate bars, and black tea, which accounted for approximately one-fourth of their total caffeine intake during pregnancy. Thus, exposure misclassification is likely.

The authors report that mean caffeine consumption of ≥300 mg/day throughout pregnancy was associated with fetal death [OR:2.3 (1.2–4.4)]. Several maternal characteristics were considered as confounders, although the criteria for confounding relied on p values, which can result in an appreciable loss of information (Dales and Ury, 1978). The crude measurement of smoking (yes/no) and pregnancy symptoms (yes/no for vomiting or nausea in first trimester) and failure to consider alcohol intake may have also contributed to incomplete control of confounding.

As noted by the authors, recall bias could have inflated the association if case mothers reported past caffeine use more accurately than controls. Another limitation of this study was the use of live births as controls, which may not accurately represent the exposure distribution in the population from which the cases arose (Signorello and McLaughlin, 2004).

In light of these limitations, this study does not provide convincing evidence that high caffeine consumption throughout pregnancy is associated with fetal death ≥20 weeks of gestation.

5. Gestational age and preterm birth

5.1. Heterogeneity

Preterm birth includes a heterogeneous group of disorders, suggesting heterogeneous etiologies (Savitz et al., 1991, 2005; Klebanoff and Shiono, 1995; Klebanoff, 1998a; Pennell et al., 2007; Berhman and Butler, 2007; McElrath et al., 2008; Savitz, 2008a). The so-called “spontaneous preterm delivery” group includes preterm labor, pre-labor premature rupture of membranes, placental abruption, and cervical insufficiency, which are associated with intrauterine inflammation. Medically indicated preterm births can be characterized by maternal and fetal origins. The maternal medical indications group is almost exclusively pre-eclampsia, and is attributed to dysfunctional placentation. Fetal indications are heterogeneous and include non-reassuring fetal testing, oligohydramnios, Doppler abnormalities of umbilical cord blood flow, or severe intrauterine growth restriction identified on antepartum ultrasound examination. Only two studies attempted to evaluate relatively homogenous outcomes (Mikkelsen et al., 2008; Haugen et al., 2008).

It is not yet clear that the processes leading to delivery near the boundary of viability (23–24 weeks) are the same as those that lead to delivery near the upper boundary of prematurity (34–36 weeks). The two studies that acknowledged the possibility that early and late prematurity might be different entities dichotomized premature deliveries at 35 weeks, rather than a considerably earlier week in pregnancy (Mikkelsen et al., 2008; Haugen et al., 2008).

5.2. Accurate estimation of gestational age

Correct classification of preterm birth is dependent on accurate estimates of gestational age. The three most commonly used methods are based on ultrasound, date of last menstrual period and neonatal assessment of physical and neurological maturity at birth (Lynch and Zhang, 2007). No method is perfect, but the use of early ultrasound is considered to provide the most accurate estimation, within 5–10 days when completed at 12–14 weeks of gestation and 9–12 days when completed at 15–20 weeks of gestation (Saltvedt et al., 2004).

Gestational age based on date of last menstrual period can be flawed due to recall errors or delayed ovulation, commonly overestimating pregnancy duration (Savitz et al., 2002). In the absence of other information, gestational age is sometimes estimated using neonatal evaluations such as the Dubowitz and Ballard examinations, which score the physical and neuromuscular development of the newborn (Dubowitz et al., 1970; Ballard et al., 1979, 1991). These postnatal estimates are less precise and accurate than the preferred prenatal methods and have a tendency to underestimate gestation for post-term deliveries and to overestimate gestation by up to 2 weeks for deliveries occurring before 40 weeks (reviewed by Lynch and Zhang, 2007).

Considering the deficiencies in each of these methods, some have recommended a hierarchy of assessments ordered by accuracy and availability of the measurements. One example preferred the dates of embryo retrieval or intrauterine insemination or ultrasound examination before the 14th week of gestation. If these were not available, the order of preference continued with ultrasound at 14 weeks or later, followed by menstrual dating without ultrasound confirmation, and finally gestational age recorded after neonatal assessment (McElrath et al., 2008).

5.3. Review of individual studies of preterm birth

Claussou, B., Granath, F., Ekblom, A., Lundgren, S., Nordmark, A., Signorello, L.B., Cnattingius, S., 2002. Effect of caffeine exposure during pregnancy on birth weight and gestational age. *Am. J. Epidemiol.* 155, 429–436. [Discussed also in 7. Fetal Growth]

In this cohort study from Sweden, 873 pregnant women were interviewed twice, between gestational weeks 6–12 and 32–34, for their recall of caffeine consumption during the previous weeks of pregnancy. Although gestational age was confirmed by ultrasound examination, spontaneous and medically indicated preterm births were not considered separately. Combining all preterm births <37 weeks of gestation could potentially obscure existing associations with caffeine intake. According to the authors, neither mean length of gestation nor mean birth weight differed for mothers grouped according to average daily caffeine intakes across the entire pregnancy (4–34 weeks of gestation) of 0–99, 100–299, 300–499 or ≥ 500 mg/day. Furthermore, average daily caffeine intake in each trimester was not associated with duration of pregnancy.

Retrospective exposure assessment may have resulted in errors in caffeine measurement given the difficulty of accurately remembering intake patterns across previous weeks and months. Since consumption patterns were assessed before delivery, however, it is unlikely that women with shorter gestations would have been influenced to systematically report more or less caffeine consumption. The authors controlled for smoking by using third trimester plasma cotinine concentrations. Pregnancy symptoms were also assessed as confounders, but had little impact on the results. These data do not support an association between caffeine and preterm birth, however caffeine exposures after 32–34 weeks could not be evaluated.

Klebanoff, M.A., Levine, R.J., Clemens, J.D., Wilkins, D.G., 2002. Maternal serum caffeine metabolites and small-for-gestational age birth. *Am. J. Epidemiol.* 155, 32–37. [Discussed also in 7. Fetal Growth]

This study measured paraxanthine, the major metabolite of caffeine, in third trimester serum samples banked for 2515 women participating in the Collaborative Perinatal Project (CPP) between 1959 and 1966. The subjects in this study served as the controls in a previous case-control study of spontaneous abortion (Klebanoff et al., 1999); thus, all had delivered liveborn infants ≥ 28 weeks of gestation. Serum samples collected after 26 weeks of gestation were stored frozen for over 30 years before caffeine

metabolite concentrations were measured. After controlling for maternal ethnicity, paraxanthine concentrations were not associated with gestational age or preterm birth.

The stability of caffeine metabolites in serum is unknown. In response to concerns about measurement error raised by Grosso et al. (2004), the authors responded by demonstrating that detected concentrations of serum paraxanthine following long-term storage were consistent with self-reported intake in another cohort assembled during the same time period (Klebanoff and Longnecker, 2004).

Because the CPP study was conducted in the early 1960s, caffeine use during pregnancy was relatively common in the study population, although actual consumption patterns were not directly assessed. Other strengths of the study include the opportunity to assess a biomarker of caffeine exposure that avoids measurement errors related to inaccurate recall and the difficulty of accounting for all sources. In light of individual differences in caffeine metabolism, paraxanthine levels may reflect biologic dose better than reported caffeine consumption. On the other hand, the paraxanthine biomarker represents recent caffeine intake (half-life = 10 h in later pregnancy) (Aldridge et al., 1981) and may reflect exposure during the relevant window of susceptibility only for those whose intake patterns remain relatively constant over time.

The data do not support an association between third trimester paraxanthine concentrations and pregnancy duration or preterm delivery.

Klonoff-Cohen, H., Bleha, J., Lam-Kruglick, P., 2002. A prospective study of the effects of female and male caffeine consumption on the reproductive endpoints of IVF and gamete intra-fallopian transfer. *Hum. Reprod.* 17, 1746–1754. [Discussed also in 2. Subfecundity and 3. Spontaneous Abortion]

Within this study of couples undergoing IVF and GIFT (described in Section 2.6), the association between caffeine intake and gestational age was assessed in 39 live births. No association was observed between caffeine intake among the male partners and gestational age. Maternal caffeine intake >50 mg/day during the week of the first fertility clinic visit was associated with a 3.5 week decrease (95% CI $-6.7, -0.3$) in gestational age when compared to women reporting 0–2 mg/day. Similar results were reported for usual “lifetime” caffeine consumption, but results for intake during pregnancy were not reported. The authors cautiously report the findings as having “borderline significance” while acknowledging the small sample size and limited precision.

Although the results were reported as adjusted, the specific control variables were not specified. Factors controlled in analyses of other outcomes in this report included smoking, alcohol use, woman's age, race, years of schooling, parity, type of infertility, type of procedure, and number of ART attempts. However, with only 39 observations, the precision of the results would be questionable with this many covariates (Feinstein 1996).

Bracken, M.B., Triche, E.W., Belanger, K., Hellenbrand, K., Leaderer, B.P., 2003. Association of maternal caffeine consumption with decrements in fetal growth. *Am. J. Epidemiol.* 157, 456–466. [Discussed also in 7. Fetal Growth]

This prospective cohort study included 2291 pregnant women enrolled by 24 gestational weeks (14.4 weeks on average). All women consuming ≥ 150 mg/day during the previous week were invited to participate as well as a random sample of those consuming <150 mg/day. Caffeine exposures were quantified by measuring caffeine concentrations in urine at the baseline interview, and by self-reported consumption during the first and last trimester of pregnancy. A major strength of this study is that it was designed to assess pregnancy outcomes in relation to caffeine use and, thus, incorporated a detailed assessment of caffeinated beverage intake including method of preparation, brand names and more accurate

reporting of serving sizes. The omission of caffeine from food and medicinal sources, however, may have underestimated exposure status.

Preterm births were not differentiated according to spontaneous or medically indicated deliveries. The frequencies of preterm birth and IUGR in this cohort were lower than those reported for the general US population within a similar time period suggesting that participants consisted of women who had generally healthier pregnancies compared to the general US population.

No associations between caffeine use and preterm birth were observed in analyses adjusted for smoking and other potential confounders. The lack of association was consistently observed for urinary caffeine concentrations as well as self-reported use.

Tough, S.C., Newburn-Cook, C.V., White, D.E., Fraser-Lee, M.J., Faber, A.J., Frick, C., Svenson, L.W., Sauve, R., 2003. Do maternal characteristics and past pregnancy experiences predict preterm delivery among women aged 20–34? *J. Obstet. Gynaecol. Can.* 25, 656–66.

This population-based case-control study of 323 preterm deliveries and 664 controls explored numerous demographic, medical and lifestyle characteristics as predictors of preterm delivery. Phone interviews were conducted 4–8 weeks after delivery. Specific details of the methods for caffeine assessment were not provided. Analyses were limited to crude odds ratios assessed separately for coffee, tea and caffeinated soft drink consumption measured as <1 cup per day compared to ≥ 1 cup per day. A crude association between coffee consumption and preterm birth was observed (OR = 1.4, 95% CI 1.0–1.9), but was not maintained in the final multivariate model reported for statistically significant predictors of preterm delivery.

Khoury, J.C., Miodovnik, M., Buncher, C.R., Kalkwarf, H., McElvy, S., Khoury, P.R., Sibai, B., 2004. Consequences of smoking and caffeine consumption during pregnancy in women with type 1 diabetes. *J. Matern. Fetal Neonatal Med.* 15, 44–50. [Discussed also in 3. Spontaneous Abortion and 6. Congenital Malformations]

In this study of 191 diabetic pregnancies, the monthly assessment of caffeine exposure was limited, measured as the average number of 8 oz cups consumed daily from all caffeinated beverages, giving equal weighting to coffee, tea and soft drinks. Analyses of preterm birth focused on caffeine exposure after 20 weeks of gestation. To assess the quality of this measurement, the authors compared the serving counts averaged across the last half of pregnancy to estimates of total caffeine consumption during this time period converted to equivalent cups of coffee per day. The agreement between methods, as measured by the Kappa statistic, was reported to be 0.61, 0.65 and 0.71 for 0, 1–2, and ≥ 3 cups/day. Thus, the analyses of preterm birth are subject to considerable exposure misclassification. The authors elected not to report results using total caffeine consumption.

In this report, no association was observed between caffeine consumption after 20 weeks of gestation and gestational age at delivery after controlling for age, smoking, glycemic control and other indicators of diabetes severity. Without a more accurate measurement of caffeine exposure, the findings offer little insight into associations with preterm birth.

Santos, I.S., Matijasevich, A., Valle, N.C.J., 2005. Maté drinking during pregnancy and risk of preterm and small for gestational age birth. *J. Nutr.* 135, 1120–1123. [Discussed also in 7. Fetal Growth]

This retrospective cohort study explored consumption of maté, a popular beverage in South America prepared by steeping leaves of *Ilex paraguayensis* in hot water. It is considered a major source of caffeine intake among women in Southern Brazil. Women ($n = 5189$) in five local maternity hospitals were interviewed within 24 h following delivery. Maté use during pregnancy was quantified as number of days per week (0, 1–6 and 7 days per week) rather than number of servings. The amount of actual caffeine consumption was not specifically investigated, but according to results

from a previous study in the same source population (Santos et al., 1998), women reporting daily consumption of maté averaged approximately 300 mg of caffeine per day.

Gestational age was measured using a clinical estimate at birth (i.e., Dubowitz score), which has been shown to have reasonable, but imperfect, agreement with ultrasound measurements (Vik et al., 1997). In light of these limitations, the authors' failure to find a relationship between maté consumption and duration of pregnancy has limited significance.

Chiapparino, F., Parazzini, F., Chatenoud, L., Ricci, E., Tozzi, L., Chiantera, V., Maffioletti, C., Fedele, L., 2006. Coffee drinking and risk of preterm birth. *Eur. J. Clin. Nutr.* 60, 610–613.

This case-control study compared 520 women who delivered at least three weeks before term to 1966 controls who delivered at term in the same hospitals in Northern Italy. Because of potential etiologic heterogeneity, subgroups of preterm births with and without small for gestational age (SGA) were evaluated separately.

It is unclear how the exposure information was originally collected and combined for analyses. For example, it is not known whether consumption patterns were reported for specific weeks or by trimester of pregnancy which were then summed or whether participants reported an overall estimate of average consumption during the entire pregnancy. Caffeine consumption during pregnancy was relatively low in this population, limiting the opportunity to assess associations with high intake levels. Given exposure assessment occurred within the 3 days following delivery, recall bias was a potential concern. Positive associations with caffeinated beverage intake, however, were not observed.

Associations with tea, cola or decaffeinated coffee were not observed for either preterm subgroup. Women who consumed two or more servings of coffee per day were at reduced risk of delivering an SGA newborn before term compared to non-consumers [OR: 0.5 (0.3–0.8)]. The reduced risk did not achieve statistical significance for preterm delivery of an appropriate for gestational age infant [OR: 0.8 (0.6–1.1)].

The authors attribute this unexpected inverse association to potential increases in coffee consumption among controls that may accompany decreased nausea during the third trimester, in conjunction with decreased coffee consumption among concerned case mothers who may have become aware of restricted fetal growth by the third trimester. Pregnancy symptoms, however, were not evaluated. Given the limitations presented above, this study does not provide convincing evidence either for or against a causal link between caffeine and preterm birth.

Bech, B.H., Obel, C., Henriksen, T.B., Olsen, J., 2007. Effect of reducing caffeine intake on birth weight and length of gestation: randomised controlled trial. *BMJ* 334, 409–414. [Discussed also in 7. Fetal Growth]

This study distinguishes itself from observational studies by using an experimental design to randomly assign 1197 heavy coffee drinkers to caffeinated or decaffeinated instant coffee during the last half of pregnancy. Mean birth weight and gestational age were compared across treatment groups. While random exposure assignment helped reduce selection biases, exposure measurement error remained, since participants in both treatment groups received no restrictions on amount of coffee consumed and were free to consume other sources of coffee or caffeinated beverages. In addition, based on interview data, more than one-third of the women in the decaffeinated group were daily consumers of caffeinated coffee (≥ 1 cup/day). This cross-over bias serves to make the caffeine distribution in the two treatment groups more similar, thereby weakening the ability to detect true differences should they exist.

Analysis of the data using the intent to treat approach (i.e., by treatment assignment) found that caffeinated coffee consumers had a distribution of pregnancy duration that was similar to that

of decaffeinated coffee consumers. This approach, however, ignores the individual level exposure data. In the absence of an analysis of actual caffeine intake, the authors do offer analyses stratified by “compliance” which was defined according to frequency of consumption of caffeinated coffee outside of that provided by the study. The lack of association between caffeine assignment and preterm birth was consistently observed across compliance groups.

Mikkelsen, T.B., Osterdal, M.L., Knudsen, V.K., Haugen, M., Meltzer, H.M., Bakketeig, L., Olsen, S.F., 2008. Association between a Mediterranean-type diet and risk of preterm birth among Danish women: a prospective cohort study. *Acta Obstet. Gynecol. Scand.* 87, 325–330.

Haugen, M., Meltzer, H.M., Brantsaeter, A.L., Mikkelsen, T., Osterdal, M.L., Alexander, J., Olsen, S.F., Bakketeig, L., 2008. Mediterranean-type diet and risk of preterm birth among women in the Norwegian Mother and Child Cohort Study (MoBa): a prospective cohort study. *Acta Obstet. Gynecol. Scand.* 87, 319–324.

These two companion studies were coordinated to assess the effects of a Mediterranean-type diet on preterm birth in two large birth cohorts in Denmark and Norway. Low coffee consumption, defined as ≤ 2 cups of coffee per day, was evaluated as part of a Mediterranean-type diet. Both studies were conducted within large existing birth cohorts and were restricted to non-smoking women between ages 21 and 38 with a BMI between 19 and 32, pregnant with singletons, with normal calorie intake and no history of >3 spontaneous abortions. Preterm deliveries were evaluated in both studies according to early (22–34 weeks) and late (35–36 weeks) preterm births as well as all preterm births combined (<37 weeks of gestation). Both studies reported results specifically for coffee consumption, but the findings for associations with preterm birth were inconsistent between the two studies.

The Danish study (Mikkelsen et al., 2008) reported data from 35,530 non-smoking women recruited from the Danish National Birth Cohort during the 25th week of gestation. Coffee consumption during the previous 4-week period was collected as part of a food frequency questionnaire. Gestational age at birth was primarily obtained from mother's reported last menstrual period. Coffee intake ≤ 2 cups per day was associated with a 26% lower odds of early preterm birth [OR:0.7 (0.6–0.9)] compared to women consuming >2 cups/day, after adjustment for other components of the Mediterranean diet and other confounders. No association with late preterm birth was observed [OR:0.9 (0.8, 1.1)].

Because these studies were designed to assess the effects of a Mediterranean diet and not caffeine intake specifically, measurements of all sources of caffeine exposure were not obtained. Thus, the results are specific to coffee consumption and not caffeine. To the extent that (1) coffee is the primary source of caffeine exposure in Danish women and (2) consumption patterns during the fifth month of pregnancy represent either the critical window of exposure or patterns of caffeine use maintained during the critical window, the results could be suggestive of a weak effect of caffeine on early but not late preterm birth.

The study published by Haugen et al. (2008) was conducted among 26,563 women participating in the Norwegian Mother and Child Cohort Study. Food frequency questionnaires were administered during mid-pregnancy (17–24 weeks), but in this study the reference period was the entire pregnancy up to that point, rather than the previous month as reported in Mikkelsen et al. (2008). Thus, coffee exposure was subject to misclassification that may occur when intake patterns are retrospectively reported over many months. Information on gestational age at birth was collected by linking to the Norwegian Medical Birth Registry. In the Norwegian sample, consuming two or less cups of coffee a day was not associated with a reduced risk of delivering before the 35th week [OR:1.11 (0.83, 1.49)] or during the 35th and 36th weeks [OR:1.15 (0.90, 1.46)].

The inconsistent results of these parallel studies by the same group of investigators may be attributed to differences in the quality of exposure and outcome measurements or they may be due to chance. Overall, convincing support for a role of caffeine in the etiology of preterm birth was not demonstrated.

6. Congenital malformations

6.1. Heterogeneity

All malformations are not etiologically identical. Even those studies limited to one organ or related structures (e.g., lip and palate) are likely evaluating heterogeneous entities. Some studies have assessed single malformations (Mongraw-Chaffin et al., 2008; Torfs and Christianson, 2000; Browne et al., 2007; Miller et al., 2009; Slickers et al., 2008) or utilized more refined sub-classifications of observed phenotypes (Browne et al., 2007; Bille et al., 2007; Johansen et al., 2009; Schmidt et al., 2009; Collier et al., 2009) in an attempt to reduce etiologic heterogeneity.

6.2. Biased exposure data

Because congenital malformations are rare, the case-control (case-referent) design is preferred. This design suffers, however, from potential recall bias, which could result in overestimating the contribution of caffeine to the occurrence of congenital malformations. Alternative strategies for control selection, such as the use of controls with other anomalies, have been proposed to assess the contribution of recall bias, but these methods do not remove the bias if it is present (Lief et al., 1999; Hook, 2000; Schlesselman, 1982).

All studies of caffeine and congenital malformations reviewed in this report selected controls from non-cases or from a random sample of all births in the population.

6.3. Relevance of time of exposure to disturbed development

While many studies define the exposure interval broadly as the first trimester, the relevant window of exposure for most congenital malformations is the period of organogenesis. Exposures after this period are unlikely to be important.

Since early first trimester exposures coincide with the appearance of pregnancy symptoms for many women, reports of caffeine consumption averaged across the entire first trimester (or longer periods) might not reflect actual exposures during the relevant period of fetal development. Furthermore, the retrospective nature of exposure assessment limits measurement precision for narrowly defined periods of gestation. Although the mean timing of symptom onset is reported to occur between 5 and 6 weeks after the last menstrual period (Gadsby et al., 1993; Lawson et al., 2004), over 13% of pregnant women have been reported to experience symptom onset as early as within the 2 weeks following the estimated time of conception (i.e., 4 weeks after last menstrual period) (Gadsby et al., 1993). Thus, concern for accurate exposure assessment in the context of symptom-related changes in caffeine consumption remains relevant for outcomes such as congenital malformations that result from very early disruptions to fetal development.

Pre-pregnancy caffeine exposure has been used as a surrogate for consumption in early stages of gestation before pregnancy symptoms develop. This approach would result in misclassification of exposure for women who changed their intake patterns either because they planned their pregnancy or experienced early pregnancy symptoms. The direction of the bias produced by these measurement errors is difficult to predict. If measurement errors are equally likely among cases and controls, misclassification would

likely underestimate caffeine-malformation associations when binary measures of exposure are assessed.

6.4. Outcome ascertainment

Recognition of congenital malformations requires the fetus to survive until birth, or at least until prenatal diagnosis. Even malformations among fetuses that survive to birth, however, are not always evident at delivery and may go undiagnosed. When studying malformations that are sometimes fatal, selection bias can occur if exposure leads to higher rates of pregnancy loss in malformed fetuses, resulting in lower proportions of exposed cases (Khouri et al., 1989). Failure to identify malformations resulting in spontaneous or elective abortions may lead to under-ascertainment of cases. Since most studies of congenital malformations are conducted in collaboration with birth defect registries, the impact of active vs. passive surveillance systems on the completeness of reporting must also be considered.

6.5. Review of individual studies of congenital malformations

Natsume, N., Kawai, T., Ogi, N., Yoshida, W., 2000. Maternal risk factors in cleft lip and palate: case control study. *Br. J. Oral Maxillofac. Surg.* 38, 23–25.

This case-control study included 306 cases of cleft lip, cleft palate, or both matched to 306 controls by “same district during the same time period”. Given the limited information provided, potential for selection bias cannot be disregarded.

The study was not designed specifically to evaluate caffeine as a risk factor; however, coffee consumption was part of an assessment of dietary preferences. Details regarding the definition of the reference periods “before” and “during” pregnancy were omitted. The authors do not offer a statistical comparison between cases and controls beyond assessing the difference in proportions (chi square test) consuming <1 cup of coffee per week. By our calculations, the crude odds ratios for coffee consumption during pregnancy were 0.8 (0.6–1.1) for 1–2 cups/week and 0.9 (0.4–2.3) for 3–6 cups per week, using the <1 cup/week group as the referent. For coffee consumed before pregnancy, the crude odds ratios were 0.7 (0.5–1.0) for 1–2 cups per week and 0.5 (0.3–0.9) for 3–6 cups per week compared to consumers of <1 cup/week. Thus, case mothers were less likely to consume coffee before pregnancy than the unaffected controls. Given the lack of consideration for confounders and great potential for exposure misclassification and recall bias, these data do not make a meaningful contribution to the body of evidence regarding caffeine and congenital malformations.

Khouri, J.C., Miodovnik, M., Buncher, C.R., Kalkwarf, H., McElvy, S., Khouri, P.R., Sibai, B., 2004. Consequences of smoking and caffeine consumption during pregnancy in women with type 1 diabetes. *J. Matern. Fetal Neonatal Med.* 15, 44–50. [Discussed also in 3. Spontaneous Abortion and 5. Gestational Age and Preterm Birth]

This study of pregnancy complications among 191 pregnant women with type 1 diabetes evaluated associations between first trimester caffeine use and all major malformations combined. Any consumption of caffeine during the first trimester (i.e., none vs. one or more cups of coffee, tea or soft drinks) was not associated with major malformations [crude OR:2.0 (0.4–11.2)]. The number of observed malformations was not described, but was reportedly too small to estimate adjusted odds ratios. This investigation was severely limited by small sample size, potential confounding, the combination of etiologically heterogeneous malformations, and inadequate exposure assessment.

Torfs, C.P., Christianson, R.E., 2000. Effect of maternal smoking and coffee consumption on the risk of having a recognized Down syndrome pregnancy. *Am. J. Epidemiol.* 152, 1185–1191.

This population-based case-control study included 997 Down syndrome cases from the California Birth Defects Monitoring Program and 1007 liveborn non-malformed controls from the general population, frequency matched to cases by hospital of birth. Women were interviewed approximately 5–6 months following delivery for consumption of coffee, tea and soft drinks “around the time of conception”. The primary analyses focused on coffee consumption, with the reference group being women who consumed 0–3 cups of coffee per day.

A protective association between heavy coffee intake (≥ 4 cups/day compared to ≤ 3 cups/day) and Down syndrome was observed among non-smokers [OR:0.5 (0.3–0.8)], but not smokers [OR:1.6 (0.8–3.4)]. The authors interpret their results as evidence that non-smoking mothers (defined as not smoking within three months of conception) consuming high levels of caffeine were more likely to miscarry a fetus with Down syndrome, thereby reducing the prevalence of cases recognized at later deliveries among heavy caffeine users. In other words, the authors suggest that the observed protective effect may indicate selection bias inherent in studies of congenital malformations such as Down syndrome which go largely undetected due to early spontaneous abortions (as described by Khouri et al., 1989). The authors speculate that the lack of a similar observed effect among smokers could be attributed to increased metabolism and caffeine clearance associated with smoking.

While the results presented in this report offer no evidence for a role of caffeine in the etiology of Down syndrome, they also do not directly evaluate contributions of caffeine use to the early loss of Down syndrome fetuses.

Bille, C., Olsen, J., Vach, W., Knudsen, V.K., Olsen, S.F., Rasmussen, K., Murray, J.C., Andersen, A.M., Christensen, K., 2007. Oral clefts and life style factors – a case-cohort study based on prospective Danish data. *Eur. J. Epidemiol.* 22, 173–181.

This study utilized prospectively collected data on coffee, tea and cola consumption in a case-cohort design, which included 134 cases of cleft lip with and without cleft palate and 58 cases with cleft palate only identified within the Danish National Birth Cohort. Controls ($n = 828$) were randomly selected from the birth cohort. The authors observed no associations with coffee intake for all oral clefts combined or by subtype. Mothers of babies with isolated cleft palate had 2.5 (1.1–5.6) times greater odds of consuming 5 or more cups of tea per day compared to mothers of controls. Weekly cola intake exceeding one liter was marginally associated with cleft lip with or without cleft palate [OR:1.5 (0.9–2.4)].

The major strength of this study is the evaluation of oral clefts by clinically confirmed subtypes (i.e., cleft lip with and without cleft palate and cleft palate only), which may be etiologically distinct.

There are several limitations, however. Analyses by subtype were limited by small numbers and lacked precision. While exposure assessment was reported to take place between gestational weeks 12–27 (for 90%), few details of the data collection are presented. It is not clear whether consumption was assessed as usual consumption since conception or as average consumption during the first trimester or during a specific reference period. There was no attempt to assess caffeine intake from all sources combined or patterns of caffeine consumption that may fluctuate with pregnancy symptoms. Closure of lip and palate structures occurs around the 8th week of gestation during normal fetal development (Burdi and Faist, 1967; Yoon et al., 2000). Thus the relevant period of exposure for oral clefts coincides with the time in early pregnancy when pregnancy symptoms begin to appear, with average onset between 5 and 6 weeks of gestation and symptoms peaking by week 8 on average (Gadsby et al., 1993; Lawson et al., 2004). Thus, reports of average daily consumption across the entire first

trimester may not reflect actual patterns of exposure during the etiologically relevant period of fetal development.

The absence of consistent results by type of beverage is not consistent with an etiologic role for caffeine in the development of cleft lip and/or palate. Although the measurement errors described above may have tended to obscure associations with caffeinated beverages, these errors would not be expected to differ by type of beverage. While the non-statistically significant protective effect of coffee may be interpreted as evidence for caffeine-related demise among malformed fetuses, we caution against this conclusion since oral clefts do not commonly result in early fetal loss and thus are not susceptible to the form of selection bias described by Khoury et al. (1989) that occurs when cases are identified from live births.

Browne, M.L., Bell, E.M., Druschel, C.M., Gensburg, L.J., Mitchell, A.A., Lin, A.E., Romitti, P.A., Correa, A., 2007. National Birth Defects Prevention Study. Maternal caffeine consumption and risk of cardiovascular malformations. *Birth Defects Res. A Clin. Mol. Teratol.* 79, 533–543.

This large case-control study from the National Birth Defects Prevention Study identified specific cardiovascular malformations ($n = 4196$) from the birth defect registries of eight states and 3957 controls randomly selected from hospital records or birth certificates within each state. This population-based study has a number of strengths including a sample size large enough to evaluate specific malformations considered etiologically homogeneous and an assessment of caffeine intake from multiple sources. The authors also evaluated a complete and well justified list of potential confounders and effect modifiers, providing specific details regarding the criteria for assessment. After conducting a thorough analysis, the authors reported no positive associations between pre-pregnancy caffeine intake and cardiovascular malformation subtypes.

The primary weakness of this study involves retrospective exposure assessment far removed in time from the critical window of susceptibility. Interviews were conducted 8–12 months after delivery on average, with some occurring as late as 24 months after delivery dates. While the burden of recall appeared to be similar for cases and controls, the excessive time gap could have led to reporting errors. Although sensitivity analyses conducted by the authors showed no difference in results when subjects interviewed more than 1 year after the estimated date of delivery were eliminated, these findings do not alleviate concerns about exposure misclassification since recall up to 1 year after delivery could be just as flawed.

The rationale provided for evaluating pre-pregnancy exposure rather than first trimester exposure is that it may better depict intake patterns during the susceptible period for fetal heart development, which tends to occur before most pregnancy symptoms develop (Lacroix et al., 2000). The authors acknowledge that exposure misclassification would likely occur for pregnancy planners or those experiencing early pregnancy symptoms who changed their intake patterns during this early period of gestation.

In the authors' words, the study "does not provide any appreciable evidence of an association between maternal caffeine consumption and risk of [cardiovascular malformations]".

Mongraw-Chaffin, M.L., Cohn, B.A., Cohen, R.D., Christianson, R.E., 2008. Maternal smoking, alcohol consumption, and caffeine consumption during pregnancy in relation to a son's risk of persistent cryptorchidism: a prospective study in the Child Health and Development Studies cohort, 1959–1967. *Am. J. Epidemiol.* 167, 257–261.

This nested case-control study of cryptorchidism was conducted among children born to mothers enrolled in the Child Health and Development Studies between 1959 and 1967. Cases ($n = 84$) were boys with an undescended testicle at birth that remained undescended until 2 years of age. Controls ($n = 252$) were selected from non-cases and matched 3:1 by race/ethnicity and

date of birth. The authors report a modest association between cryptorchidism and caffeine use equivalent to 3 cups of coffee per day [OR:1.4 (1.1–1.9)] after controlling for alcohol, smoking, body mass index and child's birth weight.

The strengths of the study include prospective data collection on health behaviors at a time when there was less stigma associated with the use of tobacco, alcohol and caffeine during pregnancy. Thus, under-reporting of such patterns would be less likely to occur in this study population and exposure reports would not be influenced by knowledge of the birth outcome.

Selection bias cannot be ruled out as an alternative explanation for the weak association observed in this study. A large proportion of cases ($33/101 = 33\%$) and controls ($40/252 = 16\%$) were excluded from analyses because of missing data. If exclusions differed from inclusions with respect to case/control status and factors that correlated with caffeine consumption, the observed association with caffeine could be distorted.

Another issue pertaining to study quality is the potential for exposure misclassification. Caffeine exposure was assessed by interview conducted "during early pregnancy", but the specific reference period was not described and the assessment did not capture fluctuations that typically occur during pregnancy. Thus, it is questionable whether the recorded data reflects accurate exposures during the relevant period of fetal development. Although the susceptible period for development of cryptorchidism is not well understood, the relevant time window could plausibly include mid to late pregnancy, given normal testicular descent occurs between gestational weeks 25 and 32 (Rotondi et al., 2001).

Potential confounding by gestational diabetes was also not considered. Mild gestational diabetes has been identified previously as a risk factor of cryptorchidism (Virtanen et al., 2006) while coffee consumption during pregnancy has been linked to a reduced risk of gestational diabetes (Adeney et al., 2007). Thus, gestational diabetes may serve as a negative confounder of the association between caffeine and cryptorchidism. Thus, in the presence of a true association, adjusting for the influence of gestational diabetes may potentially strengthen the observed association.

Given the limitations considered, the study results do not provide convincing evidence for an association between early pregnancy caffeine use and persistent cryptorchidism.

Slickers, J.E., Olshan, A.F., Siega-Riz, A.M., Honein, M.A., Aylsworth, A.S., for the National Birth Defects Prevention Study, 2008. Maternal body mass index and lifestyle exposures and the risk of bilateral renal agenesis or hypoplasia: The National Birth Defects Prevention Study. *Am. J. Epidemiol.* 168, 1259–1267.

Also originating from the National Birth Defects Prevention Study (NBDPS), this multicenter case-control study examined caffeine intake and other maternal characteristics as risk factors for bilateral renal agenesis or renal hypoplasia. The study consisted of 75 cases and 868 controls. Utilizing data on typical daily caffeine intake reported for the year preceding pregnancy, the study shares many of the main strengths and limitations of Browne et al. (2007) and other caffeine studies derived from the NBDPS. However, the authors incorporated additional data on reported changes in coffee, tea or soda consumption during pregnancy (more, same, less or no intake compared to the year before pregnancy) in an effort to more accurately classify "negligible" vs. "nonnegligible" intake during pregnancy. Intake was classified as negligible when there was no report of coffee, tea or soda consumption during pregnancy, mean daily caffeine intake was <10 mg in the year before pregnancy and no intake was reported during pregnancy, or mean daily caffeine intake was <50 mg in the year before pregnancy accompanied by decreased consumption of one or more caffeine sources and no increases in the other caffeine sources during pregnancy. In this report, contributions from chocolate or caffeine-containing medications were not included. No associations with nonnegligible

caffeine intake were observed (adjusted OR:1.01 (95% CI 0.58–1.75). The authors expressed concern that exposure misclassification due to reliance on caffeine exposure recalled for the year before pregnancy may have obscured modest associations.

Miller, E.A., Manning, S.E., Rasmussen, S.A., Reefhuis, J., Honein, M.A., and the National Birth Defects Prevention Study, 2009. Maternal exposure to tobacco smoke, alcohol and caffeine, and risk of anorectal atresia: National Birth Defects Prevention Study 1997–2003. *Paediat. Perinat. Epidemiol.* 23, 9–17.

Using data from the National Birth Defects Prevention Study, this case-control study analyzed 464 infants with anorectal atresia and 4940 controls with no major birth defects. Mothers were interviewed by telephone 6–24 months after the estimated date of delivery (mean 228 days). Caffeine consumption was measured as total caffeine derived from usual intake of beverages and chocolate reported for the year before pregnancy.

Modest borderline significant associations were observed for all categories of caffeine intake [OR:1.4 (1.0–1.9) for 10–99 mg; OR:1.3 (1.0–1.8) for 100–299 mg; OR:1.5 (1.0–2.2) for ≥ 300 mg compared to <10 mg]. It is notable that the point estimates were strengthened (but less stable) when caffeine exposure was refined according to reported changes in caffeine intake during pregnancy (i.e., restricted to those in reference group reporting same or less caffeine during pregnancy and those in the exposed groups reporting same consumption or more during pregnancy) [OR:2.6 (1.2–5.6) for 10–99 mg; OR:2.1 (1.0–4.4) for 100–299 mg; OR:2.6 (1.2–6.0) for ≥ 300 mg]. All findings are reported as unadjusted because none of the factors evaluated met the criterion for confounding (i.e., 10% change in caffeine estimate), including smoking. However, “any smoking” during the periconceptional period was found to be associated with anorectal atresia in these data, which raises the question of residual confounding by smoking due to measurement error or variable specification in the multivariate model. Recall bias and exposure misclassification resulting from the time gap for reporting or from poor representation of true periconceptional caffeine exposures are other considerations for the observed associations.

Johansen, A.M.W., Wilcox, A.J., Lie, R.T., Andersen, L.F., Drevon, C.A., 2009. Maternal consumption of coffee and caffeine-containing beverages and oral clefts: a population-based case-control study in Norway. *Am. J. Epidemiol.* 169, 1216–1222.

Cases of cleft lip with or without cleft palate ($n = 377$) and cleft palate only ($n = 196$) were identified from two surgical centers in Norway. Controls ($n = 763$) were randomly selected from the national birth registry. Mothers were interviewed 14–15 weeks after delivery for coffee, tea and soft drink consumption during the first three months of pregnancy. No associations were observed for total caffeine intake from all sources and risk of cleft lip with or without cleft palate [OR:1.2 (0.7–2.0) for ≥ 500 mg compared to 0–100 mg] or cleft palate only [OR:1.1 (0.5–2.2) for ≥ 500 mg compared to 0–100 mg]. Coffee intake, however, was weakly associated with cleft lip with or without cleft palate [OR:1.6 (1.1–2.4) for ≥ 3 cups/day], but not cleft palate only. In contrast, tea consumption appeared to protect against risk of both subtypes.

The study controlled for known or suspected confounders, including smoking and nausea. The primary limitation, however, was the possibility of recall bias. As noted for Bille et al. (2007), the lack of association with total caffeine intake and inconsistent results by type of beverage is not supportive of an etiologic role for caffeine in the development of cleft lip and/or palate.

Schmidt, R.J., Romitti, P.A., Burns, T.L., Browne, M.L., Druschel, C.M., Olney, R.S., and the National Birth Defects Prevention Study, 2009. Maternal caffeine consumption and risk of neural tube defects. *Birth Defects Res. A Clin. Mol. Teratol.* 85, 879–889.

Due to the large size of the National Birth Defects Prevention Study, this case-control study was able to separately assess associ-

ations with specific types of neural tube defects including spina bifida ($n = 459$), anencephaly ($n = 218$) and encephalocele ($n = 91$) as compared to 4143 non-malformed controls. Because total average daily caffeine intake from coffee, tea, soda and chocolate was assessed during the year prior to pregnancy, the use of caffeine-containing medications during the periconceptional period (one month before pregnancy through the first three months of pregnancy) was assessed separately. Caffeine exposure was evaluated as total mg/day and by source as cups per day. Modest associations with spina bifida were observed for any consumption of caffeine (≥ 10 mg/day; OR = 1.4, 95% CI 1.1–1.9), any caffeinated coffee (≥ 1 cup/month; OR = 1.3; 95% CI 1.0–1.6), and any caffeinated soda (>0 /day; OR = 1.2; 95% CI 1.0–1.6). When stratified by smoking, alcohol and maternal age, the associations between spina bifida and any caffeine intake were only observed among women without the high risk characteristics (i.e., among non-smokers, non-alcohol users, younger aged women). Any consumption of caffeinated tea was found to be protective for spina bifida (OR = 0.7; 95% CI 0.6–0.9). When examined across increasing categories of consumption, associations were limited to the groups with lower levels of consumption; thus, no evidence of a dose–response with increasing caffeine intake was observed. Similarly, associations with encephalocele were observed for coffee and tea, but only for categories of 1 cup/day of coffee and not greater levels of consumption. No associations with anencephaly were observed.

As with other NBDPS studies, the authors acknowledge the potential for recall bias (e.g., interviews conducted an average of 9.7 and 8.0 months after delivery for cases and controls), measurement error (e.g., exposure reported for year before pregnancy, and used only the implied serving size of “a cup”) and possible residual confounding (e.g., imprecise measurement of some covariates such as smoking (yes/no)). Although the lack of a dose–response detracts from the strength of evidence for a causal association, the authors suggest tolerance effects that develop among regular users may diminish dose effects.

Collier, S.A., Browne, M.L., Rasmussen, S.A., Honein, M.A., and the National Birth Defects Prevention Study, 2009. Maternal caffeine intake during pregnancy and orofacial clefts. *Birth Defects Res. A Clin. Mol. Teratol.* 85, 842–849.

The National Birth Defects Prevention Study was also the source for this case-control study of orofacial clefts. This study included 1531 infants with cleft lip with or without cleft palate, 813 infants with cleft palate only and 5711 controls with no major birth defects. Isolated and multiple malformations were also assessed separately. The NBDPS details concerning caffeine measurement and other study considerations have been described above. For total caffeine intake from coffee, tea, soda and chocolate, odds ratios were modestly elevated for limited amounts of caffeine intake (10–199 mg/day) relative to <10 mg/day for most outcomes (e.g., isolated cleft lip with or without cleft palate: OR = 1.2; 95% CI 1.0–1.5 for 100– <200 mg/day), but no associations were observed for higher levels of caffeine intake (200– <300 mg/day and 300+ mg/day). When intake was examined by source, three or more cups of coffee per day was protective against cleft palate only with multiple unrelated malformations (OR = 0.3; 95% CI 0.1–0.9) but an increased odds ratio was observed for similar tea intake (OR = 2.4; 95% CI 1.3–4.6). Medication containing 100+ mg of caffeine per dose was associated with isolated cleft lip with or without cleft palate (OR = 2.3; 95% CI 1.3–4.0) relative to no use of caffeine-containing medication. The authors interpret their findings as failing to provide support for an overall association between maternal caffeine intake and orofacial clefts, noting that effects with caffeine-containing medications should be further explored for the role played by other substances, confounding by indication and the possibility that users represent those with higher daily caffeine intake.

7. Fetal growth restriction

As a perinatal outcome of interest, fetal growth is considered a marker of healthy intrauterine development and a predictor of postnatal morbidity and mortality (Savitz et al., 2002). Most studies of caffeine and fetal growth have assessed intrauterine growth restriction (IUGR) (also referred to as SGA) defined as birth weight <10th centile for gestational age according to a standard growth curve from a selected reference population appropriate for the infant (by sex and race). Other measures of fetal growth explored in relation to caffeine use include birth weight, relative birth weight (Z-scores), low birth weight (<2500 g), high birth weight (>4000 g), birth length, ponderal index (birth weight (g)/birth length (cm)³ × 100), head circumference, abdominal circumference, placental weight, and placental diameter.

7.1. Defining growth restriction

The external standards of birth weight for gestational age used to define IUGR vary among studies. While it is agreed that a single standard is not appropriate for use in all populations and that the standard used should be population-specific, no population-specific standards have been adopted (Ott, 2006). Furthermore, the birth weight standards in use do not stratify the growth curves by the same population characteristics, so they may be inconsistently specified by any combination of sex, race and/or parity.

Because it is difficult to distinguish constitutionally small babies from babies who are genuinely growth restricted, births defined as IUGR using a population standard (such as the 10th centile) will capture heterogeneous outcomes. Thus, the inclusion of genetically small but otherwise normal babies as IUGR would likely attenuate associations of potential risk factors with fetal growth restriction. Alternative methods have been proposed for identifying infants who fail to obtain their inherent growth potential. These methods compare birth weight to a customized fetal growth curve, which predicts weight for gestational age according to maternal height and weight, sex, race/ethnicity and parity (Gardosi, 1997, 2006; Zhang et al., 2007). Customized growth curves have not been widely used, however, in studies of IUGR etiology.

7.2. Accurate estimation of gestational age

The issues addressed in Section 5.2 concerning accurate estimation of gestational age and preterm birth are also relevant to studies of IUGR. While ultrasound-based pregnancy dating is often preferred, this method is also derived from measurements of fetal growth (e.g., the bi-parietal diameter of the skull), which can be influenced by fetal growth restriction. Thus, IUGR infants may escape identification if this circular argument causes gestational age to be underestimated by the use of ultrasound measurements.

7.3. Relevant window of susceptibility

The timing of exposure assessment is important for studying the etiology of fetal growth restriction, but the exact window of susceptibility is unknown. Although fetal size was previously believed to be determined during the third trimester of pregnancy when weight gain is most rapid, recent evidence suggests that fetal growth restriction may be determined by conditions occurring early during the first trimester of pregnancy (Smith et al., 1998; Smith, 2004; Bukowski et al., 2007). This uncertainty underscores the importance of assessing caffeine exposure throughout the course of pregnancy when investigating possible associations with fetal growth.

7.4. Pregnancy signal

Although the role of the pregnancy signal is well acknowledged in studies of caffeine and spontaneous abortion, confounding by pregnancy symptoms has been considered less often in studies of IUGR. Yet, the pregnancy signal might be related to placental size. Fetal growth restriction is often associated with a small placenta (Naeye, 1987; Redline and Patterson, 1994; Thame et al., 2004). Still unknown is whether the process that limits fetal growth also limits placental growth, or whether a small placenta reduces fetal growth by synthesizing inadequate amounts of proteins that promote growth. Either way, the small placenta can be expected to synthesize less of the hormones needed for growth, some of which at high levels may produce the pregnancy signal (Lawson et al., 2002).

7.5. Review of individual studies of fetal growth restriction

Grosso, L.M., Rosenberg, K.D., Belanger, K., Saftlas, A.F., Leaderer, B., Bracken, M.B., 2001. Maternal caffeine intake and intrauterine growth retardation. *Epidemiology* 12, 447–455.

This analysis evaluated IUGR among 2714 women delivering singleton, live births within the Yale Health in Pregnancy Study (1988–1991). The investigators observed no association between IUGR and caffeine intake during the first or seventh month of pregnancy. Stratification by smoking status did not change the results.

Gestational age was estimated by administering the Ballard examination within the first day of life (Ballard et al., 1979), which may have contributed to nondifferential misclassification of IUGR. Although the study population was large, the study was limited by the low percentage of women consuming more than moderate amounts of caffeine (>300 mg/day) during the two time periods assessed (5% in month 1 and 2% in month 7). This limited the power to detect associations with caffeine use >300 mg/day. On the other hand, the authors acknowledge that recall bias was a possibility for month seven caffeine consumption, which was reported after delivery. In addition, pregnancy symptoms were not considered as a potential confounder.

The data presented in this study provide no evidence to support a link between caffeine use in the first or seventh month of pregnancy and fetal growth restriction.

Clausson, B., Granath, F., Ekblom, A., Lundgren, S., Nordmark, A., Signorello, L.B., Cnattingius, S., 2002. Effect of caffeine exposure during pregnancy on birth weight and gestational age. *Am. J. Epidemiol.* 155, 429–436. [Discussed also in 5. Gestational Age and Preterm Birth]

In this study, 873 controls from a case-control study of spontaneous abortion (Cnattingius et al., 2000) were followed to investigate the effects of first and third trimester caffeine consumption on pregnancy outcomes, including birth weight, Z-scores and gestational age. Weekly caffeine intake through the first 6 weeks of pregnancy was obtained from the first trimester interview (conducted between weeks 6–12), while biweekly intake from 7 weeks of gestation onward was obtained from the third trimester interview (conducted between weeks 32–34). The long recall period associated with the retrospective reports of consumption occurring up to 7 months prior may have produced nondifferential misclassification. Furthermore, averaging intake across large spans of time such as trimesters or throughout pregnancy may misrepresent peak exposures during the relevant window of susceptibility.

The authors reported no differences in adjusted mean birth weight or Z-scores across caffeine intake categories defined as 0–99, 100–299, 300–499 or ≥500 mg/day during the first, second or third trimesters or for average consumption throughout pregnancy. The authors controlled for confounding by third trimester smoking (yes/no using cotinine >15 ng/ml) and pregnancy symptoms, but this had little impact on the results. Furthermore, no

effect modification by smoking or pregnancy symptoms was observed.

Klebanoff, M.A., Levine, R.J., Clemens, J.D., Wilkins, D.G., 2002. Maternal serum caffeine metabolites and small-for-gestational age birth. *Am. J. Epidemiol.* 155, 32–37. [Discussed also in 5. Gestational Age and Preterm Birth]

This study evaluated third trimester serum paraxanthine concentrations in archived samples from Collaborative Perinatal Project participants. Caffeine metabolites might measure biologic dose better than reported caffeine consumption, but reflect only recent exposures (Aldridge et al., 1981). Because the serum samples were stored for more than 30 years before being analyzed, the stability of the caffeine metabolite in the available specimens is also questioned.

The authors reported that risk of delivering an SGA infant increased with rising third trimester serum paraxanthine concentrations, but only among smokers. The increased risk among smokers was modest (displayed graphically as ORs \approx 2.0 and lower) and only present for categories of paraxanthine concentrations exceeding the 65th percentile (>715 ng/ml). These effects were observed after controlling for self-reported number of cigarettes smoked per day within the stratum of smokers. No associations with serum caffeine concentrations were observed. The authors acknowledge that residual confounding due to under-reporting of number of cigarettes smoked could have exaggerated observed associations for paraxanthine. Furthermore, pregnancy symptoms were not evaluated. Because the modest associations observed in this study could be influenced in an unpredictable manner by sample desiccation and confounding, cautious interpretation is advised.

Balat, O., Balat, A., Ugur, M.G., Pençe, S., 2003. The effect of smoking and caffeine on the fetus and placenta in pregnancy. *Clin. Exp. Obstet. Gynecol.* 30, 57–59.

To evaluate the effect of caffeine intake on newborn and placental characteristics, the authors recruited a group of women who smoked <10 cigarettes per day ($n = 60$) and a group of non-smokers ($n = 63$) who delivered at term (37–41 weeks). The reference period for the reported caffeine intake from coffee and tea was not specified. The authors reported lower mean birth weights and placental weights for women reporting caffeine intake of >300 mg/day compared to women consuming <300 mg/day. Mean differences by caffeine intake were greater for smokers (187 g difference in birth weight and 97 g difference in placental weight) than non-smokers (128 g difference in birth weight and 81 g difference in placental weight). No differences in mean length, head circumference or placental diameter were observed in either group.

The mean differences reported in this study were based on small numbers (e.g., $n = 17$ smokers consuming >300 mg/day; $n = 19$ non-smokers consuming >300 mg/day), resulting in unstable point estimates. Other than stratification by smoking status, this study did not attempt to control for important confounders such as maternal age, alcohol use, gestational age at birth or amount smoked per day. Exposure misclassification is also likely given use of a single measurement of caffeine intake, which did not capture sources other than tea and coffee or specify cup size. Thus, the results do not offer convincing support for an effect of caffeine on fetal growth or placental development.

Bracken, M.B., Triche, E.W., Belanger, K., Hellenbrand, K., Leaderer, B.P., 2003. Association of maternal caffeine consumption with decrements in fetal growth. *Am. J. Epidemiol.* 157, 456–466. (Discussed also in 5. Gestational Age and Preterm Birth)

This prospective cohort study of 2291 pregnant women ≤ 24 gestational weeks evaluated urinary caffeine concentrations and self-reported caffeine exposures during early and late pregnancy in relation to IUGR, low birth weight and preterm birth. A major strength of this study is that it was designed to assess pregnancy outcomes in relation to caffeine consumption and, thus, incorpo-

rated a very detailed assessment of caffeine exposure from beverage sources.

First and third trimester caffeine consumption was not associated with increased risk of IUGR or low birth weight. Increased concentrations of urinary caffeine (mg/g creatinine) were also not associated with IUGR [OR:1.0 (0.8–1.1)] but appeared to be protective for low birth weight [OR:0.7 (0.5–1.0)]. When birth weight and caffeine were assessed as continuous variables, each 100 mg of caffeine consumed during the first trimester was associated with a birth weight reduction of 28 g (95% CI 10–46 g). No such associations were observed with low birth weight [OR:1.00 (0.98–1.00)]. The authors conclude that moderate caffeine consumption during the first trimester may have a modest effect on birth weight that may not be clinically important. No associations with third trimester caffeine consumption were reported. Ultimately, the authors' reasoned that urinary caffeine may not be a useful biomarker since less than 2.0% of caffeine is excreted in this form.

This study provides no evidence to suggest that caffeine consumption during early or late pregnancy is related to growth restriction as measured by IUGR and low birth weight. Only modest reductions in birth weight were observed with increasing caffeine use.

Ørskou, J., Henriksen, T.B., Kesmodel, U., Secher, N.J., 2003. Maternal characteristics and lifestyle factors and the risk of delivering high birth weight infants. *Obstet. Gynecol.* 102, 115–120.

This study of risk factors for high birth weight (>4000 g) evaluated a large cohort of women without diabetes who gave birth in Denmark (1990–1999). Women seeking prenatal care ($n = 24,093$) completed a questionnaire at approximately 16 weeks of gestation reporting average daily consumption of caffeinated beverages. The reference period for the self-reported caffeine intake was not described.

Consuming more than 200 mg/day was associated with a decreased odds of giving birth to a high birth weight infant compared to consuming <200 mg/day [OR:0.9 (0.8–1.0) for 200–399 mg/day and OR:0.9 (0.8–1.0) for ≥ 400 mg/day].

The main strengths of this study were the large sample size and control for number of cigarettes smoked per day (e.g., non-smoker, 1–4, 5–9, 10–14, 15 + cigarettes/day), alcohol intake, gestational age, and other confounders. Misclassification of caffeine, however, was possible given the single assessment at 16 weeks of gestation would not capture changing patterns of consumption during pregnancy. The modest protective odds ratio may also overestimate the relative risk given the outcome was relatively common (19%) in this study population.

Vik, T., Bakketeig, L.S., Trygg, K.U., Lund-Larsen, K., Jacobsen, G., 2003. High caffeine consumption in the third trimester of pregnancy: gender-specific effects on fetal growth. *Paediatr. Perinat. Epidemiol.* 17, 324–331.

This study population included subjects ($n = 858$) from the Norwegian site of a multi-site Scandinavian study of SGA. This study is one of few to prospectively collect caffeine exposure data through the use of food diaries. Diaries were completed across 3 weekdays in the second (17–20 weeks) and third trimesters (33 weeks). While likely to capture current intake during the two specified time points with fewer recall errors, food diaries would not necessarily reveal intake patterns that may fluctuate from week to week, month to month, or even weekday to weekend. For analyses, caffeine was dichotomized to represent intake above and below the mean value (232 mg/day at 17 weeks; 205 mg/day at 33 weeks).

No association was observed between “high” caffeine intake at 17 weeks and birth of an SGA infant [OR:1.1 (0.6–2.1)], but high consumption at 33 weeks was associated with an increased odds of SGA [OR:1.6 (1.0–2.5)]. The association for third trimester caffeine consumption was modified by infant sex [OR:2.8 (1.4–5.5)]

for males; OR:1.0 (0.5–2.0) for females]. These estimates were adjusted for smoking at conception (yes/no), pregnancy weight, education and previous SGA birth, but not for maternal age. Although the findings were consistent among smokers and non-smokers, residual confounding by amount smoked could remain if reported caffeine consumption reflected actual frequency of smoking (among smokers and inaccurately reported non-smokers alike) (Morrison, 1984).

The sex-specific effects of caffeine on SGA are difficult to explain. Fetal sex might be a marker of more severe pregnancy symptoms. For example, having a female fetus has been strongly associated with hyperemesis gravidarum (Tan et al., 2006). We do not know of any sex differences for less severe nausea, but if similar patterns exist, women with male fetuses may have had less nausea, and therefore consumed more coffee. The possibility of sex-specific effects of caffeine on SGA is intriguing, but consideration of all potential confounders including nausea and aversions would be necessary before a causal link could be legitimately considered.

Parazzini, F., Chiaffarino, F., Chatenoud, L., Tozzi, L., Cipriani, S., Chiantera, V., Fedele, L., 2005. Maternal coffee drinking in pregnancy and risk of small for gestational age birth. *Eur. J. Clin. Nutr.* 59, 299–301.

This case-control study of term singletons born in Italy selected 555 SGA babies and 1966 controls. The methods for estimating gestational age for the SGA definition were not described. Information on coffee, tea, and cola was collected after delivery, measured as number of cups per day before pregnancy and during each trimester. Total caffeine intake from all beverage sources was not assessed.

The authors observed no associations between SGA and intake of three or more cups of coffee during the first, second or third trimester of pregnancy. Likewise, no associations were observed for heavy coffee consumption (≥ 4 cups/day) before pregnancy [OR:1.3 (0.9–1.9)]. Because the control inclusion criterion for full term deliveries was not applied equally to the selection of cases, the authors repeated their analyses after restricting cases to births delivered after 37 weeks of gestation. This reduced the odds ratios for the heaviest coffee consumption categories to exactly 1.0 for all time periods assessed (i.e., before pregnancy and during each trimester). When tea, cola and decaffeinated coffee were each evaluated separately, no associations were observed.

Santos, I.S., Matijasevich, A., Valle, N.C.J., 2005. Maté drinking during pregnancy and risk of preterm and small for gestational age birth. *J. Nutr.* 135, 1120–1123. [Discussed also in 5. Gestational Age and Preterm Birth]

In addition to evaluating preterm birth, this retrospective cohort study assessed SGA births in relation to exposure to a popular caffeinated beverage consumed in South America called maté. All women ($n = 5189$) giving birth to singletons in the five local maternity hospitals were interviewed within the 24 h following delivery. Frequency of maté use during pregnancy was assessed as number of days the drink was consumed per week (0, 1–6 and 7 days per week). The authors equated daily maté consumption with a daily average caffeine intake of 300 mg (Santos et al., 1998). Gestational age was estimated using the Dubowitz score.

After controlling for maternal age, education, parity, cigarette smoking during the third trimester, previous abortion and previous low birth weight infant, the prevalence of SGA births did not differ among women consuming maté 0, 1–6 or 7 days per week. The primary limitation of this study was the non-specific measure of caffeinated beverage consumption in days per week rather than servings per day. Other sources of caffeine intake were not considered. Recall bias was also possible given women were interviewed after delivery. Confounding by alcohol and nausea was not considered in the analyses. Because of its limitations, this study should be

viewed as weak evidence that caffeine does not influence the risk of fetal growth restriction.

Grosso, L.M., Triche, E.W., Belanger, K., Benowitz, N.L., Holford, T.R., Bracken, M.B., 2006. Caffeine metabolites in umbilical cord blood, cytochrome P-450 1A2 activity, and intrauterine growth restriction. *Am. J. Epidemiol.* 163, 1035–1041.

This study was conducted within the prospective cohort described by Bracken et al. (2003) who were selected on the basis of caffeine consumption \geq or <150 mg/day. Of the 2291 participants, 1606 had cord blood samples available for analysis of serum caffeine, paraxanthine, theophylline and theobromine concentrations.

No associations were observed for the highest quartile of serum caffeine concentrations [OR:0.5 (0.1–2.0)]. Caffeine metabolites were not associated with IUGR when evaluated independently, but infants born to women with the highest concentrations of paraxanthine (fourth quartile) had a 3-fold increased risk of IUGR [OR:3.3 (1.2–9.2)], but only when also controlling for serum caffeine concentrations. This led the authors to suspect that metabolic activity rather than absolute concentrations of caffeine and its metabolites may have more relevance for fetal growth, so the remaining analyses assessed the ratio of paraxanthine to caffeine as a marker of CYP1A2 enzymatic activity. The adjusted odds ratio for a one standard deviation increase in the ratio of paraxanthine to caffeine was 1.2 (1.1–1.4). Thus, fast metabolizers experienced a higher risk of IUGR than slow metabolizers.

The major strength of this study was the use of biomarkers in cord blood measurements as indicators of biologic dose entering the fetal circulation. Given the short half-life of caffeine and its metabolites, concentrations observed at delivery may not accurately characterize exposure throughout pregnancy unless caffeine consumption habits were fairly consistent over time.

Only 55% of the population had gestational age confirmed by ultrasound. As with all studies of IUGR, errors in gestational age based on inaccurate recall of the date of the last menstrual period can lead to misclassification of IUGR. Residual confounding by smoking could have overestimated the association between caffeine metabolism and IUGR. Urinary cotinine levels (before 25 weeks of gestation) were available for the cohort and reported in a previous paper by Bracken et al. (2003), but only self-reported smoking (yes/no) during the third trimester was utilized in these analyses. This choice likely reflects the preference for third trimester smoking measurements more proximate to the timing of the cord blood measurements.

As the authors acknowledge, CYP1A2 activity may be a marker for other compounds such as tobacco smoke that induce metabolic activity and alter fetal growth (Campbell et al., 1987b). The observed effect of metabolic activity, however, was similar in self-reported smokers and non-smokers. Confounding by nausea was not evaluated, but it is unknown if pregnancy symptoms would be related to metabolic activity.

These findings are suggestive of a possible modest relationship between high metabolic activity, as measured by the ratio of paraxanthine to caffeine concentrations in cord serum, and risk of IUGR. Better control for smoking would have enhanced confidence in the results.

Tsubouchi, H., Shimoya, K., Hayashi, S., Toda, M., Morimoto, K., Murata, Y., 2006. Effect of coffee intake on blood flow and maternal stress during the third trimester of pregnancy. *Int. J. Gynaecol. Obstet.* 92, 19–22.

This study did not directly assess the effects of caffeine on fetal growth, but measured the effects of a one-cup dose of coffee on maternal and fetal blood flow during the third trimester. A group of 10 non-smoking women in their third trimester of pregnancy underwent Doppler examinations before and after coffee consumption (100 mg caffeine). All participants typically consumed 1–3 cups of coffee per day. Resistance index values were calculated

to measure blood flow in the maternal uterine artery, umbilical artery and fetal middle cerebral artery 30 min after coffee intake. Coffee had no effect on maternal or fetal blood flow. When the effects of coffee on stress levels were assessed by measuring salivary cortisol and chromogranin A (CgA), coffee intake reduced salivary cortisol levels in the 10 pregnant women but not in a comparison group of 14 women who were not pregnant. CgA concentrations, which were considered an indication of physiological stress, increased following coffee consumption in non-pregnant women, but significant increases were not detectable in pregnant women. The small study size and limited statistical power restricts confidence in the absence of associations.

Bech, B.H., Obel, C., Henriksen, T.B., Olsen, J., 2007. Effect of reducing caffeine intake on birth weight and length of gestation: randomised controlled trial. *BMJ* 334, 409–414. [Discussed also in 5. Gestational Age and Preterm Birth]

Women who regularly drank at least three cups of coffee per day ($n = 1197$) were randomly assigned to caffeinated or decaffeinated instant coffee during the last half of pregnancy. The pregnancies were followed to evaluate differences in gestational age and mean birth weight. Although participants were provided unlimited amounts of coffee, either caffeinated or decaffeinated as assigned, they were also free to consume other sources of coffee and caffeinated beverages. According to self-reports, more than one-third of those assigned to decaffeinated coffee consumed ≥ 1 cup of caffeinated coffee on a daily basis.

Using the intent to treat approach to analysis, no difference in birth weight was observed when comparing women assigned to caffeinated vs. decaffeinated coffee. This approach, however, would misclassify true exposure status given poor compliance with assigned coffee treatment. When analyses were restricted to subgroups defined as women reporting no consumption of other caffeinated coffee ($n = 283$) or women reporting <1 cup of other caffeinated coffee per day ($n = 266$), the results were unchanged. Birth weight remained similar across caffeine assignment groups within non-smokers and within women smoking 1–10 cigarettes per day. However, among women smoking ≥ 10 cigarettes per day, those randomized to caffeinated coffee had a lower mean birth weight than the decaffeinated group (mean difference: 263 g (97–430 g), adjusted for parity, pre-pregnancy BMI and length of gestation. The possible interaction between smoking and caffeine consumption is unconvincing without presentation of the results by compliance or, preferably, by actual caffeine consumption.

Diego, M., Field, T., Hernandez-Reif, M., Vera, Y., Gil, K., Gonzalez-Garcia, A., 2007. Caffeine use affects pregnancy outcome. *J. Child Adolesc. Subst. Abuse* 17, 41–49.

In this study, 750 pregnant women between 20 and 28 weeks gestation were recruited from a prenatal clinic to study depression, anxiety and substance use. Birth outcomes including birth weight and “pregnancy complications” were collected from medical charts on a sub-sample of 452 participants. Pregnancy complications were not defined. Caffeine consumption was collected from interviews conducted at enrollment and described as caffeinated drinks per day during pregnancy, but the data collection procedures were not described. It would appear, however, that all caffeinated beverages were weighted equally. The range of exposure was reported as 0–6 caffeinated drinks per day, with 38% reporting no caffeine consumption. Weak negative correlations were observed between number of caffeinated drinks and birth weight ($r = -0.11$, $p < 0.05$). In hierarchical linear regression analyses restricted to women who reported no smoking or alcohol use, caffeine intake explained 8% of the variability in birth weight when controlling for anxiety and depression. In light of the study limitations – which include questionable exposure assessment and lack of control for other important confounders such as maternal age and body mass – this study provides limited information.

Infante-Rivard, C., 2007. Caffeine intake and small-for-gestational-age birth: modifying effects of xenobiotic-metabolising genes and smoking. *Paediatr. Perinat. Epidemiol.* 21, 300–309.

This hospital-based, case-control study of SGA births identified 451 cases matched to an equal number of controls by gestational age, sex and race. Interviews were generally conducted within two days of delivery. CYP1A2 and CYP2E1 polymorphisms were genotyped using peripheral blood samples from mothers and newborns. Genotypes were dichotomized as those with one or two copies of the variant allele vs. those with the wild type.

The authors observed no association between caffeine consumption and SGA when using either continuous or dichotomous (<300 vs. ≥ 300 mg/day) measures of caffeine intake during the month before pregnancy or during the first, second, or third trimester of pregnancy. Adjustment for smoking (none vs. any) and nausea had little impact on the observed lack of associations, although stratification by smoking status revealed a 22% increased odds of SGA among non-smokers for every 100 mg/day consumed during the first trimester [OR:1.2 (1.0–1.5)]. The effect of first trimester caffeine consumption on SGA was not modified by maternal or newborn genetic polymorphisms. Birth weight was reduced by -31 (95% CI $-61, -1$) and -38 g (95% CI $-68, -8$) for every 100 mg of caffeine consumed during the second and third trimester, respectively. The effects of caffeine on birth weight were also restricted to non-smokers.

Although the study improved upon most previous studies by including measurements of nausea by trimester (presence vs. absence), residual confounding would persist if severity or aversions were the influential factors. The study does not provide evidence of effect modification of caffeine use on SGA by CYP1A2 or CYP2E1 polymorphisms in mother or child. Because the modest effects on SGA and birth weight among non-smokers could potentially be due to recall bias or inaccurately reported smoking status, the strength of the evidence for an effect of caffeine on fetal growth restriction is limited.

Xue, F., Willett, W.C., Rosner, B.A., Forman, M.R., Michels, K.B., 2008. Parental characteristics as predictors of birthweight. *Hum. Reprod.* 23, 168–177.

This cross-sectional study was conducted among the Nurses' Mother's Cohort. Mothers of the Nurses' Health Study participants ($n = 34,063$) were sent questionnaires to collect information on daughter's birth weight as well as behaviors and conditions during the pregnancy. Mothers were 60–80 years old when completing the questionnaire about events that occurred approximately 40–60 years earlier. Thus, the long recall period is the major limitation of this study. Coffee consumption was measured as intake “during pregnancy” in cups/day. Daughter's birth weight was also self-reported, although a subset were validated against birth certificates ($r = 0.85$).

Birth weight was negatively associated with coffee consumption during pregnancy, decreasing by 15, 34 and 54 g for consumption of 1–2, 3–4 and ≥ 5 cups of coffee per day. The odds of IUGR were modestly increased in a dose-response fashion with increasing coffee consumption, with the strongest association observed among women reporting ≥ 5 cups of coffee per day [OR:1.6 (1.3–2.1)]. Although many important confounders were considered, pregnancy symptoms were not accounted for. The weak associations observed in this study could potentially be attributed to measurement error and confounding.

CARE Study Group, 2008. Maternal caffeine intake during pregnancy and risk of fetal growth restriction: a large prospective observational study. *BMJ* 337, a2332.

This prospective study from the United Kingdom evaluated a cohort of 2635 women recruited early in pregnancy (8–12 weeks of gestation). A major strength of the study was its thorough assessment of caffeine exposure. Three questionnaires were

administered to capture recall of caffeine consumption for each trimester (5–12, 13–28, and 29–40 weeks of pregnancy). The questionnaire considered all beverage, food, and over-the-counter medication sources, brands, portion sizes, and methods of preparation. Caffeine half-life was measured in saliva samples as an indicator of fast or slow caffeine clearance, collected one and five hours following a 63.5 mg caffeine challenge (500 ml diet soda over 20 min) after fasting.

This study also strived to improve upon IUGR classification by applying customized fetal growth curves (i.e., identifying birth weight <10th percentile for gestational age according to maternal height, weight, ethnicity, parity and sex) in an effort to avoid misclassifying constitutionally small infants as growth restricted. Furthermore, gestational age was confirmed by ultrasound in all pregnancies. Another positive feature was the use of salivary cotinine concentrations to identify current smokers, non-smokers and those exposed to second-hand smoke. However, these measurements were taken during the first trimester recruitment visit and may not accurately reflect tobacco exposure throughout pregnancy. Caffeine consumption averaged over the entire pregnancy was modestly associated with IUGR [OR:1.2 (0.9–1.6) for 100–199 mg/day; OR:1.5 (1.1–2.1) for 200–299 mg/day; OR:1.4 (1.0–2.0) for ≥ 300 mg/day compared to ≤ 100 mg/day]. Similar associations were observed for caffeine exposure in each trimester, with slightly larger odds ratios for the 2nd and 3rd trimester. Associations between consumption >200 mg/day and reduced birth weight in the range of 60–70 g were also observed across all time periods. When the data were stratified by caffeine clearance, the association with IUGR appeared to remain only among the fast metabolizers; although, the confidence intervals in each strata largely overlapped with the exception of the 200–299 mg/day category (test for interaction $p = 0.06$).

The major limitation of this study is the potential for confounding by pregnancy symptoms and aversions. The authors mention an assessment of the effects of adjusting for nausea, but these results were not presented and no description of the nature of the nausea data was provided. A related concern is the extremely low participation rate (20%). The authors dismiss the likelihood of selection bias, but the higher prevalence of IUGR in the study population (13%) compared to the general population (10%) suggests that women with a history of fetal growth restriction may have been more motivated to participate in the study. If pregnancies destined to be growth restricted produced a weaker pregnancy signal, selection into the study would be related to both the outcome (IUGR) and the exposure (higher caffeine intake due to fewer symptoms and aversions). Thus, the more highly exposed, growth restricted group would be over-represented. Because pregnancy symptoms and aversions were not controlled in these analyses, selection bias remains a plausible explanation for the modest associations observed in this study.

The authors of the study considered that, for the first time among similar studies, the quantification of caffeine from all known sources reflected “a true picture of total caffeine intake by women during pregnancy”. However, when the validation study compared the caffeine questionnaire to a 3-day food diary and repeated salivary caffeine and paraxanthine concentrations, only fair to moderate levels of agreement (intraclass correlation for food diary = 0.5; Kappa for biomarkers 0.33–0.65) were observed (Boylan et al., 2008). Although considerable steps toward improving the assessment of caffeine exposure were taken, the data collection instrument does not succeed in eliminating all concerns about caffeine measurement error. Potential for recall bias specific to 2nd and 3rd trimester exposure reports may also exist, as women became aware of restricted fetal growth during routine mid- or late pregnancy ultrasounds. This could explain the slightly stronger

associations observed for caffeine consumption during 13–28 and 29–40 weeks of gestation.

8. Discussion/conclusions

8.1. Subfecundity

Of the nine publications since 2002, one evaluated multiple outcomes associated with fertility treatment, one considered self-reported ovulatory infertility, three addressed time to conception, and most (4) assessed the relationship between caffeine and semen parameters.

The only study to assess the effect of caffeine on endpoints of assisted reproductive technology reported no influence of previous or current caffeine intake on oocyte retrieval, fertilization, embryo transfer or the occurrence of a clinical pregnancy. The effect of limited caffeine use on failure to achieve a live birth was inconsistent for exposures reported for different time periods that reflected usual and recent exposures. Caffeine use around the week of IVF or GIFT procedures was not associated with failure to achieve a live birth, while associations with usual lifetime use and use reported during first clinic visit were observed. Replication of results in larger populations undergoing ART is needed to address concerns about statistical power, precision, residual confounding and caffeine exposures >50 mg/day.

Exposure measurement errors are a primary concern for the few recent studies addressing time to conception and ovulatory infertility. Potential recall bias and exposure misclassification may explain the modest association reported for coffee and tea consumption and increased time to pregnancy. No support for an association with infertility due to ovulation disorders was provided, but exposure measurement error was likely introduced as a result of the timing of exposure assessments.

Evaluations of semen quality have consistently failed to observe adverse effects associated with caffeine intake. Studies of DNA damage, however, have been more limited but inconsistent. Most of the studies of male reproductive outcomes have suffered from lack of detailed reporting of caffeine exposure assessment, potential exposure misclassification for the relevant etiologic window, no or limited control for confounders, potential selection bias, or restriction to fertile men, which limited the ability to detect caffeine-related abnormalities.

In summary, consistent relationships between caffeine intake and measures of subfecundity have not been observed.

8.2. Spontaneous abortion

The current evidence remains insufficient to permit conclusions regarding the potential role of caffeine in spontaneous abortion. Studies of caffeine and spontaneous abortion are complicated by the challenge of separating cause and effect.

Studies have not successfully addressed the complex interrelationship between viability, pregnancy signal symptoms and caffeine consumption patterns. Of the 15 studies that have evaluated caffeine and spontaneous abortion since the Leviton and Cowan (2002) review, 10 did not attempt to control for pregnancy signal symptoms. Although positive associations were consistently reported by these 10 studies, these consistent findings may very well be attributed to bias that persists across all studies. As a marker of pregnancy viability and a correlate of exposure, confounding by pregnancy signal symptoms may explain observed associations with caffeine use. Women with pregnancies that go to term experience more frequent and severe nausea early in pregnancy compared to women whose pregnancies end in spontaneous abortion (Weigel and Weigel, 1989). Lawson et al. (2002, 2004)

demonstrated that weekly duration of nausea (in hours) and appetite loss (in days) is positively related to human chorionic gonadotropin (hCG) levels during pregnancy, whereas hCG levels are also negatively related to coffee consumption. Of those women who decrease coffee consumption during the first trimester of pregnancy, 65% report a physical aversion to coffee (Lawson et al., 2004). Thus, women experiencing viable pregnancies tend to experience a stronger pregnancy signal that ultimately drives caffeine intake downward. The pregnancy signal is, therefore, a crucial confounder of the association between caffeine and pregnancy viability.

Results from studies that have attempted to control for nausea and vomiting during pregnancy have been less consistent. Improved assessments of relevant symptom characteristics, such as aversions to taste and smell, in addition to symptom severity, duration, frequency and timing are needed to improve study validity. The study by Wen et al. (2001) provides perhaps the best evidence for the pregnancy signal phenomenon to date, whereby increased risk of spontaneous abortion was only observed for caffeine consumed after nausea onset, but not for caffeine consumed before nausea onset or among those without nausea. Other persistent problems with the validity of studies of caffeine and spontaneous abortion include confounding by smoking and potential recall bias.

8.3. Fetal death

Three of the four studies evaluating caffeine and fetal death reported moderately positive associations of similar magnitude. Three of the four were conducted by members of the same research group using similar methodologies and similar study populations. None, however, sufficiently address concerns regarding confounding by pregnancy symptoms. Only one study attempted to control for pregnancy symptoms, but the limited assessment of the pregnancy signal defined as presence or absence of nausea or vomiting during the first trimester was insufficient to avoid residual confounding. However, Bech et al. (2005) provided a useful demonstration of how observed associations can be inflated by undetected fetal demise. As with studies of spontaneous abortion, the interpretation of this body of work, which has consistently reported modest associations across studies, needs to consider that these studies may also share common sources of bias which may explain the observed relationship with caffeine use.

8.4. Preterm birth

Larger studies considering total caffeine exposure consistently reported no increased risk of delivery before 37 weeks of gestation. No studies since 2000, however, distinguished spontaneous from medically indicated preterm deliveries. Two studies of Mediterranean-type diets (Mikkelsen et al., 2008; Haugen et al., 2008) separately evaluated early and late preterm births, with inconsistent results between studies. Coffee intake limited to ≤ 2 cups/day was linked to a lower odds of preterm delivery ≤ 34 weeks of gestation, but not late preterm delivery (35–36 weeks) in the Danish National Birth Cohort (Mikkelsen et al., 2008). A similar study conducted within the Norwegian Mother and Child Cohort observed no relationship between coffee intake and early or later preterm delivery (Haugen et al., 2008). Combining etiologically distinct outcomes could obscure associations within clinical subtypes of preterm birth.

8.5. Congenital malformations

With a few exceptions, recent studies have not reported an increased risk of malformations with greater caffeine consumption. However, the body of evidence for any single malformation or sub-

group is limited. The reports of modest associations between coffee intake and cryptorchidism and total caffeine intake and anorectal atresia could not confidently rule out potential sources of bias such as selection bias due to missing data, exposure misclassification and confounding.

8.6. Fetal growth

Studies of caffeine and fetal growth restriction are equivocal, with approximately half of the studies in this review reporting weak associations with intrauterine growth restriction or reduced birth weight and half observing no effects. The strength of the evidence for a potential effect of caffeine on fetal growth restriction is diminished by the inability to rule out alternative, credible explanations for the observed associations, namely confounding by pregnancy symptoms and aversions.

8.7. Summary

In conclusion, the weight of evidence does not support a positive relationship between caffeine consumption and adverse reproductive or perinatal outcomes. On the whole, associations with subfecundity, preterm delivery and congenital malformations are not routinely observed. Studies of pregnancy loss and fetal growth have generated more interest due to the frequency with which adverse effects are reported in connection with caffeine use. Our review identifies significant methodological weaknesses common to studies of spontaneous abortion, fetal death and fetal growth restriction, which limit confidence in causal interpretation. Consistent with the conclusion of the previous review (Leviton and Cowan, 2002), the studies available from January 2000 through December 2009 do not provide convincing evidence that caffeine consumption increases risk of any reproductive adversity. Future studies addressing the methodological limitations of current research may alter this conclusion. In particular, quantitative methods are available for adjustment for measurement error in the absence of a gold standard (Joseph et al. 1995) and would be especially useful for estimating the impact of errors in the assessment of caffeine exposure.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Review

A review of the literature relating caffeine consumption by women to their risk of reproductive hazards

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Abstract

From this detailed review of the literature, several conclusions can be drawn: (a) An association between caffeine consumption and a reproductive hazard is more likely to be seen in lower-quality studies than in studies that come closer to approximating the ideal. This is especially evident for “lower” birthweight and congenital anomalies. (b) The association between caffeine consumption and spontaneous abortion may well reflect the Stein–Susser epiphenomenon (women with prominent nausea tend to reduce caffeine consumption and nausea appears to be a marker of good implantation, perhaps reflecting a favorable balance of hormones produced by a healthy placenta). (c) The claim that caffeine consumption by women delays conception has not been followed by convincing support. (d) Reproductive hazards associated with cigarette smoking tend to be associated with caffeine/coffee consumption. Sometimes this appears to be a consequence of residual confounding associated with inadequate adjustment for cigarette smoking, which is over-represented among those who drink the most coffee/caffeine. Sometimes this reflects the tendency of women to underreport socially undesirable behaviors (e.g. smoking) while accurately reporting socially neutral behaviors (e.g. coffee and caffeine consumption). Thus, it seems reasonable to conclude that no convincing evidence has been presented to show that caffeine consumption increases the risk of any reproductive adversity. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Caffeine; Coffee; Birth defects (or congenital malformations); Low birthweight (or small for dates); Miscarriage (or spontaneous abortion); Subfecundity (or time to conception); SIDS (sudden infant death syndrome)

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Abbreviations: Anom, anomaly (i.e. malformation); IUGR, intrauterine growth retardation; Low BW, low birthweight; OR, odds ratio; RR, risk ratio; Prem, prematurity; Spon Abort, spontaneous abortion.

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1. Introduction

Meta-analysis is the name given to the technique of summarizing all the published work in a narrow field (Glass et al., 1981). This review resists the desire to offer a meta-analysis of caffeine exposure and each possible reproductive adversity. This reflects a desire not to (a) incorporate into any meta-analysis studies that the author considers worthless and (b) give potentially equal importance to studies that differ in quality (Greenland, 1987; Fleiss and Gross, 1991; Spitzer, 1991; Felson, 1992; O'Brien, 1993). Recent meta-analytic techniques that allow weighting of a study, not only by sample size, but also by overall quality (Powell et al., 1987; Berlin and Colditz, 1990; Detsky et al., 1992; Viscoli et al., 1993; Greenland, 1994; Olkin, 1994), are not yet adequately developed to be used to evaluate the relationship between caffeine consumption and each reproductive hazard.

Each of the most relevant studies has been critically reviewed so that readers will be able to appreciate when claims made by authors are justified, and when, and to what extent, caution is appropriate in evaluating the inferences drawn. These reviews were created over more than a decade. Some contain more details about the design and results than others. The varied length of the reviews reflects not only this author's response, but also what else had been published recently in the scientific or lay press.

There exist 20 reviews of the relationship between caffeine (or coffee) consumption and the risk of adverse reproductive outcomes (FDA, 1980; Morris and Weinstein, 1981; Dews, 1982; Briggs et al., 1983; James and Stirling, 1983; Ernster, 1984; Leviton, 1984, 1988, 1993; Brown and Scialli, 1987; Heller, 1987; Berger, 1988; Myers and Miwa, 1988; Nash and Persaud, 1988; Nolen, 1988; Al-Hachim, 1989; Narod et al., 1991; Diugosz and Bracken, 1992; Nehlig and Debry, 1994; Golding, 1995). Each reviewer's perspective/expertise appears to account for many of the differences in inferences drawn. This review differs from most of these in providing (a) greater detail about each study and (b) identifying the specific deficiencies that limit each study's usefulness for drawing inferences. The issues that most deserve consideration are:

1.1. Ascertainment of exposure

Some studies measured coffee consumption, whereas others evaluated caffeine. Often, the exposure was estimated after the occurrence of the event evaluated. Few studies have assessed the quality of the data collected. For example, did the woman occasionally drink much more than usual (Barr et al., 1981)? Is a coffee cup 5.5 ounces or 8 ounces (Schreiber et al., 1988a,b)? Did the woman always consume the entire contents of a cup/

mug/container? Is a serving of cola 6, 8 or 12 ounces? Did the woman actually drink less than a full serving because much of each service was ice? Were the caffeine consumption changes that accompany pregnancy considered (Hook, 1976, 1978; Diugosz and Bracken, 1992; C14)? Specifically, was the consumption measured pregnancy, during the first trimester, during the third trimester, or after delivery?

1.2. Sample studied

All too frequently, the sample was one of convenience with all of the negative attributes that term brings to mind. Was selection bias avoided? The sample should, as much as possible, be representative of all women the investigators purport to study. Invariably, the investigators should be able to say that they can generalize (with few caveats or limitations) from their sample to the universe of all pregnant women, or of all women trying to become pregnant. Is the sample representative of all women who consume any caffeine, high levels of caffeine (however defined), or no caffeine? Similarly, if the study is of the case-non-case design, are the two samples comparable, and are they approximations of the universe of women with and without the reproductive adversity?

1.3. Analyses/inferences

Those who consume relatively high amounts of caffeine appear to differ from their peers who consume less caffeine, or who consume no caffeine (Jacobsen and Thelle, 1987; Schreiber et al., 1988a,b; Puccio et al., 1990; Zavela et al., 1990; Fortier et al., 1994; Leviton et al., 1994). Unfortunately, some investigators do not consider the possibility that caffeine/coffee consumption is an indicator/predictor of exposures that place the fetus at risk (e.g. cigarette smoking, alcohol consumption, maternal age, lead exposure). Failure to consider the possibility that caffeine consumption is an epiphenomenon (i.e. a marker of a "cause" of the outcome, but not itself in the causal chain) can distort the true relationship between caffeine consumption and reproduction adversities. Some investigators, probably reflecting either naivete or sophisticated awareness of the limitations of logistic regression analyses, have avoided using multivariable models to deal with potential confounders. Others do not seem to be aware that information about beverage consumption can provide added information about some covariates that have been measured or classified inadequately (Morrison, 1984; Savitz and Baron, 1989; Kimball and Friedman, 1992; Leon, 1993), or ignored (Stein and Susser, 1991).

The principles of drawing inferences about causation based on epidemiologic studies of humans, suggested by Hill in 1965 (Hill, 1965), have not always received the

attention they deserve. Perhaps the most important principle relevant to this discussion is that of consistency. As you read each section of this review, note that every one of the five reproductive hazards evaluated fails the consistency criterion of consistently showing a relationship between caffeine/coffee consumption and the adversity evaluated.

Rather than giving each of the studies a summary score that measures its quality in all the areas considered, this document provides detailed commentary about the strengths and weaknesses of each study. The reports are ordered chronologically by date of publication (see Table 1) because other ordering or grouping schemes (e.g. by outcome) would have been less suitable for studies that evaluated multiple reproductive adversities. In addition, this document has grown as reports of new studies have been published.

Two recent reports claim that coffee consumption is associated with multi-fetal pregnancies (Kapidaki et al., 1995; Parazzini et al., 1996). Because twins and triplets tend not to be viewed as reproductive hazards, these studies are not evaluated here. The irony of such claims is appreciated in the light of other claims that coffee/caffeine consumption is associated with *subfecundity* (i.e. infertility and delayed conception).

Sudden infant death¹ should also probably not be viewed as a reproductive hazard. Nevertheless, a recent report linking caffeine consumption to the risk of sudden infant death is included here, more to demonstrate how obvious are some faults that plague the epidemiologic literature about the relationship between caffeine consumption (just before conception and during pregnancy) and reproductive and later adversities.

2. Reviews of individual reports

C1. Mau G., Netter P., 1974. Are coffee and alcohol consumption risk factors in pregnancy? *Geburtshilfe Frauenheilkd* 34, 1018–1022.

Mau and Netter were pioneers in the field of evaluating the relationship between coffee consumption and pregnancy adversities. They recruited 5200 women who were questioned during the first 3 months of pregnancy about health, personal habits and living conditions. Coffee and alcohol consumption were assessed by asking whether they were used “never”, “rarely” or “frequently”. Because only 0.1% of women said they used alcohol frequently, this variable was dichotomized for analysis as “yes” and “no”. The outcomes of interest

were low birthweight (defined as <2.5 kg) and preterm delivery (defined as gestation before day 260).

“Frequent” consumption of coffee was associated with an increased frequency of low birthweight, and of birthweights below the 10th percentile for gestational age, but was not significantly related to preterm delivery. “Frequent” consumption of tea or cola was not related to low birthweight.

This study has a number of methodologic limitations. While the investigators attempted to control for a number of potentially confounding variables, such as alcohol use, cigarette smoking and maternal age, they apparently only controlled for one confounder at a time. This approach is not appropriate when a number of interrelated exposures are being considered, and one can conclude that the results are not adequately controlled, even for known confounders. If tea and cola drinkers are not as likely as coffee drinkers to be consumers of alcohol and cigarettes and to be older, the reported absence of an association between drinking of tea and colas and low birthweight also suggests that the coffee relationship is confounded.

Quantification of beverage consumption was also less than ideal. Interpretations of such qualitative terms as “rarely” and “frequently” are likely to vary from woman to woman, resulting in misclassification of exposure. As the investigators acknowledge, they also did not have information about changes in consumption during the course of pregnancy, although they speculate that most women probably reduced consumption then.

The results of this study are similar to many others in that the observed association between low birthweight and coffee consumption could be entirely explained by inadequate adjustment for confounding variables. Failure to adjust for confounders severely limits the usefulness of these results in understanding whether or not a relationship exists between coffee/caffeine use and low gestational age and low birthweight.

C2. van den Berg B.J., 1977, Epidemiologic observations of prematurity: effects of tobacco, coffee and alcohol. In: Reed D.M., Stanley F.J. (eds.) *The Epidemiology of Prematurity*. Urban and Schwarzenberg, Munich, pp. 157–176.

This paper presents data from the Child Health Development studies, which recruited pregnant women who were members of the Kaiser Health Plan in the San Francisco Bay area between the years 1960 and 1967. Data were obtained from medical records and interviews, which included information on reproductive history, socioeconomic factors, smoking habits and beverage consumption. A total of 15,000 women were interviewed. Endpoints of interest for the white, singleton, live-born infants were low birthweight (i.e. <2.5 kg), preterm delivery (i.e. before week 37), combinations of gestational

¹ Sudden infant death syndrome, the topic of this paper, is strictly not a reproductive hazard. This paper is included here because of the claim that caffeine consumption during pregnancy was associated with an increased risk of death of the infant months after delivery.

age and birthweight, infant mortality, and severe congenital anomalies (not defined) diagnosed within the first 5 years of life. The only source of caffeine considered was coffee, with consumption divided into four groups.

Fully 65% of women consuming coffee at the highest level (i.e. ≥ 7 cups) were categorized as smokers, compared to 38% in the total cohort. Although the frequency of low birthweight in preterm delivery was highest among women consuming the most, the increased frequency of both outcomes was seen in the smokers among the high consumers of coffee, but not among the non-smokers. This prompted the conclusion that “the coffee effect on prematurity appears to be entirely explainable by the association with smoking”.

The interpretation offered by van den Berg was challenged by Hogue (1981), who re-analyzed the data comparing within smoking strata the percent of low-birthweight infants born to women consuming seven or more cups of coffee per day vs those drinking less than 7 cups per day. In this reanalysis, the risk of low birthweight was significantly increased [risk ratio (RR) = 1.2] in offspring of women drinking 7 or more cups per day, after controlling for smoking status. This reanalysis is a more appropriate approach to assessing the association of coffee and low birthweight than that used by van den Berg. In the original paper, the analysis deals with the effect of smoking only among “high” coffee users. The re-analysis by Hogue, however, suffers from the failure to adequately control for smoking. She compared smokers to non-smokers, ignoring the possibility that the risk of low birthweight increases incrementally with the number of cigarettes smoked per day. The Hogue re-analysis also does not consider preterm delivery as an endpoint.

Van den Berg's univariate analyses of the associations of coffee drinking and infant mortality compared high coffee consumers (7 or more cups per day) to those who consumed less. The slightly higher rates of infant mortality (1.7 vs 1.4 per 100 single liveborn infants) and severe congenital malformations (4.4 vs 3.5%) did not achieve statistical significance, even in these analyses unadjusted for covariates.

The limitations of this study reflect the lack of multi-variable analyses, and the rather crude categorization of both exposure and potential confounding variables. The other major limitation of this study was that the only source of caffeine considered was coffee. The contribution from soft drinks and from over-the-counter medications was not considered.

C3. Weathersbee P.S., Olsen L.K., Lodge J.R., 1977, Caffeine and pregnancy: a retrospective survey. Postgraduate Medicine 62, 64–69.

Weathersbee and his colleagues appear to be the first to report an association between caffeine consumption

and an increased risk of spontaneous abortion. Many of the limitations were identified for the Food and Drug Administration (FDA, 1980).

The study population consisted of 800 households selected by random sampling of the medical records of women who had been obstetric patients at one of seven hospitals in Utah and southern Idaho in 1974 and 1975. A questionnaire was mailed to each household requesting information about the level of consumption by household members of a variety of beverages. Completed questionnaires were received from 489 households (61%). Caffeine intake was estimated from daily consumption of coffee, tea and cola. Outcomes were spontaneous abortion, stillbirth, and premature birth. Of the 489 respondents, 1% of the women consumed at least 600 mg of caffeine per day. In this group of 16 women, only one had an uncomplicated delivery. A total of eight had a miscarriage, five had stillbirths, and two gave birth prematurely.

This study has a number of major design flaws, and thus the results do not contribute to our understanding of the association between caffeine consumption and reproductive outcome. The response rate of 61% is low, making it likely that respondents might differ from non-respondents in important ways. The investigators did not report any comparison of responders to non-responders. In addition, because data on beverage use were collected considerably after the birth outcome, information about this important exposure variable might have been subject to recall bias and might not have reflected beverage consumption during pregnancy (see below).

The majority of women in these communities were Mormons. The investigators contend that because of the religious tenets of these women, other potentially adverse exposures were not likely to be elevated in women consuming 600 or more mg of caffeine per day. This view was considered inappropriate by reviewers in 1980 (FDA, 1980). Subsequently, investigators studying Utah Mormon women have shown that women who deviate from church teachings in one area are likely to deviate from church teachings in other areas (Gardner and Lyon, 1982). In the light of the evidence that other exposures might be correlated with caffeine consumption, failure to control for such confounding factors as cigarette smoking, alcohol consumption and socioeconomic status make the results of the Weathersbee et al. study uninterpretable. The investigators' use of beer consumption as a surrogate measure of total alcohol consumption in women is viewed as inappropriate.

Another major problem of this study is that caffeine use was not ascertained in reference to the index pregnancy. The reader has no way of knowing whether the amounts reported at the time the questionnaire was completed bear any relationship to the consumption patterns during pregnancy. It is even possible that high

caffeine consumption resulted from the outcome rather than preceding it (Conway et al. 1981). In addition, the definitions of consumption categories shown in the authors' table pose problems, in that some groups relate to exposure only in the mother, and some to either the mother or father or both. For example, what inference should be drawn from the heading of a table "300–450 milligrams by man, woman, or both"?

C4. Borlee I., Lechat M.F., Bouckaert A., Misson C., 1978, Le café facteur de risque pendant la grossesse? Louvain Medical 97, 284–297.

This case-control study included all children with congenital malformations, defined as "any morphological malformation which is detectable upon birth", born during a 2-year interval in a region of Belgium ($N=202$). Controls were 175 (normal) children born in the same region during the same time interval and matched to cases by age (gestational?), sex and socioeconomic status. The mechanism for control selection was not described. Both fathers and mothers were questioned about possible risk factors, including beverage consumption.

The investigators reported that the average consumption of coffee per week was significantly higher in the mothers of infants with congenital anomalies than in control mothers (28 cups vs 25 cups). Consumption of 8 or more cups of coffee per day was nearly twice as common in case mothers as in control mothers.

This study has several strengths. First, an attempt was made to include all cases of congenital malformation born within a specified time period in a geographically defined area. Participation rates are not given, however, and thus the reader does not know to what extent response bias may have accounted for the results. The method of control selection was also not described, leaving the possibility of bias here, too. The investigators were appropriately cautious in their interpretation of the results, and in a subsequent letter published in *Science* (Lechat et al., 1980), they noted that testing multiple hypotheses or indirect associations may have accounted for the observed association.

In addition to the issues raised by the authors, a number of other limitations plague this study. The investigators state that they excluded other risk factors, including maternal age, tobacco and medications. Unfortunately, the basis for these exclusions is not presented. Another major difficulty is the failure to adjust for alcohol consumption. In addition, data were collected retrospectively and may have been subject to recall bias. Information about the timing of exposures is not present. This is especially relevant in assessing the plausibility of a biologic basis for the wide variety of congenital malformations grouped together in this report.

C5. Warburton D., Stein Z., Kline J., Strombino B., 1980, Environmental influences on rates of chromosome anomalies in spontaneous abortions. American Journal of Human Genetics 32, 92A.

This abstract, reporting an association between caffeine consumption and triploid abortions, has not been followed by a full report despite an interval of 17 years. Indeed, the monograph about the epidemiology of prenatal development written by her colleagues and published 9 years after the abstract did not make any mention of this association (Kline et al., 1989). It seems reasonable to conclude that this careful group of investigators is not convinced they have explained their possibly chance observation.

C6. Berkowitz G.S., Holford T.R., Berkowitz R.L., 1982, Effects of cigarette smoking, alcohol, coffee and tea consumption on preterm delivery. Early Human Development (7), 239–250.

Berkowitz and her colleagues evaluated the contribution of coffee and tea consumption to birth before week 37 of gestation. They compared 175 women who delivered an infant before that gestational age to 313 women who delivered an infant after it. Women were classified by daily coffee and tea consumption, but not by total daily caffeine consumption. Mothers of preterm infants were not appreciably more likely than mothers of full-term infants to consume four or more cups of coffee per day.

This is one of the few reports to include information about power of the study. The investigators had designed and carried out a study that had a power of 0.9 to appreciate an estimated RR of 3.0, and 0.8 to appreciate a RR of 2.5. Thus, the investigators can state with confidence that consumption of four or more cups of coffee per day during pregnancy does not appear to increase the risk of preterm delivery by a factor of 2.5.

C7. Linn S., Schoenbaum S.C., Monson R.R., Rosner B., Stubblefield P.G., Ryan K.J., 1982, No association between coffee consumption and adverse outcomes of pregnancy. New England Journal of Medicine 306, 141–145.

A total of 12,205 non-diabetic, non-asthmatic women delivering a single child were interviewed within 2 days of delivery concerning a variety of factors that might be associated with pregnancy outcome. Women were asked about their consumption of coffee and tea in cups per day during the first trimester of pregnancy, and about smoking and alcohol consumption in each trimester. Outcomes of interest were: low birthweight (<2.5 kg), short gestation (<37 weeks), and congenital

malformations (classified as total number, major, minor, and selected types). Beverage consumption was categorized as: no coffee or tea, and 0, 1, 2, 3 and 4 or more cups of coffee per day. Information was also collected about a variety of potentially confounding variables, including cigarette smoking, alcohol consumption, previous pregnancy history and socioeconomic characteristics.

The authors reported an increased risk of premature rupture of membranes in women drinking 4 or more cups of coffee per day, after statistical adjustment for potential confounders [odds ratio (OR)=1.5]. After adjustment for potential confounders, coffee consumption was not significantly associated with risk of low birthweight, short gestation, or congenital malformations.

This study has a number of strengths. First, the investigators included information on a wide variety of potential confounders. Second, the sample size was large, and thus the study had sufficient statistical power to detect relatively small increases in risk for the major outcomes of interest.

One limitation of the study is that coffee consumption was assessed only for the first trimester, while several important confounders were determined for each trimester. The coffee consumption for the first trimester only is certainly appropriate for assessing the contribution of coffee/caffeine consumption to the risk of congenital anomalies. The appropriateness of the first trimester coffee consumption variable to the risk of low birthweight and short gestation are less clear, however. In addition, information about exposures was collected retrospectively. The authors did not describe how they handled data on confounders that varied by trimester.

The possibility that women who did not drink coffee or tea were exposed to other sources of caffeine is discussed by the investigators. This lack of information about additional sources of caffeine is highly unlikely to account for the negative results, mainly because coffee is invariably the major source of caffeine consumption, and no dose-response relationship was observed among coffee drinkers.

C8. Rosenberg L., Mitchell A.A., Shapiro S., Slone D., 1982, Selected birth defects in relation to caffeine-containing beverages. *Journal of the American Medical Association* 247, 1429–1432.

This is a retrospective study of the association of maternal caffeine use in six groups of congenital malformations (i.e. inguinal hernia, cleft lip with or without cleft palate, isolated cleft palate, cardiac defect excluding isolated heart murmur, pyloric stenosis, and neural tube fusion defect). Case groups were selected based on sample size ($N \geq 100$) from among 2030 malformed infants born between 1976 and 1980 in participating

hospitals in the Greater Boston area, Philadelphia and Toronto. Controls were all other malformed infants ($N=712$). Within 6 months of the child's birth, mothers were interviewed about medical and obstetrical history, personal characteristics and habits, and use of medications and beverage consumption during the index pregnancy. Total daily consumption of caffeine was estimated for each mother by summing the intake from tea, caffeine-containing coffee, and cola. In the analysis, caffeine was treated as a categorical variable in units: 0, 1–199 mg per day, 200–300 mg per day, and 400 or more mg per day. Some comparisons were also made between women consuming none and any caffeine. The investigators reported no statistically significant associations between caffeine consumption at any level and any of the six malformations studied. No trends in dose-response were observed.

In general, this is a well-designed study that has a number of strengths. Sample size in each of the malformation groups was sufficiently large to detect two- to three-fold increases in risk associated with caffeine consumption. Selecting mothers of children with other malformations as controls is seen as a strength because this manoeuvre reduces the likelihood of recall bias. The investigators argue persuasively against the generalized teratogenic effect of caffeine. They also examined within the control group the frequency of caffeine use by type of defect and found no consistent evidence of association with any of the malformations.

Another attribute of this study is that a number of factors that might have confounded an association between caffeine consumption and the risk of selected malformations were controlled in the analyses. Although perhaps not all of the potential confounders should have been dichotomized, inadequate control for confounders would most likely have produced an association with caffeine, rather than obscured a true relationship. Likewise, alcohol consumption, for which data were not available, is probably a positive rather than a negative confounder. Thus, the fact that alcohol use was not controlled in this study and that other covariates were not optimally controlled cannot account for the lack of an association between caffeine and the selected malformations investigated.

The major limitation of the study is that case groups were selected on the basis of the adequacy of the sample size rather than a biologic mechanism by which caffeine might produce the given malformation. Thus, the results of the study are informative and useful with respect to a number of major, frequently occurring malformations. However, they do not address limb-reduction defects, a very rare malformation, for which animal data suggest a possible relationship with exposure to very high doses of caffeine. The authors acknowledge this, but also point out that the relevance of these animal data to human experience is questionable.

C9. Kuzma J.W., Sokol R.J., 1982, Maternal drinking and decreased intrauterine growth. Alcohol Clinical and Experimental Research 6, 396–402.

Pregnant women who delivered a live-born infant in the mid-1970s at one of four hospitals in the Riverside-San Bernardino-Ontario metropolitan area of Southern California were recruited for this study of maternal alcohol consumption and intrauterine growth. A self-administered questionnaire was completed at the time of the first prenatal visit by 63% of the 5093 women in the sample. Data from the remaining 37% of women were collected postpartum. Those recruited early in pregnancy are reported to have had the same number of prenatal visits as those recruited postpartum.

In the authors' previous paper, 12,349 women completed not only the initial questionnaire, but also provided additional data (Kuzma and Kissinger, 1981). Never explained is why this paper deals with less than 40% (5093/12,349) of the original sample.

Another suggestion that selection bias may have occurred comes from the observation that 29% of women consume the equivalent of 6 or more cups of coffee per day. This is somewhat higher than seen in other series, and raises the issue of the generalizability of the authors' findings.

In their previous paper, the authors state that many participants completed their questionnaire about "health related habits... relative to her life *prior* to the pregnancy." Nowhere in this report have the authors assessed caffeine consumption *during* pregnancy. One must conclude, therefore, that the reported relationship is between caffeine consumption before pregnancy and birthweight.

Instead of using the actual estimate of caffeine consumed by each woman, three categories were used. This degradation of data results in some loss of potentially important information.

The authors selected 17 maternal and paternal characteristics to be independent variables in stepwise multiple regression analyses of birthweight. The coffee consumption variable explained two-tenths of one percent of the variance, compared to the three-tenths of one percent explained by frequency of beer use and the failure of frequency of wine and liquor use to explain any of the birthweight variance. The wisdom of using stepwise multiple regression techniques has been questioned (Leigh, 1988).

Because the mean daily intake of absolute alcohol did not relate significantly to adjusted infant birthweight as expected, the authors hypothesized that some women who consumed alcohol during pregnancy did not reliably report their alcohol consumption. If the authors are right, then the possibility needs to be considered that correlates of alcohol consumption (e.g. caffeine consumption) provide discriminating information not conveyed by the alcohol consumption variables them-

selves. The caffeine consumption variable might carry with it information about the residual confounding of such inadequately measured adverse exposures as tobacco and alcohol (Morrison, 1984; Savitz and Baron, 1989; Leon, 1993). This is in keeping with the findings of others (Jacobsen and Thelle, 1987; Schreiber et al., 1988a,b; Puccio et al., 1990; Zavela et al., 1990; Fortier et al., 1994; Leviton et al., 1994).

In the light of its multiple problems, inferences should not be drawn from this study about the relationship between caffeine consumption and intrauterine growth.

C10. Kurppa K., Holmberg P.C., Kuosma E., Saxen L., 1983, Coffee consumption during pregnancy and selected congenital malformations: a nationwide case-control study. American Journal of Public Health 73, 1397–1399.

This case-control study included all children born in Finland between January 1980 and April 1982 who had defects of the central nervous system ($N=112$), orofacial clefts ($N=241$), structural defects of the skeleton ($N=210$) or cardiovascular malformations ($N=143$). Controls were deliveries immediately preceding the index case and born in the same district. Information on coffee consumption during pregnancy was obtained by interview, along with information about family history, previous pregnancies, cigarette smoking and alcohol consumption. All interviews were conducted within 3 months of delivery. The participation rate was 95%. Excluded from analyses were 35 pairs that included habitual tea drinkers and 14 pairs with "inadequacies in the forms".

The investigators found no association between coffee drinking during pregnancy and the risk of all the malformations included in the study (adjusted OR = 1.1, 95% CI = 0.8, 1.3). This lack of association with coffee consumption was seen whether coffee was analyzed by mean number of cups per day or by categories of consumption. The risk of each of the subgroups of malformations was also not related to coffee drinking. In addition, no significant dose-response relationship was observed.

This study was well designed and has a number of strengths. First, cases and controls were identified from the entire country of Finland and response rates were very high. Thus, it is unlikely that bias in the selection of cases or controls could explain the failure to observe a significant association between coffee consumption and the risk of specific congenital malformations. Second, analyses controlled for differences between case and control mothers in regard to maternal age, cigarette smoking and alcohol consumption. While information was collected retrospectively, recall bias is more likely to produce a positive than a neutral result. In addition, as the authors point out, the interview included questions on a wide variety of behaviors, and the study was conducted prior to any public concern about coffee drinking and reproductive outcome.

The major concern with this study is that because coffee drinking is so prevalent in Finland, statistical power might not be sufficient to detect relatively small increases in risk. This was, in fact, the case for those analyses restricted to specific types of malformations. The power to detect a 1.5-fold increase in risk associated with drinking 4 or more cups of coffee per day was approximately 25% for central nervous system malformations, 51% for orofacial clefts, 46% for structural defects of the skeleton, and 32% for cardiovascular malformations (when the two-tailed alpha error was set at 0.05 and the exposure rate in controls was set at 0.29). This study did have power of 94% to detect a 1.5-fold increased risk for all malformations. In addition, the power was approximately 91 and 95% to detect a two-fold increase in risk of structural skeletal defects and orofacial clefts, respectively.

This large, well-designed case-control study provides some of the best evidence available refuting the hypothesis that coffee consumption is associated with any important increase in risk of major congenital malformations. While the study does not address the issue of caffeine per se, coffee is probably the major source of caffeine in this population.

C11. Tebbutt I.H., Teare A.J., Meek J.H. et al., 1984, Caffeine, theophylline and theobromine in pregnancy. Biological Research on Pregnancy and Perinatology 5, 174–176.

Tebbutt and colleagues evaluated 59 “unselected” women between 6 and 22 weeks pregnant attending an antenatal clinic in England in 1981. Seven women moved and were lost to follow-up. Of the remaining 52 women, 29 had both plasma studies and dietary survey, 13 had plasma studies only, and 10 dietary survey only. Women were asked about intake of coffee (whether instant or ground), tea, cocoa, soft drinks containing caffeine, and chocolate. Amounts of caffeine ingested were calculated using standard conversions. Blood samples were analyzed for caffeine, theophylline and theobromine levels.

Of the 39 women with dietary data, four (10%) reported low intakes of caffeine (<100 mg per day), 23 (59%) had “medium” intake (100–399 mg per day), and 12 (31%) reported “high” intake (400 mg or greater per day). The correlation coefficient for dietary and plasma caffeine was 0.6 ($P < 0.01$). Three first-trimester abortions occurred in this group, but no fetal abnormalities or perinatal deaths. Women with preterm labor did not differ from women who did not go into preterm labor in either dietary or plasma levels of the three methylxanthines evaluated. None of the babies was “small for dates” (i.e. had intrauterine growth retardation).

The major strengths of the study are: (1) the assessment of multiple dietary sources of caffeine; and (2)

obtaining plasma caffeine levels (although this only assesses levels at a single point in time). The major weakness of this study is the very small sample size. No valid conclusions about the association of caffeine and pregnancy outcome can be based on the results of this study. Another limitation of the study is that the investigators did not include potential confounding variables, such as smoking or alcohol consumption.

C12. Barr H.M., Streissguth A.P., Martin D.C., Herman C.S., 1984, Infant size at 8 months of age: relationship to maternal use of alcohol, nicotine and caffeine during pregnancy. Pediatrics 74, 336–341.

From the 1529 women who presented for prenatal care before month 5 at two Seattle hospitals during one year, a follow-up sample was selected prior to delivery to consist equally of women who consumed the most alcohol and tobacco and women who abstained or consumed minimal amounts of alcohol (and tobacco?). The 462 babies followed to age 8 months constitute the sample for this report. Caffeine consumption was not related to length, weight or head circumference at 8 months of age when the multiple regression analyses included terms for the baby's sex, gestational age and examination age and the mother's parity, height and consumption of alcohol and nicotine. Although no mention is made in the results section of any relationship between caffeine consumption and birth measurements, item 3 in the summary states, “Caffeine use during pregnancy is not significantly related to...birth size.”

The major limitation of this study is the nature of the sample. Half the women represent the top 15–20% of alcohol consumers. People who consume the most alcohol tend to consume the most caffeine (Morrison, 1984; Schreiber et al., 1988b; Puccio et al., 1990; Leviton et al., 1994). Thus, the alcohol consumption variable will carry information about caffeine consumption. Because the alcohol variable overwhelms all others, the effects of correlates of alcohol consumption might not be identified as conveying unique information about their influences on birth size.

C13. Furuhashi N., Sato S., Suzuki M. et al., 1985, Effects of caffeine ingestion during pregnancy. Gynecologic and Obstetric Investigation 19, 187–191.

This is a prospective study of the pregnancy outcomes among 9921 “healthy” women after week 24 of pregnancy. Women were divided into five groups based on their average daily beverage consumption: less than 5 cups of coffee ($N=3815$), greater than 5 cups of coffee ($N=53$), less than 5 cups of coffee and green tea ($N=473$), tea only ($N=348$), and neither tea nor coffee ($N=5232$). Outcomes of interest included mean birth-

weight, length of labor, mean amount of intrapartum hemorrhage, and incidence of intrauterine growth retardation, spontaneous abortion, premature labor, congenital malformations and chromosomal abnormalities. Women who consumed more than 5 cups of coffee per day had an increased frequency of heart failure, anemia, impending abortion and premature labor, and a significantly increased frequency of babies small for gestational age. Compared to women who drank no coffee, all coffee drinkers combined had a significantly higher rate of spontaneous abortion, and increased incidence of offspring with chromosomal anomalies or multiple congenital malformations. The overall risk of congenital anomalies among offspring of coffee drinkers, however, was not significantly increased.

The major strengths of this study are its prospective design and the large number of pregnancies studied. Unfortunately, the authors do not appear to have taken advantage of these strengths.

This investigation has a number of problems. No estimates of the range of caffeine from tea are provided. Did some tea drinkers consume “large amounts” of caffeine? No description is given of how information on either the exposures or the outcomes was ascertained. Many outcomes, such as chromosomal abnormalities, impending abortion, and premature labor, are not defined. If medical record information was used to determine exposure status, a large number of women were misclassified.

The major limitation of the study (assuming the data were of high quality) is the failure to control for potential confounders. While little is known about the causes of congenital malformations or chromosomal abnormalities, adjustment for maternal age and other potential covariates seems important. The potential confounding effects of cigarette smoking and alcohol consumption in analyses of outcomes such as birthweight and gestational age are well known (Stein, 1984), but were not considered in this study. In a country such as Japan, where tea drinking predominates, the investigators should have considered the possibility that the coffee drinkers were different in many ways from tea drinkers, or from women who drank neither beverage. In the absence of adjustment for potential confounders, this study provides little useful information regarding the association of coffee consumption and adverse pregnancy outcome.

C14. Watkinson B., Fried P.A., 1985, Maternal caffeine use before, during and after pregnancy and effects upon offspring. Neurobehavioural Toxicology and Teratology 7, 9–17.

Another paper published in 1985 did not find an association between maternal caffeine consumption and spontaneous abortion. Like the report from Furuhashi

and his colleagues, this one from Watkinson and Fried evaluates a number of outcomes. As part of the Ottawa prenatal prospective study, a questionnaire was sent to 371 women who had participated in the study and whose infants were at least 1 year of age. Responses were received for 284 mother/child pairs, and two mother/twins sets. Exposure information was collected retrospectively about demographic factors, feeding patterns, use of alcohol, nicotine, marijuana and caffeine. Women were asked to report their typical use of coffee, tea, caffeinated soft drinks, chocolate bars, chocolate drinks and caffeine-containing medications. The seven time periods consisted of the third, second and first years before pregnancy, the first, second and third trimesters, and the year after pregnancy. Details were obtained about frequency of use, portion size, decaffeinated vs caffeinated and, where applicable, method of beverage preparation and beverage strength.

Study 1 was designed to develop a method for accurately estimating the amount of exposure to caffeine based on method of preparation of coffee and tea, and including all sources of caffeine. Samples of coffee and tea prepared by a subset of women were collected and analyzed for caffeine content. The caffeine content of these samples varied widely, and was lower than that reported in previous studies in which the coffee had been prepared in a laboratory according to the manufacturer's instructions. Caffeine content was highest in percolated coffee, followed by drip and instant. Subjects' subjective estimates of strength of coffee were reasonably correlated with caffeine content. An algorithm was developed to estimate total exposure to caffeine derived from coffee, tea, chocolate bars and drinks, caffeine-containing soft drinks, and medications, including serving size, frequency per day, and for coffee and tea, an estimate of the strength.

Study 2 evaluated the patterns of caffeine exposure before, during and after pregnancy. Upon becoming pregnant, 62% of women decreased their caffeine consumption. Most of the decreased caffeine ingestion could be attributed to decreased coffee consumption. No important changes during pregnancy were observed in consumption of soft drinks, or chocolate bars or drinks. Both before and during pregnancy, caffeine use was positively correlated with alcohol and nicotine use, and both alcohol and nicotine use declined during pregnancy. Among respondents, about 2% consumed more than 400 mg of caffeine per day during pregnancy.

Study 3 examined the association between caffeine exposure and reproductive outcome. Including birthweight and length, head circumference, ponderal index, Apgar score, length of labor, and gestational age. For these analyses, caffeine was treated both as a continuous and a categorical (dichotomous) variable. Consumption ≥ 300 mg per day was considered “heavy use”. Approximate fetal caffeine exposure levels were also

computed by dividing average pregnancy caffeine intake for each mother by her pre-pregnancy weight.

Of the 284 mother/child pairs, 12 women reported an average consumption of more than 300 mg of caffeine per day during pregnancy. Less education, heavier pre-pregnancy weight, greater number of pregnancies and births, and nicotine use were associated with heavier consumption of caffeine. When caffeine was treated as a continuous variable, no significant relationships were found between pre-pregnancy or pregnancy consumption and birthweight or length, head circumference, ponderal index, Apgar score, length of labor, or length of gestation.

When caffeine use *before* pregnancy was dichotomized, no significant associations were noted with any outcome variables. Heavier caffeine users *during* pregnancy gave birth to babies with smaller head circumference than did women who consumed less caffeine (33.5 vs 34.6 cm). This association remained significant after adjustment for nicotine and for mother's education. The mean birthweight was also significantly lower in infants born to mothers in the heavy caffeine group (3158 vs 3537 g). This difference remained significant after adjustment for nicotine, and approached statistical significance after adjustment for mother's education. When fetal caffeine exposure levels were used to establish the two caffeine groups, the lowered birthweight, but not the head circumference reduction, was apparent in the more heavily exposed group, and persisted after controlling for parity and for maternal nicotine use during pregnancy. Heavy caffeine use was not associated with an increased incidence of miscarriage. The authors conclude that the present results suggest that daily caffeine intake of 300 mg or more can interfere with normal fetal growth.

The major strengths of this study relate to the measures of caffeine exposure. The first study, which was designed to evaluate the caffeine content of coffee and tea prepared in different ways, provides useful information about the variability of caffeine content depending on method of preparation and dilution with cream or milk. The caffeine variable takes into account a variety of sources of exposure and considers other factors that may affect total level of exposure. The second study provides useful information on changes in caffeine use during pregnancy, and points to the importance of considering changes in exposure over time.

Despite these important strengths, the study has several weaknesses. First, exposure information was obtained retrospectively. While the authors argue that recall bias is not likely to be a problem, the possibility exists that this bias did occur. Second, adjustment for potential confounders appears to have been done one at a time, so that it is possible that any single analysis is still confounded by all the other variables not included. Finally, only 12 of the 284 mothers were in the "heavy"

use category. Thus, comparisons that are made using caffeine as a dichotomous variable are likely to be affected by the small sample size in the "heavy" use group. A single outlier will greatly affect mean values in the small group, perhaps creating the appearance of an association where none exists. Thus, extreme caution is advised in extrapolating from these 12 women to the universe of all women who consume 300 mg of caffeine per day during pregnancy.

C15. Martin T.R., Bracken M.B., 1986, Association of low birthweight with passive smoke exposure in pregnancy. American Journal of Epidemiology 124, 633–642.

In this report from the Yale Pregnancy Outcome Interview Study, passive smoking was defined as "being exposed to someone else's cigarette smoke for at least two hours per day, either at home or at work, during pregnancy." Women were categorized into four groups based on their exposure to cigarette smoke: none, passive only, direct only, and both and direct. The outcomes of interest included: low birthweight (i.e. <2.5 kg), preterm delivery (<37 weeks), mean birthweight, mean gestational age, and intrauterine growth retardation (defined as birthweight <2.5 kg in term deliveries). Potential confounders of the association between passive exposure to cigarette smoke and adverse birth outcome included: maternal age, marital status, ethnicity, education, employment status, cigarettes smoked per day in smokers, alcohol consumption, use of marijuana, parity, previous spontaneous abortion, previous induced abortion, previous stillbirth, body mass index, weight gain during pregnancy, and caffeine consumption in milligrams per day grouped as none, 1–150, 151–300 and >300.

The distribution of caffeine consumption was significantly different among the four smoking groups. For example, 72% of women exposed to no smoke ingested caffeine, whereas 84% of those exposed to both passive and direct smoke consumed caffeine. Similarly, only 4% of those not exposed to any cigarette smoke consumed 300 mg or more of caffeine per day, compared to 19% of women who themselves smoked and were also exposed to passive smoke.

Caffeine consumption was not significantly related to the risk of delivery before week 37. In addition, in multiple logistic regression models designed to identify factors related to low birthweight among term infants born to non-smokers, caffeine consumption variables did not provide risk information. Similarly, caffeine consumption did not improve linear regression models designed to identify factors related to mean birthweight in term infants.

The results of this study suggest that caffeine consumption is not significantly related to the risk of low

birthweight or to mean birthweight in term infants born to non-smokers. Caffeine was also not significantly related to the risk of preterm birth in any of the four smoking groups.

The comparisons shown in Table 1 of this paper suggest that cigarette smokers are very different from non-smokers in characteristics other than caffeine consumption, including higher alcohol consumption, lower education, much greater use of marijuana, greater frequency of previous induced abortion, and a smaller weight gain during pregnancy. Although data are not presented discriminating between those who consumed the highest amount of caffeine from those who consumed less, the implication of Table 1 is that many potential confounding variables related to cigarette smoking may also apply to a correlate of cigarette smoking, such as caffeine consumption. The irony raised by Table 1 is that some of the results regarding caffeine effects in pregnancy reported by Martin and Bracken in their other paper (C19) might reflect inadequate control for smoking.

C16. Srisuphan W., Bracken M.B., 1986, Caffeine consumption during pregnancy and association with late spontaneous abortion. American Journal of Obstetrics and Gynecology 155, 14–20.

This is the most recent paper assessing the relationship between caffeine consumption and late miscarriage. Addressing the topic of “late spontaneous abortion”, this report from the Yale Pregnancy Outcome Interview Study enrolled women at the time of their first prenatal visit. Each woman was interviewed about demographic characteristics, previous medical and obstetric history, smoking and drinking habits during pregnancy, and occupational exposure. Caffeine consumption was calculated from caffeinated coffee, tea, colas and drugs. The outcome of interest was spontaneous abortion, defined as non-deliberate interruption of an intrauterine pregnancy of less than 28 weeks gestation in which the fetus was dead when expelled. Sixty-eight spontaneous abortions were identified from medical records and follow-up contacts with obstetricians and respondents. All miscarriages included in the study occurred after interview and between eight and 26 weeks gestation. The final sample size was 3135 women. In the analyses, average caffeine consumption was either trichotomized as zero, 1–150 mg per day, and >150 mg per day, dichotomized as ≤ 150 mg per day and >150 mg per day, or entered in 50 mg per day increments.

The investigators reported a non-significant elevation in risk of spontaneous abortion ($OR=1.95$, $P=0.07$) among women consuming greater than 150 mg caffeine per day compared to non-users, adjusted for gestational age at interview, maternal age, prior gynecological sur-

gery, Jewish religion, and spontaneous abortion in the previous pregnancy. No increase in risk was observed for women in the 1–150 mg per day category, except for women who also had a history of spontaneous abortion in their last pregnancy ($OR=4.2$, $P=0.04$). When caffeine was treated as a dichotomous variable, women consuming more than 150 mg per day had a significantly increased adjusted OR of spontaneous abortion of 1.7 ($P=0.03$), compared to women consuming less. Caffeine consumption showed a “marked increase in the risk” at levels greater than the 150 mg, but no further increase at consumptions greater than 200 mg per day.

This study has several strengths. Because the data were collected prospectively, reported caffeine consumption cannot be biased by knowledge of the outcome. Multiple sources of exposure were considered, including coffee, tea, colas and drugs. Efforts were also made to collect information on a number of factors that could confound the association of caffeine use and risk of spontaneous abortion.

This study also suffers from a number of limitations. First, “late spontaneous abortion” may very well be a different entity than “early spontaneous abortion”. In this regard, caution is advised in comparing the results of this study to those of studies evaluating spontaneous abortions that tend to occur before women seek prenatal care.

The specification of caffeine exposure poses problems. No rationale is given for the cut points used to trichotomize caffeine. It is possible to both create and eliminate associations, depending on the manner in which a variable is categorized. The failure to observe a dose-response relationship between caffeine and the risk of abortion further suggests that the findings in the categorical analyses are spurious. In fact, only women in the 150–199 mg per day category had a higher risk of abortion.

A third major problem in this study is the selection of candidate confounders for inclusion in the multivariable models. The investigators selected covariates on the basis of the probability of their association with both caffeine and abortion ($P=0.10$). To quote Dales and Ury (1978), “To assess the confounding potential by... using a ‘traditional’ critical level is inadequate. Such a preliminary test places the burden of proof in the wrong direction, ignores a major aspect of confounding potential, and may be inappropriately influenced by other factors. If it must be used at all, the significance level should be 0.25 or 0.50, possibly even higher.”

Because Srisuphan and Bracken are the first and only people to report an association between caffeine consumption and the risk of late spontaneous abortion, and because of the methodologic weaknesses of their study, caution is advised in drawing inferences from their data.

Table 1
Tabulation of studies by the reproductive adversity evaluated

Bibliographic citation	First author	Reproductive adversity					
		Low BW	Prem	Spont abort	Fetal death	Anom	Delayed conception
C1	Mau, 1974	X	X				
C2	van den Berg, 1977		X				
C3	Weathersbee, 1977		X	X	X		
C4	Borlee, 1978					X	
C5	Warburton, 1980			X			
C6	Berkowitz, 1982		X				
C7	Linn, 1982	X	X			X	
C8	Rosenberg, 1982					X	
C9	Kuzma, 1982	X					
C10	Kurppa, 1983					X	
C11	Tebbutt, 1984		X				
C12	Barr, 1984	X					
C13	Furuhashi, 1985	X	X	X		X	
C14	Watkinson, 1985	X		X			
C15	Martin, 1986	X	X				
C16	Srisuphan, 1986			X			
C17	Beaulac-Baillargeon, 1987	X					
C18	Fried, 1987	X					
C19	Martin, 1987	X	X				
C20	Tierson, 1987	X					
C21	Wilcox, 1988						X
C22	Munoz, 1988	X					
C23	Christianson, 1989						X
C24	Brooke, 1989	X					
C25	Caan, 1989	X					
C26	Joesoef, 1990						X
C27	Williams, 1990						X
C28	Hansteen, 1990			X			
C29	Fenster, 1991a	X	X				
C30	Fenster, 1991b			X			
C31	Olsen, 1991						X
C32	Peacock, 1991	X					
C33	Kline, 1991			X		X	
C34	Parazzini, 1991			X			
C35	Armstrong, 1992			X			
C36	McDonald, 1992a		X				
C37	McDonald, 1992b					X	
C38	Godel, 1992	X					
C39	Williams, 1992		X				
C40	Mills, 1993	X		X			
C41	Grodstein, 1993						X
C42	Fortier, 1993	X	X				
C43	Larroque, 1993	X					
C44	Infante-Rivard, 1993			X	X		X
C45	Little, 1993				X		
C46	Hatch, 1993						X
C47	Al-Ansary, 1994	X					
C48	Florack, 1994	X					
C49	Parazzini, 1994			X			
C50	Dominguez, 1994			X			
C51	Pastore, 1995		X				
C52	Shu, 1995	X					
C53	Stanton, 1995						X
C54	Alderette, 1995						X
C55	Peacock, 1995		X				
C56	Windham, 1995	X					
C57	Rondo, 1996	X					
C58	Cook, 1996	X					
C59	Wisborg, 1996		X				
C60	Dlugosz, 1996			X			

Table 1 (continued)

Bibliographic citation	First author	Reproductive adversity					
		Low BW	Prem	Spont abort	Fetal death	Anom	Delayed conception
C61	Bolumar, 1997						X
C62	Vlajinac, 1997	X					
C63	Fenster, 1997			X			
C64	Curtis, 1997	X					
C65	Ford, 1998 ^a						
C66	Klebanoff, 1999		X				
C67	Cnattingius, 2000		X				
C68	Grosso, 2001	X					

^a Sudden infant death syndrome, the topic of this paper, is strictly not a reproductive hazard. This paper is included here because of the claim that caffeine consumption during pregnancy was associated with an increased risk of death of the infant months after delivery. Low BW, low birthweight; Prem, prematurity; Spont abort, spontaneous abortion; Anom, anomaly (i.e. malformation).

C17. Beaulac-Baillargeon L., Desrosiers C., 1987, Caffeine-cigarette interaction on fetal growth. American Journal of Obstetrics and Gynecology 157, 1236–1240.

This retrospective study included all women who delivered at two hospitals in Quebec City during a 5-month interval in 1985. Excluded were 101 women with multiple pregnancies, pre-eclampsia, chronic hypertension, diabetes, heart disease and other conditions, plus deliveries resulting in stillbirth or major malformations. Data concerning obstetric and health histories, and consumption patterns during pregnancy of caffeine, cigarettes and alcohol were obtained from a self-administered questionnaire. Outcomes of interest included: birthweight, head circumference, length, placental weight and placental ratio (i.e. placental weight/birthweight $\times 100$). Data collection was completed by review of the medical and obstetric records of both the mother and baby. The investigators trichotomized alcohol and cigarette consumption and dichotomized caffeine consumption (i.e. <300 mg per day and $300+$ mg per day). Most analyses adjusted for length of gestation and sex of the infant.

Birthweight was lowest for offspring of women who consumed 15 or more cigarettes per day and 300 or more milligrams of caffeine per day. Placental weight, head circumference and body length were not significantly associated with caffeine consumption.

This study has a number of strengths. The investigators chose to focus on caffeine consumption rather than coffee, and therefore included measures of consumption from coffee, tea, cola and chocolate. They also considered whether the coffee was caffeinated or not, and the methods of preparation of coffee and tea. The initial sample size was relatively large, although stratification of subjects by cigarette consumption resulted in reduced statistical power in some subgroups.

A number of weaknesses limit the value of this study. First, the investigators chose to categorize exposure

variables rather than treating them as continuous. This potential loss of information can be justified when a rationale is provided. Unfortunately, the investigators did not provide any rationale for the cut points used to define each category. Second, the investigators state that alcohol had no significant effect on the outcome variables, and thus apparently dropped this variable from further consideration. The possibility exists, however, that the cigarette/caffeine interaction is confounded by alcohol. Unfortunately, the investigators did not describe the alcohol consumption of women classified by their cigarette/caffeine consumption. In addition, the authors did not adjust for maternal age, pre-pregnancy weight or weight gain, each of which may also have confounded the associations observed. Finally, data regarding exposures were collected after delivery. Women with low-birthweight infants may have differentially recalled a variety of behaviors, compared to women whose infants were of normal weight. Thus, while the results of the study are provocative, they may still be confounded and/or biased.

C18. Fried P.A., O'Connell C.M., 1987, A comparison of the effects of prenatal exposure to tobacco, alcohol, cannabis and caffeine on birth size and subsequent growth. Neurotoxicology and Teratology 9, 79–85.

This paper presents results from the Ottawa Prenatal Prospective Study, which recruited women from obstetricians' offices and from prenatal clinics in three of the largest hospitals in Ottawa. Each participating woman was interviewed once during each of the trimesters remaining in her pregnancy. Data were collected about socioeconomic factors, her general health, obstetrical history, the father's medical history, the mother's 24-h recall of her diet, as well as about past and present consumption of alcohol, cigarettes and cannabis. Average daily caffeine consumption was calculated according to the number and size of servings of coffee, tea, cola

beverages, and other dietary sources of caffeine consumed during pregnancy. A weighted mean based on a maximum amount of caffeine was also calculated. The follow-up cohort included the babies born to women who consumed cannabis, and those who were the heavier consumers of alcohol and cigarettes. The comparison cohort consisted of the babies born to 50 women who did not consume cannabis and who abstained or consumed a small amount of alcohol and cigarettes. Outcome measures were weight, length/height, and head circumference measured at birth, 12 and 24 months.

Caffeine consumption did not significantly contribute to reduced growth of any of these outcome measures, as evaluated in regression models that included important covariates. "When the caffeine variable was analyzed by dividing the women into groups of those who consumed more than 300 mg per day ($N=22$) and the rest of the sample, a non-significant decrease in birthweight of 173.9 g was associated with heavier caffeine use." No effects of caffeine consumption were observed on outcomes at 12 or 24 months.

This study has a number of strengths. These include: assessment of caffeine rather than coffee only, follow-up at 12 and 24 months for evaluation of longer-term outcomes, blind assessment of outcome variables, inclusion of four periods of gestational exposure, and statistical adjustment for many potential confounders. Nevertheless, the study also has some limitations. These include: failure to control for previous pregnancy history, use of 24-h recall as a measure of usual caffeine consumption, and post hoc dichotomization of the caffeine variable. The sample size was too small to have sufficient statistical power for demonstrating an effect of caffeine. Thus, the absence of associations between caffeine consumption and parameters of infant growth should be interpreted with caution.

C19. Martin T., Bracken M.B., 1987, The association between low birthweight and caffeine consumption during pregnancy. *American Journal of Epidemiology* 126, 813–821.

This study comes from the Yale Pregnancy Outcome Interview Study [see also Srisuphan and Bracken (C16)]. Women were recruited at the time of their first prenatal visit and often were interviewed within a few weeks of that time. For this report, outcomes of interest were mean birthweight, percentage low birthweight (defined as <2.5 kg), mean gestational age, percentage preterm (defined as <37 weeks), and intrauterine growth retardation (defined as <2.5 kg birthweight in term infants). Average daily caffeine consumption was the main exposure of interest.

Lower birthweight was seen in the offspring of women consuming more than 300 mg of caffeine per day, but this relationship was observed only among infants with

gestational age greater than 36 weeks. ORs, adjusted for gestational age, race, parity and smoking, of low birthweight in term infants by level of caffeine use were 1.4 (<150 mg of caffeine per day), 2.3 (151–300 mg per day), and 4.6 (>300 mg per day) compared to women who consumed no caffeine. The adjusted mean decrease in birthweight was 105 g in infants of women consuming more than 300 mg of caffeine per day. No significant associations were observed between caffeine consumption and either preterm delivery or mean gestational age.

This study has several strengths. Because it was prospective, data on exposures were not biased by knowledge of the outcome. Several sources of caffeine intake were considered in arriving at an estimate of total consumption. Caffeine consumption was treated as both a continuous and a categorical variable, although only data from the categorical analyses were presented. Attempts were made to adjust for a number of important potential confounders, although the specification of variables (e.g. smoking: yes/no) was not always sufficient.

The utility of this study is probably limited by a number of the problems, especially in analysis. First, as stated earlier in the quote from Dales and Ury, selecting potential confounders based on low P values is fraught with problems. Indeed, some of the discarded covariates may, nevertheless, be important confounders of the risk-indicator/outcome association. Second, the report does not make it clear whether data on some variables, such as cigarette smoking, were specified in the multivariable models as dichotomous variables (their Table 3), or whether dichotomies were used only to calculate the associated OR estimates. If the former is true, it is unlikely that these potential confounders were adequately controlled in the models.

Perhaps the most important limitation of the study was the very reasonable decision to confine the analyses to term deliveries. The authors state that in preterm infants the ORs of low birthweight by category of caffeine use were less than 1.0, although non-significant. This result would be consistent with a protective effect for caffeine in infants of less than 7 weeks' gestation. Another possible interpretation is that caffeine is not associated with birthweight at any gestational age, but that it prolongs the gestation of low-birthweight infants. [This would be consistent with the observation of Berkowitz and colleagues of a reduced risk of preterm deliveries in women consuming 4 or more cups of coffee per day (C6).] In such circumstances, low-birthweight infants would be over-represented among caffeine consumers in comparisons confined to term infants. Information on the mean gestational age within birthweight strata for consumers and non-consumers of caffeine could certainly be helpful.

Another limitation of this study is that the investigators define intrauterine growth retardation as occurring

only in term infants. The authors do not seem to be aware of the tendency of black children to be of lower birthweight than white children, and of the usual definition of intrauterine growth retardation as less than the 10th percentile of birthweight for gestational age.

C20. Tierson F.D., Hook E.B., 1987, The effect of some embryotoxins in the maternal diet on pregnancy outcome. American Journal of Physical Anthropology 72, 262–263.

This abstract reports data from a prospective study of 400 “well-nourished” women in Albany whose pregnancies were ascertained by week 13 of gestation, and who were followed until delivery. Data on diet and cigarette smoking were obtained retrospectively for the month before the last menstrual period, and prospectively for weeks 12, 16, 20, 30 and 38 of pregnancy. These 400 pregnancies resulted in 372 live births and 28 fetal deaths. The outcomes of interest were birthweight and fetal death. When controlling for cigarette and alcohol consumption, the investigators found a significant negative association between infant birthweight and coffee, as well as total caffeine consumption. Smoking, coffee and alcohol consumption accounted for 4.5% of the variance in birthweight. A strong inverse association was seen between fetal death and the consumption of “some caffeine” during the pregnancy.

Because this is an abstract, insufficient detail is provided about many aspects of the study, including measurement of exposure, timing of exposure, coffee vs caffeine, and inclusion of covariates other than smoking and alcohol, such as maternal age, socioeconomic status and previous pregnancy history. Although this abstract was published 7 years ago, the full manuscript has yet to be published.

C21. Wilcox A., Weinberg C.R., Baird D.D., 1988, Caffeinated beverages and decreased fertility. Lancet 2, 1453–1455.

In this study of 104 women, those who daily consumed the amount of caffeine in 1 cup of coffee were less likely to get pregnant than women who consumed smaller amounts. This provocative report suffers from a number of limitations, some of which have been acknowledged (Weinberg and Wilcox, 1990). First, this study should not be viewed as testing a hypothesis, but rather it should be looked on as generating a provocative hypothesis. Prior to this study, no basis existed for the hypothesis that low levels of caffeine consumption delayed fecundability.

Second, this study fails to meet the basic criterion of having all participants at comparable risk of conception. Obviously the best documentation that a couple is fertile is a previous conception. Nevertheless, one-third of the women recruited by Wilcox and his associates

had not conceived previously. The authors provide no assurance that parity was independent of caffeine consumption in this sample, nor did they evaluate nulliparous women separately from their previously-pregnant peers.

Third, Dr. Wilcox and his colleagues eliminated 117 women who became pregnant in the first 3 months from their total sample of 221 women who agreed to collect daily urine specimens during the time they wanted to conceive. In essence, the investigators have carried out a subsample analysis, ignoring more than half the total sample. Looking only at those who did not get pregnant within 3 months is not a design limitation, but rather, an inferential quagmire (Holland, 1986; Stallones, 1987).

Fourth, the authors have assumed that the pattern of consumption during the first months of trying to conceive continued until conception occurred. The inappropriateness of this assumption is documented by evidence that coffee and cola consumption do have obvious seasonal fluctuations (Holland, 1986; Schreiber et al., 1988a,b; Guenther, 1986). In the United States, the birth rate peaks in or close to September, virtually the opposite of what might be expected if a sizable proportion of delayed fecundability could be attributed to consumption of caffeine-containing coffee (National Center for Health Statistics, 1988).

Fifth, obtaining caffeinated-beverage consumption during the fourth month of trying to conceive, and not during the first 3 months, poses another problem. A body of literature supports the hypothesis that anxiety is associated with reduced fertility (Peyser et al., 1973; Nijs et al., 1984; Adams et al., 1985; Harrison et al., 1986; Paulson et al., 1988). Anxiety might impair some process involved in fertilization (e.g. ovulation), although more plausible is the hypothesis that failure to conceive, despite prolonged attempts, results in anxiety about ever conceiving. Because some people during periods of heightened anxiety increase their food and beverage consumption, caffeine/coffee consumption might be a consequence, and not a cause of delayed fecundability. In the light of its limitations, this study does no more than generate a provocative hypothesis.

Cramer has called attention to the possibility that tannic acid, and not caffeine, might account for any association between decreased fertility and consumption of coffee, tea and cocoa (Cramer, 1990). This prompted Wilcox and Weinberg to re-analyze their data (Wilcox and Weinberg, 1991). They found that “tea had a negligible association with fertility, whereas soft drinks were strongly related to lower fertility”. After controlling for coffee consumption, frequency of intercourse, and age in the linear regression model, one caffeinated soft drink per day was associated with a 50% reduction in the monthly probability of conception. The magnitude of the association so exceeds what would be

anticipated if caffeine was responsible for the fertility reduction that the re-analysis is difficult to offer as support for the caffeine/decreased fertility hypothesis.

C22. Munoz L.M., Lonnerdal B., Keen C.L., Dewey K.G., 1988, Coffee consumption as a factor in iron deficiency anemia among pregnant women and their infants in Costa Rica. American Journal of Clinical Nutrition 48, 645-651.

In a sample of low-income breast-feeding Costa Rican women who denied tobacco and alcohol consumption during the pregnancy leading to the birth of their full-term babies, 22 coffee drinkers were compared with 26 women who did not drink coffee (but did tend to drink panela, a beverage made from sugar cane). The mean birthweight of babies born to coffee drinkers was lower than that of the babies born to coffee abstainers (3189 vs 3310 g).

This very small study has a number of limitations. Most prominent is the small sample size. In addition, bias may have resulted from selective recruitment. Intended as a study of iron deficiency, 110 women were recruited to provide three separate 24-h dietary recalls, a completed food frequency questionnaire, and a venous blood sample during the third trimester. The drop-out rate of close to 60% (62/110) raises the possibility of additional selection bias.

A third problem is that no attention was paid to potential confounding of the coffee/birthweight relationship. Coffee drinkers differed from abstainers in higher parity, lower education, lower income, later initiation of prenatal vitamin and mineral supplementation, and lower energy intake. The authors did carry out multivariate analyses for hemoglobin and hematocrit, which were the foci of this report, but for reasons that are not clear, they describe no multiple regression analyses for birthweight.

Prudence dictates that this study not be viewed as supporting the hypothesis that coffee/caffeine consumption lowers birthweight.

C23. Christianson R.E., Oechsli F.W., van den Berg B.J., 1989, Caffeinated beverages and decreased fertility. Lancet 1, 378.

This letter to the editor of the *Lancet* was written in response to the report by Wilcox and his colleagues (C21), published just 7 weeks before. In a sample of 6303 women, the probability of "difficulty conceiving" increased with the daily number of cups of coffee consumed prior to pregnancy. Unfortunately, their analyses, which controlled for whether or not women smoked, did not quantify cigarette smoking. Classifying the women who smoked one cigarette per day and those who smoked 40 cigarettes per day as being at similar

risk of tobacco-associated adversity leaves much to be desired. Because people who drink the most coffee have tended to smoke the most cigarettes, and people tend to under-report such socially undesirable habits as smoking cigarettes, Morrison may well be right that information about coffee consumption improves the precision of measures of cigarette consumption (Schreiber et al., 1988a,b). On this basis alone, the letter by Christianson and her associates should not be viewed as supporting the hypothesis of Wilcox and his colleagues.

C24. Brooke O.G., Anderson H.R., Bland J.M., et al., 1989, Effects on birthweight of smoking, alcohol, caffeine, socioeconomic factors and psychosocial stress. British Medical Journal 298, 795-801.

This study recruited 1513 white women from a consecutive series of 1860 who presented for prenatal care at a district general hospital in London. To investigate the effects of smoking, alcohol, caffeine, socioeconomic factors and psychosocial stress on birthweight, the authors collected data at three points during gestation about these antecedents.

After birthweight was adjusted for gestational age, maternal height, gender and nulliparity, the investigators found that coffee, tea and caffeine consumption were significantly associated with reduced birthweight, but no significant dose-response trend was seen with caffeine. Once adjustment was also made for smoking, the coffee, tea, and caffeine effects became non-significant.

The strengths of this study include relatively unbiased recruitment of subjects, prospective ascertainment of exposures and characteristics, minimal degradation of data collected as continuous variables, and appropriate adjustment for potentially confounding covariates. Although power estimates based on assumptions about caffeine consumption are not provided, the authors claimed that their study had a high power to detect an effect of smoking on birthweight, even in subsamples. Power to detect effects of coffee, tea and caffeine, however, are probably less than for effects of tobacco.

In light of the major strengths of this study, its results should be emphasized in any evaluation of the literature that addresses the issue of birthweight and caffeine consumption.

C25. Caan B.J., Goldhaber M.K., 1989, Caffeinated beverages and low birthweight: A case-control study. American Journal of Public Health 79, 1299-1300.

The authors collected birth outcome data from 9564 pregnancies followed for a study of the effects of aerial malathion spraying on pregnancy outcomes. They identified 97 women whose infant had a birthweight less than two standard deviations below the mean for gestational age, and an additional 34 women whose

babies weighed less than 2500 g. These 131 women were the cases, and 136 randomly selected women served as controls. Data about caffeine consumption during the first trimester was obtained postpartum.

Even after adjusting for important covariates, women who consumed more than 300 mg of caffeine per day did not have a significantly increased risk of delivering a low birthweight baby when compared with women who consumed no caffeine. Unfortunately, the authors view their data as demonstrating “a modest effect of caffeine consumption on fetal growth”. Their preference for that interpretation is only one of many difficulties with this report. Some of the more prominent limitations follow.

First, not only is this a study of a heterogeneous group of cases, but they are not representative of the universe of low-birthweight babies. Intrauterine growth retardation (IUGR; especially in full-term infants) is not the same entity as less extremely low birthweight, especially if many of the low birthweight infants are preterm (Arbuckle and Sherman, 1989). Combining these etiologically disparate groups is at best not good form, and at worst is a serious flaw.

Indeed, IUGR may itself be a heterogeneous group of entities (Villar and Belizan, 1982). What is also disturbing is the authors’ claim that they grouped the 97 IUGR babies with the 34 low-birthweight babies because their mothers did not differ in caffeine consumption. Are they not aware of the enormously low power with such small groups?

Second, with a base of approximately 9000 pregnancies, the authors could have defined IUGR based on the birthweight distribution in their own sample, rather than on the less appropriate birthweight distribution in the high-altitude population of Denver.

Third, approximately 6.5% of all babies born to white mothers are less than 2.5 kg (Eisner et al., 1979). In California, with a sizable population of Hispanics, blacks, and Orientals, the rate should be higher (Shiono et al., 1986). With 9000 pregnancies, approximately 630 low-birthweight babies should have been identified. Even with an 85% response rate, the investigators should have identified and recruited considerably more than 500 mothers. In essence, the number of cases represents less than a quarter of the expected [$131/(9564 \times 0.95 \times 0.07 \times 0.85)$]. This immediately leads to the supposition that this is a biased case series.

Fourth, collecting data retrospectively, especially from women who have recently given birth to a baby with obvious difficulties poses problems (Joffe and Grisso, 1985; Tilley et al., 1985; Werler et al., 1989). Indeed, they can be of such magnitude that one group recently advised against comparing data collected retrospectively from mothers of malformed infants to data collected retrospectively from mothers of babies without malformations (Shiono et al., 1986).

Fifth, the authors seem not to understand that some impairments of weight gain may have their origin in the

second and third trimesters (Villar and Belizan, 1982). They collected data about caffeine consumption during the first trimester, and yet women reduced their coffee consumption during pregnancy. Perhaps not all women reduced their consumption equivalently. Is it not possible that in addition to biased recall, the authors have misclassified some women based on first trimester rather than on second or third trimester caffeine consumption?

Sixth, adjustment was made for smoking 10 or more cigarettes per day, but not for the obvious dose-response relationship known to exist between smoking and birthweight (Hebel et al., 1988). As is discussed elsewhere in this review, caffeine consumption data may provide supplemental information about residual confounders (Savitz and Baron, 1989; Leon, 1993) such as cigarette consumption (Morrison, 1984). On the basis of inadequate attention to confounding, a caffeine–low birthweight relationship (especially if small/weak) can be expected.

Seventh, the authors trichotomized (i.e. “degraded”) their caffeine consumption data (without justification) rather than viewing caffeine consumption as a continuous variable. Perhaps they might have seen a more convincing caffeine–birthweight relationship if they did not use an ordinal scale.

Eighth, the authors reveal their bias in the inferences they draw from their data. Even when comparing “heavy” consumers to women who consume no caffeine, the lower bound of the CI of the adjusted OR was less than one. Nevertheless, the authors conclude in their abstract that their “data support previous findings of a modest effect of caffeine consumption on fetal growth”. They could, just as readily, have viewed their data as supporting previous findings of no relationship between caffeine consumption and birthweight.

The ideal way to study the effects of caffeine on low birthweight is to obtain information about consumption and covariates at multiple times during pregnancy and to follow these women until they give birth. Then the analyses should be limited to full-term babies to avoid issues related to prematurity (e.g. premature rupture of membranes, premature onset of labor, third trimester bleeding, rapidly worsening pre-eclampsia, etc.). Finally, birthweight should be treated as a continuum. Brooke and his colleagues (C24) carried out such a study, but Caan and Goldhaber did not.

C26. Joesoef M.R., Beral V., Rolfs R.T., et al., 1990, Are caffeinated beverages risk factors for delayed conception? *Lancet* 335, 136–137.

This brief report consists of two tests of the hypothesis that caffeine consumption diminishes a woman’s ability to conceive. The two samples consist of women recruited for another purpose. In the first sample of 2817 women who planned a pregnancy, the average

time to conceive did not vary with reported consumption of coffee, tea, cola or caffeine. The fecundability ratio comparing women who consumed more than 7 g of caffeine per month to those who consumed no more than 0.5 g per month was 1.03. Thus, among fertile women in this study, caffeine consumption did not appear to delay conception.

The investigators identify a number of ways their first study differed from that of Wilcox et al. (C26). Weinberg and Wilcox have also done so (Kline et al., 1989).

	Wilcox et al. (C25)	Joesoef et al. (C26)
Prospective	Yes	No
Recruitment	Volunteers planning to conceive	Women who delivered a liveborn child
Sample size	104	2817
Caffeine consumption: measured	When trying to conceive	After delivery
of interest	When trying to conceive	When not pregnant

Joesoef and his colleagues found that caffeine consumption and the time to conceive both varied with age, weight and cigarette and alcohol consumption. Even when these potential confounders were included in multivariate models, the fecundability ratio did not vary with caffeine consumption. In the second sample, caffeine consumption was no higher among 1818 women with primary infertility compared with their 1765 primiparous controls. In this extreme test of the hypothesis that caffeine consumption contributes to fertility "delay" after adjustments for potential confounders, women who sought medical care for infertility had a caffeine consumption distribution that did not differ from that of fertile women. Both of these studies, therefore, provide no support whatsoever for Wilcox's hypothesis that caffeine consumption delays/diminishes a woman's ability to conceive.

C27. Williams M.A., Monson R.R., Goldman M.B., Mittendorf R., 1990, Coffee and delayed conception. *Lancet* 335, 1603.

The studies raising the possibility that caffeine consumption delayed conception prompted Williams and her colleagues to re-evaluate data collected for another study. Compared with women who did not drink, those who consumed 4 or more cups daily were 81% as likely to become pregnant each cycle. When the median number of months to conceive was the measure of fecundability and cups of coffee consumed per day was the

exposure, no dose-response relationship was seen. These results, presented briefly in a Letter to the Editor of the *Lancet*, are not readily evaluated. For example, "Women with a history of infertility... were excluded," but infertility here is not defined. (The authors do define infertility among those included in their sample.)

C28. Hansteen I.-L., 1990, Occupational and lifestyle factors and chromosomal aberrations of spontaneous abortions. *Mutation and the Environment Part B*, pp. 467-475.

This is a report of preliminary results from the Telemark Central Hospital in Porsgrunn, "situated in the most industrialized part of Norway". Obviously, occupational exposures are the focus of this study of the antecedents of spontaneous abortion. Cases were women hospitalized for spontaneous abortion during the 2-year interval beginning in September 1985. The referent population consisted of women recruited from the antenatal clinic at weeks 17 and 18 of pregnancy, and matched on age only.

Details about the "partly self-administered" questionnaire are lacking. For example, the reader does not even know if the coffee-drinking variable refers to consumption before pregnancy or during the first trimester.

Another limitation of this report is that results of univariate analysis only are presented. No mention is made of any attempts to deal with potential confounders.

In light of these limitations alone, the observation of an over-representation of "heavy" coffee drinkers among aborters has virtually no meaning.

C29. Fenster L., Eskenazi B., Windham G.C., Swan S.H., 1991, Caffeine consumption during pregnancy and spontaneous abortion. *Epidemiology* 2, 168-174.

Cases were women who were at least 18 years of age and resided in Santa Clara County, California, who also had a spontaneous abortion by week 20 of gestation between June 1986 and February 1987 that was documented by histologic examination of aborted tissue. Two controls who delivered a liveborn infant were frequency matched to each case by last menstrual period and hospital. Data about caffeine and other potential antecedents were collected by telephone interview of 607 cases and 1284 controls, usually within 1 year after the pregnancy ended.

The crude OR for the 9% of cases who were considered "heavy" consumers of caffeine (i.e. they consumed 300 mg or more of caffeine per day) was 1.6 (95% CI=1.0,2.3), which decreased to 1.2 (95% CI=0.8,1.9) after adjusting for potentially confounding and other variables. Thus, support was not found for the main hypothesis that women who aborted were

more likely to be “heavy” consumers of caffeine than were women who did not abort.

Unless it is an explicit component of the hypothesis before data collection, subsample analysis should be viewed as a venture in hypothesis generation (Stallones, 1987). This caveat should be kept in mind when trying to make sense of the finding that, among women with early pregnancy nausea, “heavy” caffeine consumption was associated with “a doubled risk for spontaneous abortion” (adjusted OR = 2.1, 95% CI = 1.2, 3.7) and a halved risk among those who did not report nausea (adjusted OR = 0.5, 95% CI = 0.3, 1.0).

Caveats are always in order when the risk is doubled in one stratum (i.e. those with nausea), but halved in the other (i.e. those without nausea). Would the authors claim that caffeine consumption by pregnant women who have no nausea reduces the risk of spontaneous abortion?

Stein and Susser, in an accompanying editorial (1991), raise questions about the inferences to be drawn from the findings of Fenster and her colleagues. A minor modification of their argument follows:

1. First trimester nausea is more frequent in pregnancies carried to term than in those that end in early spontaneous abortion.
2. Nausea reflects a favorable balance of hormones produced by the placenta. When this hormone balance is disturbed (e.g. by diminished placental synthesis of these hormones), nausea subsides and pregnancy continuation is threatened. Although Stein and Susser suggest that chorionic gonadotropin might be the main placental hormone contributing to reduced caffeine consumption, estradiol and other estrogens deserve consideration (Petridou et al., 1992; Buyalos et al., 1992).
3. If nausea is accompanied/followed by diminished caffeine consumption, then nauseated women with viable fetuses will be more likely to reduce/eliminate caffeine consumption than will women who are not nauseated and whose pregnancies are in jeopardy. Caffeine consumption then becomes an epiphenomenon or marker of impending pregnancy failure. In this view, unchanged (i.e. “heavy”) caffeine consumption is a consequence, and not a cause, of the underlying circumstances resulting in late first trimester pregnancy loss.

Stein and Susser end their editorial with a list of study characteristics that are needed to avoid the error of assigning consumption occurring after the outcome event to the antecedent hypothesized causal exposure. The kind of precision needed to avoid this error “remains to be achieved”.

Nausea is only one explanation of how elevated placentally produced hormones reduce caffeine consumption. Early in pregnancy, even women who are not nauseated reduce their caffeine consumption (Caan and Coates, 1994; C44, C51). Thus, it seems plausible that nausea might not be the only “pregnancy signal” a woman receives that influences her beverage consumption.

Another way in which placental hormones might reduce caffeine consumption is by way of reduced rate of caffeine clearance. Beginning sometime before the middle of the pregnancy, the half-life ($t_{1/2}$) of caffeine tends to double and may even triple (Aldridge et al., 1981; Knutti et al., 1982; Brazier et al., 1983). Because the rate of caffeine clearance will influence blood levels, longer clearance can be expected to be accompanied by reduced caffeine consumption.

C30. Fenster L., Eskenazi B., Windham G.C., Swan S.H., 1991, Caffeine consumption during pregnancy and fetal growth. American Journal of Public Health 81, 458–461.

The controls selected for the authors’ study of spontaneous abortion (C29) served as the subjects of this study of three outcomes, low birthweight (defined as birthweight less than 2500 g, intrauterine growth retardation (defined as birthweight less than the tenth percentile for gestational age) and prematurity (defined as less than 37 weeks gestation). Among the 1230 women, consumption of 300 or more mg of caffeine per day during the month before pregnancy was associated with a significantly increased risk of intrauterine growth retardation, but not of low birthweight or of prematurity.

This study has a number of limitations. First, exposure information (i.e. caffeine consumption), confounder information (i.e. cigarette and alcohol consumption) and outcomes were all degraded. The authors assure the reader that findings similar to those presented in the paper were obtained when caffeine was modeled as a continuous variable. No mention, however, is made of evaluating tobacco consumption, alcohol consumption, birthweight, or gestational age as continuums.

Second, data were collected on average 9 months after delivery. Third, changes in caffeine were limited to the first 6 weeks of pregnancy (because this began as a spontaneous abortion study). This is unfortunate because some women first reduce their caffeine consumption later in pregnancy. Fourth, the authors, who felt nausea was so critical to their study of spontaneous abortion, neglect the potential confounding of this early pregnancy characteristic.

In light of these limitations, the authors’ findings might reflect the Morrison phenomenon (i.e. the caffeine

variable provides unique information about important, but inadequately measured covariates such as alcohol and tobacco; Morrison, 1984) or the Stein and Susser postulate (i.e. unchanged/"heavy" caffeine consumption may be a marker of suboptimal placental hormonal synthesis; Stein and Susser, 1991). In addition, the attributes of this study more closely approximate those of the study of Caan and Goldhaber (C25) than those of the far superior study of Brooke and his colleagues (C24). Thus, the authors have not provided convincing evidence that first trimester caffeine consumption in any way contributes to intrauterine growth retardation.

C31. Olsen J., 1991, Cigarette smoking, tea and coffee drinking, and subfecundity. *American Journal of Epidemiology* 133,734–739.

A total of 11,888 pregnant women who were residents of Odense or Aalborg, Denmark, during their third trimester completed a questionnaire about social conditions and lifestyle characteristics. Caffeine consumption was estimated based on number of cups of coffee and tea consumed per day before becoming pregnant. Two cups of tea were considered equivalent to one cup of coffee. Time to conception was estimated based on responses to the question, "For how many months did you and your partner attempt to achieve conception?"

A number of the study's limitations are identified by the author. Caffeine consumption before pregnancy was assessed during the weeks just prior to delivery, and not during the time before conception. Nevertheless, misclassification of coffee and tea consumption was deemed to be non-differential. In addition, not recruiting women who had not yet conceived limits the generalizability of the findings.

This study, however, has a number of strengths, including a large size and a sample relatively free of selection bias. More than 85% of all the women in a given region were recruited if they were pregnant and had not received treatment for infertility.

In 5602 non-smokers, subfecundity (whether defined as not conceiving within 6 or 12 months) was *not* associated with coffee/tea consumption. (An association between coffee/tea consumption and subfecundity was seen only for smokers and only among heavy caffeine consumers (8 cups of more per day)... (and) is statistically significant at the 5% level only when subfecundity is defined as a wait of more than 12 months before becoming pregnant. Among smokers, the OR of not conceiving in 12 months was 1.35 (95% CI = 1.02,1.48) for those who drank the equivalent of 8 cups of coffee per day relative to women who consumed 0–3 cups per day.

This study offers no support for the hypothesis that coffee/caffeine consumption substantially delays conception.

C32. Peacock J.L., Blasid J.M., Anderson H.R., 1991, Effects on birthweight of alcohol and caffeine consumption in smoking women. *Journal of Epidemiology and Community Health* 45, 159–163.

This re-analysis of data presented previously (C24) evaluated the interaction between caffeine consumption and cigarette smoking on birthweight. The mean birthweight ratio (i.e. observed birthweight relative to an external standard for that gestational age) did not decline with increasing caffeine consumption in non-smokers, but did among both light and heavy smokers (dichotomized at 13 cigarettes per day).

The authors seem to think that consumption of caffeine exacerbates the birthweight decline associated with cigarette smoking. An equally plausible interpretation is that the Morrison phenomenon is working here. Indeed, it may be especially likely because the authors have degraded their continuous variable of number of cigarettes smoked to tertiles (i.e. non-smoker, "light" smoker and "heavy" smoker). The caffeine variable may provide supplemental information about numbers of cigarettes smoked rather than information about any other ill-effect.

C33. Kline J., Levin B., Silverman J., Kinney A., Stein Z., Susser M., Warburton D., 1991, Caffeine and spontaneous abortion of known karyotype. *Epidemiology* 2, 409–417.

This study is unique in not considering all spontaneous abortions as a homogeneous entity. Each spontaneous abortion was classified as chromosomally normal or by the type of chromosomal aberration (e.g. trisomy, monosomy X, triploidy and other). Because the chromosomal aberrations differ in underlying genetic mechanisms, the authors began with the hypothesis that an adverse exposure would result in an association with a single karyotype group, but not the others.

Between 1982 and 1986, 927 women were interviewed who presented to the Columbia Presbyterian Medical Center in New York City with a spontaneous abortion and whose abortus was retrieved and karyotyped. Controls registered for prenatal care before week 22 of gestation and delivered after week 28. If caffeine were to contribute to chromosomal aberration, consumption would have to be very close to the time of conception. The authors classified two time periods of caffeine consumption. The first, "perifertilization", included the interval between 2 months before and 1 month after the last menstrual period. ORs adjusted for maternal age were calculated comparing those whose consumption was in the highest quartile (i.e. 225 mg or more per day) to women in the lowest quartile of caffeine consumption (?27 mg per day). The OR for chromosomally normal abortions was 1.0, and 0.8 for all chromosomally aberrant karyotypes.

The only elevation seen was for monosomy X. Relative to women in the lowest quartile of caffeine consumption, the odds of monosomy X for women in the highest quartile was 1.6 (95% CI=0.7,3.8). With 54 monosomy X cases, the authors had limited power. Nevertheless, the absence of a dose-response relationship leads to the inference that caffeine does not contribute to the occurrence of monosomy X, or of any other chromosomally-aberrant spontaneous abortion.

The second time interval of interest for caffeine consumption was “during pregnancy”, which included the interval from 1 month after the last menstrual period until the time of loss for cases, or of interview for controls. The authors hypothesized that if caffeine consumption were to play a role in chromosomally-normal abortions, the caffeine would have to be consumed in this “during pregnancy” interval. The maternal age-adjusted OR for chromosomally-normal spontaneous abortions was 1.9 (95% CI=1.3,2.6). This would lead to the inference that caffeine consumption contributes to spontaneous abortion. However, the authors also found that the OR of chromosomally-aberrant spontaneous abortions was also elevated for women whose caffeine consumption *during* pregnancy was in the highest quartile [OR = 1.6 (95% CI = 1.1,2.3)]. Because caffeine consumption after the first month of gestation is unlikely to influence the risk of chromosomal aberration, the authors considered the possibility that the greater average reduction in caffeine intake level in controls compared with cases reflected phenomena now contributing to intrauterine fetal loss. Although the authors offer a number of explanations for their observations, the most intriguing is offered elsewhere by two of the authors of this study (Stein and Susser, 1991). These authors considered the possibility that because reduced consumption of caffeine during pregnancy is associated with nausea, and nausea is more common in women who do not abort, that the non-viable fetal/placental unit produces less of a nausea-promoting substance than does a surviving conceptus. In this explanation, caffeine consumption is an epiphenomenon.

C34. Parazzini F., Bocciolone L., Fedele L., Negri E., La Vecchia C., Acaia B., 1991, Risk factors for spontaneous abortion. *International Journal of Epidemiology* 20, 157–161.

In this case control of spontaneous abortion, 94 women who had two or more unexplained miscarriages before the end of the first trimester were compared with 176 women admitted to the same facility for a normal delivery and who never had a miscarriage. Questions about coffee consumption and other lifestyle characteristics were asked for “the last miscarrying pregnancy for cases and...the first trimester of the index

pregnancy for controls. Coffee drinkers were not deemed at increased risk of recurrent abortion (OR = 1.4, 95% CI = 0.7, 2.6).

This is an unusual study in several ways. First, the cases had not simply had a miscarriage, but were recurrent aborters who never had a normal pregnancy. Second, women were dichotomized into those who drank any coffee and those who did not. This is a classical example of what Leon calls overaggregation (Leon, 1993). The amount of coffee/caffeine consumed is not described, and so the reader has no sense of the magnitude of the potential errors that might be expected because of this overaggregation. No attention is given to nausea, anorexia, or any other aspect of the SteinSusser postulate (Stein and Susser, 1991).

C35. Armstrong B.G., McDonald A.D., Sloan M., 1992, Cigarette, alcohol, and coffee consumption in spontaneous abortion. *American Journal of Public Health* 82, 85–87.

During a 2-year period in the early 1980s, 56,067 women who were admitted to the obstetrical services of 11 Montreal hospitals for either a miscarriage (spontaneous abortion), or delivery were interviewed shortly after termination of the pregnancy. The authors acknowledge that women treated in hospital for spontaneous abortion are not representative of all women who have a spontaneous abortion. Thus, they chose to focus on spontaneous abortions in previous pregnancies. The OR for spontaneous abortion was 1.2 (95% CI=1.0,1.3) for women who consumed 5–9 cups of coffee during the index pregnancy and 1.2 (95% CI=0.97,1.5) for women who consumed 10 or more cups of coffee per day. In the next-to-last paragraph of the discussion, the authors acknowledge “the small risk associated with coffee consumption might be explained by residual confounding.”

If, as the authors acknowledge, women who come to hospital for vaginal bleeding associated with a spontaneous abortion are not representative of all women who have a spontaneous abortion, then why did they include approximately 7000 women who were admitted for a current spontaneous abortion? Looking at their previous pregnancies does not eliminate selection bias. In 1987 the authors wrote, “Substantial and statistically significant excesses on spontaneous abortion were observed in nursing aides, women in sales occupations and food and beverage service” (McDonald et al., 1986). Nevertheless, the authors ignored this and divided their sample into those employed and not employed. An “employment during pregnancy” variable was included in the logistic regression analyses, but the authors did not identify those occupations at increased risk of spontaneous abortion. Perhaps the most important limitation of this paper and the two papers that follow (C34 and C35) is the low quality of

exposure information. Can one really expect a woman to accurately recall her coffee consumption years ago, especially during the first week of pregnancy, especially when she may not have known or been certain she was pregnant? If the ascertainment of coffee, alcohol and cigarette consumption years before is fraught with problems, they are magnified when the woman has experienced a pregnancy adversity (Werler et al., 1989).

C36. McDonald A.D., Armstrong B.G., Sloan M., 1992, Cigarette, alcohol, and coffee consumption and prematurity. American Journal of Public Health 82, 87–90.

This paper is drawn from the same population described in the study above (C35). In this sample of 40,000 women, the risk of prematurity, defined as less than 37 weeks' gestation, was 1.1 (95% CI=0.9,1.3) for women who consumed 5–9 cups of coffee per day and 1.2 (95% CI=0.9,1.8) for women who consumed 10 or more cups per day during this pregnancy.

Although the title would lead one to think that prematurity was the only focus of interest, the authors also looked at low birthweight (2.5 kg) and low birthweight for gestational age, defined as the bottom 5%. The risk of delivering a low birthweight infant was 1.4 (95% CI=1.0,2.0) for women who consumed 10 or more cups of coffee per day. For women who consumed 5–9 cups of coffee per day, the odds of delivering a child who was in the bottom 5% of birthweight for gestational age was 1.3 (95% CI=1.1,1.7). For women who consumed 10 or more cups of coffee per day, the odds were 1.4 (95% CI=0.97,2.0).

Although this study has the advantage of a large sample, it does have a number of limitations. First, the authors group all pregnancies before week 37 of gestation as reflecting a single entity. The phenomena that lead to delivery during the earliest part of the third trimester will not be the same as those that occur during the middle of the third trimester. Second, premature delivery probably reflects at least four separate groups of disorders, including premature rupture of membranes, premature onset of labor, maternal illness (usually pre-eclampsia/toxemia), and vaginal bleeding (often placenta previa or abruption).

The exposure information is not detailed. Thus, the reader does not know if coffee consumption reflects third trimester consumption or consumption in the first trimester as was identified in C34.

In this sample, the risk of low birthweight was increased among chamber maids, cleaners and janitors, and women employed in the manufacture of food and drink, metal and electrical goods and clothing (Armstrong et al., 1989). In this paper, however, as they do in their other related papers, the authors ignored their own findings. Indeed, they make no adjustment whatsoever in their analyses for employment of any kind.

C37. McDonald A.D., Armstrong B.G., Sloan M., 1992, Cigarette, alcohol, and coffee consumption and congenital defects. American Journal of Public Health 82, 91–93.

In this, the third article in a series, "Congenital defects were ascertained (blind) in current pregnancies from pediatric records." For reasons that are not clear, the authors classify coffee consumption as 1–2 cups per day and 3 or more cups per day. What happened to the 5–9 cups per day and 10+ cups per day groups? Of the eight groups of congenital defects evaluated, only the cardiovascular group showed a statistically significant association with consumption of three or more cups per day [OR=1.5 (95% CI=1.1, 2.2)].

Having the pediatric examination at the time of discharge serve as the source of information about congenital defects poses two problems. First, routine clinical examination tends to underestimate the occurrence of congenital defects (Mills et al., 1983). Second, assessing malformations only at the time of discharge from the birth admission may fail to identify malformations that become biologically important weeks to months later. This is especially important for cardiovascular malformations. A less common problem is the misclassification of a child as having a defect, when in fact the physiologic disturbance is a transient one and of little, if any, biologic significance.

In this sample, congenital defects were associated with employment in the child-care field, certain service occupations, and the manufacture of electrical goods (McDonald et al., 1988). Nevertheless, here again, the authors ignored their own findings in this sample, and did not consider these occupations at all in their analytic strategy.

"No specific type of heart defect was overrepresented in the 58 cases born to women who drank 3 or more cups of coffee per day compared with the distribution of cardiovascular defects in babies born to the 101 women who consumed no coffee". If a coffee constituent disturbed heart development, then a specific type or group of malformations would be expected. In the absence of any specificity, a biologically plausible link is unlikely. Perhaps the most revealing phenomenon is that the authors chose not to mention the heart defect/coffee consumption association in the abstract.

C38. Godel J.C., Pabst H.F., Hodges P.E., Johnson K.E., Froese G.J., Joffres M.R., 1992, Smoking and caffeine and alcohol intake during pregnancy in a northern population: effect on fetal growth. Canadian Medical Association Journal 147, 181–188.

This is a study of 162 women who presented for prenatal care in 10 communities of the western region of the northwest territories of Canada and subsequently gave birth in Inuvik during the years 1987–1990. As

might be expected, this is a unique sample of women, with 35% Inuit, 38% Native Indian, 23% White and 19% classified as “mixed race”. Information about “caffeine intake was estimated through the use of the questionnaire and dietary assessment.” The authors acknowledge that there was good correlation between the two methods ($r=0.84$), but failed to clarify how they incorporated information from both sources.

To control for gestational age, the authors used a weight ratio comparing measured birthweight to standard weights for gestational age, very similar to the approach used by Brooke, Peacock and colleagues (C24, C32). In essence, this is equivalent to calculating a Z score.

Once smoking was controlled for, the birthweight was not associated with caffeine intake. Similarly, infant length and head circumference were not associated with caffeine consumption.

The authors acknowledge most of the potential limitations of the study, including quality of exposure data, small sample size, and the uniqueness of their sample. Nevertheless, in the three tables and one figure in this manuscript, nowhere are data presented for the relationship between caffeine consumption and birthweight. The most logical interpretation of this absence is that the authors did not consider it important.

C39. Williams M.A., Mittendorf R., Stubblefield P.G., Lieberman E., Schoenbaum S., Monson R.R., 1992, Cigarettes, coffee, and preterm premature rupture of the membranes. *American Journal of Epidemiology* 135, 895–903.

The subjects of this study were 307 women who had preterm premature rupture of the membranes and delivered before week 37 of gestation, 488 women who delivered before week 37 of gestation because of premature onset of labor, and 2252 randomly selected women who delivered at the same institution during the same 4 years, 1977–1980. Information about exposures and potential confounders was obtained by personal interview during the immediate postpartum period and by review of medical records. Compared with women who consumed no coffee, those who consumed 3 cups of coffee per day during pregnancy had an OR for premature rupture of membranes of 2.4 (95% CI = 1.5, 4.0), but the risk did not increase with increasing number of cups of coffee consumed per day. The increased risk of premature onset of labor associated with coffee consumption was less prominent, did not achieve nominal significance for any level of coffee consumption, and did not increase with increasing number of cups of coffee consumed per day.

The sample for this study is the sample that was used for the 1982 paper of Linn et al. (C7). For reasons that are not clear, the authors have made this a nested case-

control study, rather than the prospective study it could have been.

Clearly, the authors did not adjust for smoking as a continuous variable (i.e. number of cigarettes per day). They do not, however, make it clear whether they used the interval scale reported in parts of this manuscript (0, 1–9, 10–19, 20+ cigarettes smoked per day) or the dichotomous variable (i.e. smoking: yes/no). Either way, it is possible that the Morrison principle applies here, with coffee consumption variable conveying additional information about smoking, as well as about other risk factors for premature rupture of membranes.

Premature rupture of membranes and premature onset of labor increasingly appear to be related to sub-clinical infection of the membranes and amniotic fluid (Gibbs et al., 1992; Seo et al., 1992). The failure of the authors to acknowledge that there are presumed risk factors other than cigarettes and coffee poses a problem. Consider the possibility that in 1980, health-conscious women were not reducing their coffee consumption during early pregnancy. They might, at that time, have reduced their cigarette smoking. If women with the most sexual partners or with other lifestyle characteristics that would increase their risk of uterine infection at any time in pregnancy were those most likely to have smoked cigarettes, then coffee consumption as a correlate of cigarette smoking would be identified as a risk factor of premature rupture of membranes, when in fact neither coffee nor cigarettes played a role in the causal chain. More important than this conjecture is the observation that no dose–response relationship was seen between coffee consumption and either premature rupture of membranes or premature onset of labor.

C40. Mills J.L., Holmes L.B., Aarons J.H., Simpson J.L., Brown Z.A., Jovanovic-Peterson L.G., Conley M.R., Graubard B.I., Knopp R.H., Metzger B.E., 1993, Moderate caffeine use and the risk of spontaneous abortion and intrauterine growth retardation. *Journal of the American Medical Association* 269, 593–597.

In this study, 431 women enrolled within 21 days of conception as non-diabetic controls in the Diabetes in Early Pregnancy Study and were monitored throughout pregnancy to determine exposures, fetal loss, fetal growth as assessed by ultrasonography and birthweight. The OR of intrauterine growth retardation (i.e. birthweight less than the tenth percentile for gestational age) for women who consumed 300 or more mg of caffeine per day was 1.1 (95% CI = 0.9, 1.4).

The women, who were planning to get pregnant, were recruited from corporations, medical centers and pre-paid health plans, thereby avoiding some selection biases. In retrospect, one quarter were already pregnant for 3 weeks or less when recruited.

Accurate dating of pregnancy was assured by assessment of human chorionic gonadotropin. Information about caffeine consumption was obtained by asking the question, "How many cups or glasses (8 oz.) of each of the following are you now drinking on an average day?" This was asked for regular coffee, tea, cocoa and cola drinks.

The adjusted OR for spontaneous abortion among women in the highest caffeine group was 1.2 (95% CI = 0.9, 1.5). Early fetal growth, as assessed by crown-rump length on ultrasonographic examination did not appear to be affected by caffeine.

The main limitation of this study is that only 13 women consumed more than 300 mg of caffeine per day. Thus, the study had a low power to detect even a tripling of the risk of intrauterine growth retardation associated with the highest level of caffeine consumption (Eskenazi et al., 1993; Hatch and Bracken, 1993). Nevertheless, this study did have a power of more than 90% to identify a doubling of the risk of all outcomes with each 100 mg increase in daily caffeine consumption. The two unusual strengths of this study are that the authors were able to identify all spontaneous abortions occurring after day 21 of gestation, and the prospective design and repeated ascertainment of caffeine consumption at weeks 6, 8, 10, 12, 20, 28 and 35, thereby avoiding biased recall.

C41. Grodstein F., Goldman M.B., Ryan L., Cramer D.W., 1993, Relation of female infertility to consumption of caffeinated beverages. American Journal of Epidemiology 137, 1353–1360.

In this contribution to the literature assessing the relationship between caffeine consumption and delayed conception, the cases are 1880 women who sought care at one of seven infertility clinics in the United States and Canada. They were compared with 4023 controls who were admitted for delivery of a live birth to hospitals adjacent to the infertility clinics. One of the unique characteristics of this study is that the infertile women were each given a primary diagnosis. This allowed the authors to subdivide the infertile women into five case groups: ovulatory factor, tubal disease, cervical factor, endometriosis and idiopathic. The OR of tubal disease was 1.5 (95% CI = 1.1, 2.0) for women who consumed more than 7 g of caffeine per month. The RR for endometriosis was 1.6 (95% CI = 1.1, 2.4) for women in this highest caffeine consuming group of women. Women who consumed 5.1–7 g of caffeine per month were not at increased risk of tubal disease, but were at increased risk of endometriosis. The authors offer the inference that in both cases a threshold effect was seen.

Infertility attributed to ovulatory and cervical factors was not associated with coffee or caffeine exposure. Similarly, and perhaps more important than any other

finding in this study is the observation that consumption of the largest amounts of caffeine did not appear to place a woman at increased risk of idiopathic infertility.

The authors acknowledge a number of limitations themselves. Perhaps the most important is that pregnant controls may have reduced or underreported their caffeine consumption (Hook, 1976, 1978; Diugosz and Bracken, 1992; C14). The authors also raise the possibility that they might have failed to adequately control for potential confounders. Although they feel they controlled adequately for cigarette smoking, others might disagree. Classifying women as non-smokers, current smokers and former smokers does not adequately discriminate between women who consume five cigarettes per day and those who smoke two packs per day. Thus, the authors' findings may reflect nothing more than the Morrison (1984) effect.

In their final paragraph, the authors contort themselves in trying to explain how caffeine consumption might contribute to tubal disease or endometriosis. The absence of a plausible biologic explanation should add to the skepticism with which these data are considered.

C42. Fortier I., Marcoux S., Beaulac-Baillargeon L., 1993, Relation of caffeine intake during pregnancy in intrauterine growth retardation and preterm birth. American Journal of Epidemiology 137, 931–940.

The 7025 women who are the subject of this study resided in the territory of six community health departments in and around Quebec City and gave birth in 1989 to a liveborn singleton weighing at least 500 g. Information about caffeine consumption and potential confounders were obtained postpartum by telephone interview. Caffeine intake was not related to preterm delivery or low birthweight. Compared with babies of mothers who consumed less than 10 mg of caffeine per day, those who consumed between 11 and 150 mg of caffeine per day had an adjusted OR of intrauterine growth retardation of 1.3 (95% CI = 1.0, 1.6). The OR increased to 1.4 (95% CI = 1.1, 1.9) for babies whose mothers consumed 151–300 mg of caffeine per day and to 1.6 (95% CI = 1.1, 2.3) for newborns whose mothers consumed more than 300 mg of caffeine per day. Although consumption of the largest amounts of caffeine was associated with an increased risk of intrauterine growth retardation, caffeine intake was not related to a reduction in adjusted mean birthweight (Shiono and Klebanoff, 1993). The authors defend this inconsistency by suggesting that caffeine did not shift the overall distribution of birthweight, but only skewed the distribution to the left toward smaller babies (Marcoux et al., 1993).

Shiono and Klebanoff, in their commentary about this paper, appeared to extend the Stein–Susser hypothesis to include not only miscarriages, but also intrauterine

grown retarded infants (Shiono and Klebanoff, 1993). They did not discuss the issue that babies destined to have intrauterine growth retardation might have placentas that produce less human chorionic gonadotropin than do babies without any growth retardation. Nevertheless, they suggest that nausea and vomiting might have been more common among the mothers of babies without any growth retardation than among mothers of babies who were small for dates. In their response to this criticism, the authors pointed out that vomiting was reported by approximately 20% of women regardless of their caffeine consumption (Marcoux et al., 1993).

Although the authors did adjust for number of cigarettes smoked per day (0, 1–5, 6–15 and 16+), they did not adjust for mother's weight, weight gain during pregnancy, or the occurrence/severity of maternal hypertension. This is unfortunate because such maternal risk factors appear to influence the risk of intrauterine growth retardation (Levkoff et al., 1982; Ounsted et al., 1985; Lang and Lieberman, 1992).

C43. Larroque B., Kaminski M., Lelong N., Subtil D., Dehaene P., 1993, Effects on birthweight on alcohol and caffeine consumption during pregnancy. *American Journal of Epidemiology* 137, 941–950.

The women recruited for this study sought prenatal care at a public hospital in northern France during an 8-month interval during 1985 and early 1986. The 684 women were interviewed at their first prenatal visit. The referent group consisted of the 73% of women who consumed 400 mg or less of caffeine per day. The highest exposure group consumed more than 800 mg per day and represented 7% of the sample. This was a unique sample, however, by virtue of the levels of alcohol consumption. Fully 27% of women consumed more than one drink per day during pregnancy. The relation observed between maternal caffeine consumption and baby's birthweight disappeared after adjustment for maternal smoking.

Because caffeine consumption is widespread in this community, only 6% of the pregnant women did not consume any caffeine. Thus, the authors felt compelled to group non-consumers with those who consumed what others might consider moderate amounts of caffeine during pregnancy. The result may have been some caffeine "exposure" of women in the referent group (Wynder and Stellman, 1992). In responding to this criticism (Shiono and Klebanoff, 1993), the authors pointed out that they grouped all women who consumed less than 400 mg caffeine per day only after convincing themselves of no dose-response relation between the risk of low birthweight and caffeine consumption of 0, 1–100, 101–200, 201–400 mg per day (Larroque et al., 1993).

Because the authors wanted to recruit as many women who consumed moderate levels of alcohol, they

allowed recruitment to include women who presented in the third trimester. Although the reference period for the study was the first trimester of pregnancy, for some women the information about exposures was collected then, whereas for other women it was collected in the third trimester. As a consequence, perhaps those at highest risk of giving birth to a low-birthweight baby (i.e. those who present late for prenatal care) may have been most likely to provide the lowest quality information about antecedents. The authors responded to this criticism (Shiono and Klebanoff, 1993) by pointing out that when they examined data limited to women who presented in the first trimester, they found no caffeine-birthweight relationship (Larroque et al., 1993). Similarly, they compared first and third trimester interviews in a sample of 131 women and found "no difference between the average caffeine consumption in the first trimester and that of the third".

C44. Infante-Rivard C., Fernandez A., Gauthier R., David M., Rivard G.-E., 1993, Fetal loss associated with caffeine intake before and during pregnancy. *Journal of the American Medical Association* 270, 2940–2943.

This study was designed to evaluate the relationship between lupus antibodies and death of a fetus at any gestational age. Almost three-quarters of the cases were miscarriages before week 17 (mean gestational age = 10.9 weeks). Fully 10% of the cases were stillbirths in the third trimester. The adjusted ORs for fetal loss associated with caffeine intake greater than 321 mg per day during pregnancy were 2.6 (1.4, 5.0). The authors also report a dose-related linear trend in which the OR was increased by a factor of 1.2 for each 100 mg of caffeine ingested daily during pregnancy.

	Mills et al. (C40)	Infante-Rivard et al. (C44)
Prospective	Yes	No
Original interest	Diabetes	Lupus antibodies
Recruitment	Volunteers planning to conceive	Women who experienced a fetal loss and their pregnancy controls
Sample size	431	331 cases 993 controls
When caffeine consumption was assessed	0–3, 6, 8, 10, 12 weeks of gestation	After the fetal death

This manuscript has quite a number of limitations, a few of which were identified by Brenda Eskenazi (Eskenazi, 1993) in her editorial accompanying this paper. Additional limitations are identified, but discuss only the most important here.

2.1. Heterogeneity of cases

The authors began this study looking for a relationship between lupus antibodies and the death of a fetus *any* gestational age (thus, they prefer the term *fetal loss* to *miscarriage*). Others who study pregnancy in women with lupus consider spontaneous abortion separately from fetal death in utero (Nicklin, 1991). Miscarriages occurring during weeks 10–12 of gestation do, indeed, tend to have a risk profile that differs considerably from that of stillbirths occurring in the third trimester (Modvig et al., 1990). Although the authors did describe the number of cases and controls at each gestational age, they did not describe the relationship between caffeine consumption and fetal loss in each of these gestational age strata.

2.2. Selection bias

The authors recruited 98% of eligible controls but only 64% of eligible cases. Apparently this discrepancy reflects the added requests for repeated blood samples from the cases. The low rate of recruitment of cases is disturbing, but even more so in light of the discrepancy between the rates of case and control recruitment.

The authors matched controls to cases on gestational age. The problem with this is that the cases came to medical attention because of an emergency situation, most commonly vaginal bleeding. Apparently they had not yet presented for routine prenatal care. Controls, on the other hand, did present at a comparable gestational age. Thus, controls differed from cases in a number of ways, including the socioeconomic characteristics identified by the authors. Better educated, more attentive, and perhaps more anxious women, such as the controls, may be more likely to alter their diet during early pregnancy. Adjusting for mother's level of education may not be sufficient to eliminate such biases.

2.3. Confounders

It is now almost 20 years since Alan Morrison first offered the hypothesis that inadequate adjustment for smoking will allow a coffee or caffeine variable to carry information about such covariates as the number of cigarettes smoked per day (Morrison, 1984). In this paper, the authors claim to have adjusted for smoking and alcohol, but it appears that these were yes/no variables and did not provide any information about the number of cigarettes smoked per day or the number of ounces of alcohol consumed per day or week. Thus, if smoking or alcohol conveys any information about the risk of fetal loss, and the authors do not provide us with any such information, then failure to adequately adjust for the amount of cigarettes smoked or alcohol consumed would provide the caffeine variable with the

opportunity to compensate for this deficiency (Savitz and Baron, 1989; Leon, 1993).

Stein and Susser (the authors' citation No. 18) pointed out that pregnancies with early nausea were more likely to come to term than pregnancies without appreciable nausea during the first trimester (Stein and Susser, 1991). Thus, the nausea appears to be a marker of a good implantation, and might very well reflect some hormone produced in good quantities by a healthy placenta (e.g. human chorionic gonadotropin). Women with prominent nausea tend to reduce caffeine consumption and usually subsist for large portions of the day on dry crackers and sips of water. By and large, no other beverage is consumed when the woman is nauseated.

The authors nominally cite the Stein–Susser hypothesis, while trying to minimize the possibility that the Stein–Susser phenomenon accounts for their observations. Nevertheless, the authors do report that controls reduced their mean caffeine consumption by 144 mg per day, whereas, cases reduced their mean caffeine consumption by only 114 mg. This difference is compatible with the Stein–Susser postulate.

In light of these limitations, the authors have not provided convincing evidence that caffeine consumption by pregnant women in any way contributes to a heightened risk of miscarriage or stillbirth.

C45. Little R.E., Weinberg C.R., 1993, Risk factors for antepartum and intrapartum stillbirth. *American Journal of Epidemiology* 137, 1177–1189.

This set of case-control studies is based on data from the 1980 National Natality Survey and the National Fetal Mortality Survey (Placek, 1984). Approximately 50% of a sample of married mothers of fetal deaths and live births completed and returned a mailed questionnaire about demographic and lifestyle characteristics. This information was supplemented with data abstracted from the mothers' hospital records. The fetal deaths were divided into two groups according to the time of death.

In univariate analyses, 20% of mothers of live births, 23% of antepartum deaths, and 20% of intrapartum deaths daily consumed 3 or more cups of caffeinated coffee and/or tea during pregnancy. The OR for antepartum death adjusted for eight potential confounders was 1.4 for fetuses whose mothers consumed 5 or more cups of caffeine-containing coffee and or tea. The comparable OR for intrapartum death was 1.2.

Smoking more than one and a half packs per day was associated with reduced risk of both antepartum and intrapartum death. Alcohol consumption was also associated with reduced risk of intrapartum death. In light of the low RRs associated with caffeine consumption and the unexpected relationships between stillbirth

and maternal consumption of alcohol and tobacco, caution is advised in drawing inferences from this report.

C46. Hatch E.E., Bracken M.B., 1993, Association of delayed conception with caffeine consumption. American Journal of Epidemiology 138, 1082–1092.

Data for this report came from a study originally designed to estimate to what extent induced abortions influenced subsequent pregnancies (Bracken et al., 1986). Analysis was restricted to 1909 women who had not conceived while using a method of birth control, and who conceived while married. Information about caffeine consumption was restricted to the interval between conception and the date of interview, although cigarette smoking was assessed for the year before conception, and alcohol consumption was assessed for the month before conception.

The mean time to conception among women who denied caffeine consumption was 4.5 menstrual cycles, whereas the means were 5.0 cycles for “light” caffeine consumers (1–150 mg/day), 5.1 cycles for moderate consumers (150–300 mg/day) and 5.5 for heavy consumers (>300 mg/day). “There was no overall association between the levels of caffeine intake and time to conception according to the log rank test.”

The unadjusted ORs for conception delay of more than 12 cycles were 1.3 for light, 1.6 for moderate, and 1.5 for heavy caffeine consumers. Only after adjusting for last method of birth control, parity (0, 1, 2+) and cigarette smoking (none/any? or none/1–10/11–20/21+ cigarettes per day?) did the lower bound of the two highest consumption groups exceed one (i.e. 1.4 for light, 1.9 for moderate and 2.2 for heavy caffeine consumption). The authors attribute this increase in ORs only when adjusting for parity and cigarette smoking to the negative confounding conveyed by these variables. This is plausible for parity because the relationship between parity and fecundability ratio appears to be linear. Nevertheless, the nominal statistical significance for the relationship between conception delayed 12 cycles or more and heavy caffeine consumption was achieved only after the cigarette smoking variable was added to the logistic regression equation. This would be plausible if those who smoked the most cigarettes were at lowest risk of delayed conception. In Table 1, however, the unadjusted probability of conception delay beyond 12 cycles was 10.3% for caffeine abstainers, 11.4 for light, 9.1 for moderate and 11.2 for heavy caffeine consumers. Nevertheless, the authors wrote, “Level of cigarette smoking prior to conception, while not significantly associated with delayed conception, had estimates that were indicative of a possible protective effect.” This is in contrast to other studies, which have found that cigarette smoking is associated with reduced

fertility (Suonio et al., 1990; Olsen, 1991; Laurent et al., 1992; Van Voorhis et al., 1992; Tzonou et al., 1993). When the primary data do not satisfy the inferences drawn by the authors, one needs to question their inferences or their data.

Also disturbing is the observation that the fecundability ratio (i.e. the probability of conception in each cycle in caffeine consumers relative to that in caffeine abstainers) was significantly reduced when parity was considered and cigarette smoking (the great enigma) was not.

The tendency of the authors to go beyond their data, and the unexpected negative confounding associated with cigarette smoking, pose inferential problems. As with many other studies reviewed in this document, caution is advised in drawing inferences from this study.

The authors acknowledge the criticism of Caan and Coates (1994) that caffeine consumption during early pregnancy was a suboptimal surrogate for consumption during the time prior to conception (Hatch and Bracken, 1994). Caan and Coates also pointed out that they have data in support of the hypothesis that women with short conception times are more likely to reduce their caffeine intake than are women who need more time to conceive. They offer two explanations for their findings, both of which have the potential to explain away the findings of Hatch and Bracken. In one explanation, the most fertile women experienced stronger and earlier pregnancy symptoms. In the other, the most fertile women are more health conscious, and their decision to reduce caffeine intake is a marker of other behaviors that increase the probability of a short conception time.

C47. Al-Ansary L.A., Babay Z.A., 1994, Risk factors for spontaneous abortion: A preliminary study on Saudi women. Journal of the Royal Society of Health, August, 188–193.

This case-control study of spontaneous abortion from King Khalid University Hospital in Riyadh, Saudi Arabia, compared 226 women who were hospitalized for spontaneous pregnancy loss before the end of week 24 of gestation (86% of whom aborted before week 13 of gestation) to 226 women admitted consecutively for normal delivery and who had no history of bleeding during that pregnancy. Caffeine consumption was calculated on the basis of the content assumed in each beverage serving (i.e. instant coffee = 70 mg, Arabic coffee = 25 mg; tea = 40 mg and cola = 40 mg).

Approximately 30% of cases (67/226) and 18% of controls (41/226) consumed more than 150 mg of caffeine in beverages every day [OR = 1.9; 95% CI = 1.2, 3.0]. Although data collection for this study began October 1992, more than 1 year after the publications of Kline et al. (C33), and Stein and Susser

(1991), no attention was addressed to the Stein-Susser postulate. Specifically, no assessment is presented of changes in caffeine consumption from prepregnancy to near the end of the first trimester. Similarly, no attention was drawn to the implications of chromosomally normal and chromosomally abnormal spontaneous abortions. This report also suffers from overaggregation of caffeine consumption and from a lack of attention to potential confounders.

C48. Florack E.I.M., Sielhuis G.A., Rolland R., 1994. Cigarette smoking, alcohol consumption, and caffeine intake and fecundability. *Preventive Medicine* 23, 175–180.

During the years 1987–1989 women who performed non-medical functions at 39 Dutch hospitals were invited to participate in an observational study if they were planning to become pregnant during the forthcoming year. The study was designed to evaluate the contribution of occupational (and not behavioral) factors to early pregnancy failure.

The authors used a caffeine index that had been used previously (C21). A caffeine drink is defined as containing 100 mg (with 1 cup of coffee viewed as equivalent to 2 cups of tea or 2.5 glasses of cola). Among the 259 women who participated in this study, only 48 consumed less than 300 mg of caffeine per day. Women in this study who consumed 700 or more mg of caffeine each day did not have a decreased fecundability ratio.

The major strength of this study is the prospective design. According to the authors, the major weakness of this study is the lack of information about the frequency of sexual intercourse. In stable relationships this the frequency of intercourse does not appear to confound the relationship between behavioral risk factors and fecundability (C21).

C49. Parazzini F., Tossi L., Charenoud L., Restelli S., Lucini L., La Vecchia C., 1994, Alcohol and risk of spontaneous abortion. *Human Reproduction* 9, 1950–1953.

In this study, 462 women who experienced documented fetal loss during the first 12 weeks of gestation were compared with 814 women who carried to term. These data were collected in the years following 1990, which differentiates this manuscript from the authors previous search for the antecedents of fetal loss (C34; see also Parazzini et al., 1994). The focus here is alcohol consumption. Coffee is addressed as a dichotomous variable (i.e. yes/no), and caffeine is not considered at all. In all the years since publication of this manuscript, no publication from this group has addressed coffee/caffeine as a separate topic of interest as the authors had done so previously.

In this sample, 70% of women who experienced a fetal loss, but only 51% of women who carried to term

consumed coffee during the first trimester. After adjustment for the confounders they considered important, including cigarette smoking as a dichotomous variable (with all its problems; Leon, 1993), the OR of spontaneous abortion associated with coffee consumption was 2.1, 95% CI = 1.7, 2.7).

Unfortunately, the authors did not present data about coffee consumption before delivery. If they had, then perhaps data for consumption before pregnancy might have been compared to consumption after conception as they had previously. Then they found minimal difference between cases and controls in consumption before delivery, and a modestly higher consumption among cases during the weeks before fetal loss (110).

C50. Dominguez-Rojas V., de Juanes-Pardo J.R., Astasio-Arbiza P., Orteg-Molina P., Gordillp-Florencio E., 1994, Spontaneous abortion in a hospital population: are tobacco and coffee intake risk factors? *European Journal of Epidemiology* 10, 665–668.

This retrospective cohort study recruited 711 women employed at one hospital in Madrid who were pregnant between January 1989 and June 1991. Spontaneous abortion was defined as fetal loss before week 20 of gestation.

This study suffers from a number of methodologic problems which limit the references that can be drawn from it. First, the investigators excluded women who did not consume coffee. Second, the possibility of selection bias is raised by the very high rate of clinically-defined spontaneous abortion in this sample (24% overall and 71% among women who consumed more than 420 mg of caffeine per day during the first trimester). Third, the authors rely exclusively on exposure information obtained for clinical purposes. More detailed information, especially about changes during the first weeks of pregnancy, was not obtained. Fourth, the authors did not adjust for smoking, alcohol consumption, occupational exposures, or other potentially confounding factors.

C51. Pastore L.M., Savitz D.A., 1995, Case-control study of caffeinated beverages and preterm delivery. *American Journal of Epidemiology* 141, 61–69.

This study recruited 408 women who delivered before week 37 of gestation and compared them with 490 women (matched by race and hospital) who delivered a full-term, normal-weight infant. All were interviewed by telephone (with a median time following birth of 6 months). Caffeine consumption was assessed for a time 3 months before pregnancy and for each trimester. Caffeine content was estimated based on 146 mg per cup of percolated coffee, 110 mg per cup of filtered coffee, 66 mg per cup of instant coffee, 107 mg per cup of coffee

with an method of preparation, 34 mg per cup of tea, and 42 mg per 12 oz. serving of cola. Unlike most other studies, this one also considered non-cola caffeinated soft drinks (46 mg per 12 oz.). Caffeine from medications was not assessed.

Approximately 9% of controls consumed 300 or more mg of caffeine per day during the third trimester. The biggest decrease in coffee consumption occurred between prepregnancy and the first trimester. In both cases and controls, the second trimester mean was identical to the third trimester mean.

Caffeine consumption in the third trimester was associated with a slight reduction in risk of delivery before week 37. Fortunately, the authors appreciate that prematurity is not a dichotomous variable. What contributes to premature delivery at 36 weeks may differ from contributions to delivery even earlier. Unfortunately, they defined their very preterm group as less than 34 weeks, rather than an even earlier gestational age.

Because these very premature cases had only 2.7 weeks of the third trimester, their second trimester caffeine exposure was the focus. Moderate consumption (150–300 mg per day) was more strongly associated with very preterm birth than was heavy consumption (greater than 300 mg per day).

Prematurity is a heterogeneous group of disorders and the final set of analyses separately evaluated premature rupture of membranes, premature onset of labor, and medically induced labor (not stated, but probably most often severe pre-eclampsia). Here, too, prematurity subtypes were not associated with third trimester consumptions. “The lack of a doseresponse relation in both trimesters reduces the likelihood that observed increases and decreases in risk reflect a causal association between caffeinated beverages and preterm delivery.”

The authors point out the limitations of their study, including the lack of information about some sources of caffeine (including chocolate and nonprescription drugs) and the retrospective collection of coffee consumption information more than a year later. They modestly do not emphasize the strengths of their study. Their classifications of prematurity, however, may be the major strength.

C52. Shu X.O., Hatch M.C., Mills J., Clemens J., Susser M., 1995, Maternal smoking, alcohol drinking, caffeine consumption, and fetal growth: Results from a prospective study. *Epidemiology* 6, 115–120.

A total of 712 women were recruited in early pregnancy for studies of the reproductive consequences of problems at the Three Mile Island Nuclear Power Plant. Women were interviewed “at entry into care” (12.9 ± 4.3 weeks of gestation) and again at 28 and 36 weeks of gestation. Sources of caffeine included coffee (1

cup = 100 mg), tea (1 cup = 45 mg) and cola (12 oz. = 45 mg). Caffeine from pills and other medications was not considered.

The number of women who consumed 3 or more cups of coffee per day declined progressively from the first ($N=19$) to the third ($N=7$) trimester. The number of women who consumed at least 2100 mg of caffeine per week also declined from 34 in the first trimester to 21 in the third.

Those who were “heavy caffeine consumers” (i.e. 3 or more cups of coffee per day or 2100+ mg of caffeine per week) in the first and second trimesters gave birth to babies whose birthweight was lower than that of women who did not consume any caffeine at all. No decrease in fetal growth was seen, however, after adjustment for parity, prior adverse pregnancy outcome, prepregnancy weight, income, smoking and nausea. Other analyses also adjusted for gestational age and first trimester bleeding. The four women who reported heavy caffeine consumption throughout pregnancy gave birth to babies whose mean birthweight was 108 g heavier than that of the 65 babies born to women who consumed no caffeine in the third trimester.

If caffeine has any effect on birthweight, it should be expected to produce its effect mainly during the third trimester. The failure to see any relationship between caffeine consumption in the first, second or the third trimester and birthweight reduction is viewed as support for the null hypothesis that caffeine consumption throughout pregnancy is not associated with any reduction in fetal growth.

C53. Stanton C.K., Gray R.H., 1995, Effects of caffeine consumption on delayed conception. *American Journal of Epidemiology* 142, 1322–1329.

This study of 1430 women employed in 1989 and 1990 at two companies that manufactured semi-conductors found an increased risk of conception delay. One year associated with caffeine consumption greater than 300 mg/day, but only in non-smokers. Stanton and Gray did not find any relationship between caffeine consumption and delayed conception in the *entire* sample.

In describing the results of randomized clinical trials, Peto wrote in 1977 “Believing that a treatment effect exists in one stratum of patients, even though no overall significant treatment effect exists, is a common error.” (Peto et al., 1977). Stallones was also critical of subset analyses, especially when there was no prior hypothesis that an effect would be seen (at all or even preferentially) in one subgroup. “The difference between prediction and ‘postdiction’ is often ignored.” (Stallones, 1987) It appears that Stanton and Gray had expected that the caffeine consumption/delayed conception relationship would have been most prominent in smokers. Their failure to find this relationship appears

to have left them baffled. Indeed, they offered the following explanation, "It is possible that the detrimental effect of smoking on fertility in our data overwhelmed the negative impact of caffeine use by smokers."

In an editorial pointing out some problems with subgroup analyses, Hatch wrote, "Skepticism increases when the reported association is not simply stronger in a subgroup than in the population overall, but is observable only in a subgroup." (Hatch, 1995) Stanton and Gray did *not* find an overall effect. The OR for delayed conception comparing those women who consumed 300 or more mg of caffeine per day to those who consumed none was 1.4, with the 95% CI including one (i.e. 0.9–2.4).

Non-smoking women who consumed 1–150 mg of caffeine per day and non-smoking women who consumed 151–300 mg of caffeine per day were *not* at increased risk of delayed conception. The absence of an increasing risk of delayed conception with increasing level of daily caffeine consumption should raise doubts about a claim that an exposure (e.g. coffee consumption) causes an outcome (e.g. delay in conception; Hill, 1965).

C54. Alderette E., Eskenazi B., Sholtz R., 1995, Effect of cigarette smoking and coffee drinking on time to conception. *Epidemiology* 6, 403–408.

This study of 1341 primigravidas is based on data collected in the years 1959–1966 from women enrolled in the Child Health and Development Studies of the Kaiser Permanente Health Plan (C2, Hogue, 1981). Conception was delayed among smokers, but not among non-smoking coffee drinkers. Similarly, an increased risk of conception delay was not associated with coffee drinking among smokers.

This study did not address the issue of caffeine, nor did it have information about frequency of intercourse. Another characteristic of this study can be viewed as a limitation by some observers and a strength by others (Baird et al., 1986). That is the restriction to women who conceived. This would be a problem if caffeine consumption increased the risk of sterility. In light of what is in the literature (C27, C41, C61, C64), this is highly unlikely.

What is intriguing about this study is that the data were collected more than 30 years ago, at a time when smoking was considered neither socially undesirable, nor inappropriate for pregnant women. Thus, it is likely that women fully acknowledged their cigarette smoking, thereby reducing the likelihood of residual confounding when evaluating coffee consumption (Leviton, 1996).

C55. Peacock J.L., Bland J.M., Anderson H.R., 1995, Preterm delivery; effects of socioeconomic factors, psychological stress, smoking, alcohol, and caffeine. *British Medical Journal* 311, 531–536.

St. George's Hospital in London has been home of a group of investigators who reported previously on their study of caffeine consumption and low birthweight (C24, C32). Birth before 37 weeks of gestation is the outcome in this report, rather than measures of reduced birthweight. For this study, 1513 women were recruited at the time of booking and interviewed then and at 17 and 28 weeks of gestation. Socioeconomic and psychological factors were emphasized.

Women who consumed ≥ 2801 mg/week of caffeine did not have a higher rate of prematurity than women who consumed less or no caffeine. Cigarette smoking was not associated with prematurity, and perhaps this explains why coffee consumption is not implicated as an antecedent. If this is the reason, then it supports the hypothesis that caffeine consumption is implicated in the etiology of smoking-related pregnancy hazards, because the caffeine variable in these studies carries (residual) information about smoking (Baird et al., 1986).

C56. Windham G.C., Fenster L., Hopkins B., Swan S.H., 1995. The association of moderate maternal and paternal alcohol consumption with birthweight and gestational age. *Epidemiology* 6, 591–597.

This study only peripherally addresses the contribution of caffeine consumption to birthweight. The subjects of this study were recruited as the randomly selected controls for a study of the contribution of consuming bottled water to the risk of spontaneous abortion. Caffeine's contribution to spontaneous abortion was evaluated in C30, and to fetal growth in C29.

This report differs from C29 mainly in that the focus here is alcohol consumption. The authors also more clearly separate fetal growth impairments into two components, birthweight reduction and premature delivery. Indeed, one of the improvements in this report is the acknowledgement that birthweight is measured as a continuum. Unfortunately, gestational age is still treated as a dichotomous variable.

The methodologic weaknesses described for C29 apply here, including degrading of exposure, confounder, and to some extent outcome information, and collection of data on average 9 months after delivery. Here, too, changes in caffeine consumption, as well as alcohol and cigarette smoking were limited to the first 20 weeks of pregnancy.

What prompts this alcohol study to be included here is the authors' comments in a letter to the editor about C52 (Windham et al., 1995). In citing their alcohol paper the authors claimed that "moderate consumption of caffeinated beverages (i.e. ≤ 150 mg per day) appeared to exacerbate the joint effects of smoking and alcohol drinking on birthweight." The claim seems unjustified in light of the limitations of this study, especially the possibility of residual confounding due to the

caffeine consumption variable carrying information about cigarette smoking even after “adjustment for cigarette smoking”.

C57. Rondo P.H.C., Rodrigues L.C., Tomkins A.M., 1996, Coffee consumption and intrauterine growth retardation in Brazil. *European Journal of Clinical Nutrition* 50, 705–709.

This is a study of 356 full-term singletons classified as growth retarded (i.e. in the lowest decile of birthweight) and an unmatched comparison group of full-term singletons classified as having a birthweight appropriate for gestational age (i.e. in the top nine deciles). The authors interpret their data to indicate that women who consumed 3 or more cups of coffee each day were twice as likely as women who did not consume coffee to deliver a term infant in the lowest birthweight decile.

This study has a number of limitations. First, the authors do not describe when women were interviewed, nor when during pregnancy was coffee consumption the focus. Second, the authors ignore some of the very confounders they identify. For example, maternal body weight (presumably, prepregnancy), weight gain (presumably during pregnancy), and prior low-birthweight infant were each identified as predictors of growth retardation. Indeed, the literature supports these as predictors of birthweight (Stein, 1984; Nieto et al., 1994; Neggers et al., 1997). The authors neglect to discuss whether any of these was associated with coffee consumption. This is unfortunate because diet is associated with coffee/caffeine consumption (Schreiber et al., 1988a,b; Puccio et al., 1990; Leviton et al., 1994). Their multivariate analyses, however, do not include any of these potential confounders.

Third, the authors classify tobacco consumption as none, <5, 5–9, >9 cigarettes per day. The variation between 10 and 40 or more cigarettes per day gets lost in this classification. This provides an opportunity for residual confounding.

Finally, the authors calculate attributable risk percentages. This is a controversial topic (Northridge, 1995; Greenland and Robins, 1988). Unfortunately, the authors do not address the controversy, nor do they address the very source of the controversy, which is the tendency to violate the assumption of *ceteris paribus* (i.e. all else being equal; Leviton, 1995). Indeed, in light of the probability of residual confounding, the authors should have been much more cautious in interpreting their findings and in attaching importance to them.

C58. Cook D.G., Peacock J.L., Feyerabend C., et al., 1996, Relation of caffeine intake and blood caffeine concentrations during pregnancy to fetal growth: prospective population based study. *British Medical Journal* 313, 1358–1362.

This study is a re-evaluation of data collected for C24. Of the 1513 white women recruited for that study, fully 1500 had provided one blood specimen available for measurement of cotinine and caffeine, and 640 provided three specimens (one each at booking, 28 weeks and 36 weeks). In this report “adjusted birthweight was unrelated to blood caffeine concentrations overall”.

The strengths of this study have been identified before. The discussion here focuses on biomarkers, which are coming to be recognized as measures of exposures (and response modifiers) that are potentially superior to those obtained from interview or review of industrial and medical records (Schulte, 1993). The idea of considering measures of caffeine in blood as superior to reports of coffee/caffeine consumption is, therefore, attractive. Unfortunately, in this sample “blood specimens (obtained for cotinine and caffeine levels) were not collected at standard times”. This is especially important for beverages whose consumption can vary considerably with the time of day. Seasonal and other variations also deserve attention. In this study “the correlations (*r*) between any two of the three measurements was in the 0.5 range”.

Reasonably good, but not as high as one would have hoped.

In light of these considerations, this study is a good beginning. Future studies will be needed to address to what extent caffeine blood levels at a set time of day discriminate better than interview-obtained caffeine exposure information between those with and without a reproductive hazard.

C59. Wisborg K., Henriksen T.B., Hedegaard M., Secher N.J., Smoking during pregnancy and preterm birth. *British Journal of Obstetrics and Gynaecology* 1996, 103, 800–805.

This study recruited 4260 nulliparae who presented to Aarhus University Hospital for routine prenatal care during 1989 and 1991. Preterm delivery was defined as delivery before 37 completed weeks.

Unlike the Peacock report (C55), the risk of preterm delivery in this study was associated with cigarette smoking, but only among women who consumed more than 400 mg/day of caffeine. In the entire sample 4.0% of women who drank less than 400 mg/day of caffeine delivered preterm, whereas the rate was 4.7% among women who consumed more caffeine.

This minor difference might be due to the cigarette smoking that is overrepresented among the highest consumers of coffee/caffeine. Unfortunately, the association between caffeine consumption and preterm delivery is not as well studied in this sample as it should have been.

C60. Dlugosz L., Belanger K., Hellenbrand K., Holford T.R., Leaderer B., Bracken M.B., Maternal caffeine

consumption and spontaneous abortion: a prospective cohort study. *Epidemiology* 1996 7, 250–255.

This prospective cohort study recruited and followed 2849 women who presented for prenatal care during 1988–1992 and planned to deliver at Yale–New Haven Hospital. The mean gestational age at interview was 9.1 weeks for women who experienced a fetal loss and 10.1 weeks for women who delivered a liveborn singleton. The authors defined spontaneous abortion as fetal loss before week 28 of gestation.

The authors sought information about caffeine consumption during the first month of pregnancy. This is an obvious asset, as is the prospective cohort study design. Unfortunately, this study also has a number of problems. First, the rate of spontaneous abortion in this sample is 4.5%. In part this reflects the authors decision to exclude 200 women who aborted after being informed of the study, but before interview. When the expected rate is closer to 15%, the possibility of selection bias needs to be considered. Second, the authors view a late second trimester stillbirth the same as a first trimester fetal loss. Some obstetricians would find fault with this, and suggest that the phenomena leading to early fetal loss are not the same as those leading to stillbirth after the threshold of fetal viability (at 23–24 weeks). Third, the authors seem to go beyond their data. The increased risk of fetal loss associated with consumption of 3 or more cups of coffee/day was not seen with consumption of more than 300 mg of caffeine/day, nor was a linear trend seen with either increasing coffee or caffeine consumption. Fourth, in their discussion, the authors view nausea as the earliest expression of the pregnancy signal that might influence beverage consumption, a view questioned by others (Stein and Susser, 1991; C63).

C61. Bolumar F., Olsen J., Rebagliato M., Bisanti L., and the European Study Group on Infertility and Subfecundity. Caffeine intake and delayed conception: A European Multicenter Study on Infertility and Subfecundity. *American Journal of Epidemiology* 1997, 145, 324–334.

This paper from the European Multicenter Study on Infertility and Subfecundity randomly selected 3187 women who were sexually active and not using any contraception. The first paper from this study (Bolumar et al., 1996) reported delayed conception among smokers. Despite the authors claims in the abstract that they found an association between consumption of more than 500 mg caffeine/day and conception delay ≥ 9.5 months, their Table 5 does not show any statistically significant increased risk for the most recent waiting time. Even Cox regression analyses failed to reveal any statistically significant increased risk of conception delay associated with consumption of ≥ 500 mg/day of caffeine.

1. Residual confounding

Cigarette smoking was classified categorically using a small number of broad groups (none, 1–11, 11+), rather than a continuous variable or variables defined by the relationship with the outcome and with caffeine/coffee exposure (Leon, 1993). The consequence of this is that the coffee/caffeine variable conveys some information about smoking that supplements the smoking category variable (Baird et al., 1986). The low magnitude elevations in risk of subfecundity are similar to those seen in the bladder cancer literature, which has tended to have this same problem with residual confounding (Morrison, 1984). The authors acknowledge that residual confounding might account for some of their findings, but list it with a number of other explanations for their findings, almost, but not quite dismissing it.

2. Suboptimal information about exposures

Information was collected retrospectively, in some cases years after the exposures and events. How well should a 40-year-old woman recall how much coffee she drank when she conceived her first child (one to two decades earlier)? The authors do not see this as a problem. The authors think that by following the admonitions of Schreiber et al. (1988a) to record size of cup/mug, etc. they have obtained high quality information. When evaluating the risks associated with subfecundity, a study needs to address issues associated with other risks. The authors did not present information about a history of venereal disease or about the number of sexual partners, both of which might convey information about the risk of subfecundity. If women who smoke are more likely than women who do not to have a venereal disease, and women who smoke are more likely to consume higher levels of caffeine daily, then consumers of higher levels of caffeine might be expected be less fertile than their peers who consume less caffeine. In this situation caffeine consumption does not cause subfecundity; rather it is a marker of causal risk factors.

3. Different pregnancies

The authors asked 3187 women (who eventually conceived) about their first pregnancy, and 3092 women about their most recent “waiting time”. The latter group includes some women who are probably infertile, but the authors do not describe how large this group is. This point is important because the caffeine/coffee relationships with “prolonged waiting time” achieve statistical significance in the first pregnancy sample, but not with the most recent “waiting time” sample. The authors suggest that their inclusion of

infertile women in this latter group accounts for their failure to find a statistically significant relationship between “prolonged waiting time” and coffee/caffeine exposure. Others might disagree with this interpretation (Baird et al., 1986). Perhaps the failure represents nothing more than better recall (for instance, about cigarette smoking).

4. Nine and a half months as definition of “prolonged waiting time”

The authors defend their choice of this definition by saying that it avoids end digit preference, and that it is below the eligibility criterion for treatment of ≥ 12 months. Some might not consider this convincing. If only the authors stated that the effect they found with a 9.5 cut-off was also seen when they used a 12-month cut-off.

5. Biased investigators

“When data were broken down by smoking status, the effect of drinking more than 500 mg (of caffeine per day) was relatively stronger in smokers (OR = 1.56) than in non-smokers (OR = 1.38).” This quote documents that the authors assume the relationship between coffee/caffeine exposure and prolonged waiting time is causal. The perception of naivete or bias is reinforced by the authors’ citing the 1978 publication of Palm et al. (1978; with all the problems associated with gavage) in support of their claim that caffeine prolongs time to pregnancy in rodents. On the other hand, the authors wrote, “Our results should be taken with caution given the low magnitude of the difference in ratios” (p. 331).

C.62. Vlainjac H.D., Petrovic R.R., Marinkovic J.M., Sipetic S.B., Adanja B.J., Effect of caffeine intake during pregnancy on birthweight. American Journal of Epidemiology 1997, 145, 335.

This study from Belgrade interviewed 1011 women during the first 3 days after delivery. The main interests were caffeine consumption during the last trimester (viewed as a categorical variable) and birthweight (viewed as a continuum). The authors claim that “a significant reduction in birthweight was found to be associated with an average caffeine intake of ≥ 71 mg per day”.

To identify confounders, most papers will have a table that shows the relationships between candidate confounders and both the exposure and outcome. No such table is provided. Rather, the authors offer snippets of statements such as “Caffeine intake (how classified?)

was significantly, positively related to parity and occupational and housekeeping activities during pregnancy and negatively related to maternal height. Smokers were shorter and leaner, with longer duration of occupational activity during pregnancy. . .”

The authors write, “Variables associated with both the birthweight and caffeine intake or smoking (i.e. maternal height, weight and parity) were considered to be potential confounders.” Not enough information is provided the reader to understand the nature of the confounding.

The authors fail to consider important items. For example, maternal weight gain during pregnancy is one of the strongest predictors of birthweight (Eisner et al., 1979; Stein, 1984; Nieto et al., 1994). Nevertheless, Vlainjac and colleagues do not even consider weight gain important, even though it has been recognized as important since at least 1968. In a similar vein, the authors do not explore maternal body mass index, which appears to convey more information than either height or weight as individual variables.

The authors did not deal with the issue of maternal disease known to be associated with reduced birthweight. The best known and most common example is pregnancy-induced hypertension/pre-eclampsia (Stein, 1984; Nieto et al., 1994).

Related to maternal weight gain is the issue of diet (Neggers et al., 1997). The authors do not deal with non-beverage elements of the diet. This might be important, especially since the births that are the subjects of this study occurred in 1992 and 1993, when Belgrade and outlying Serbian territories were involved in a state of war with their neighbors, which had been part of the confederation of Yugoslavia. The Dutch famine during the Second World War did not seem to have an adverse effect on fetal well-being, presumably because pregnant women received special attention (Susser and Stein, 1994). Nevertheless, when people feel under a state of siege, they can be expected to feel under stress, and perhaps consume more tobacco and alcohol. The authors do acknowledge the potential for “differential misclassification” (i.e. biased reporting of consumption), “since it is likely that the women were aware of the harmful effects of smoking”. They do not, however, acknowledge the consequences, including residual confounding.

The authors explain a coffee relationship with birthweight only among non-smokers as a consequence of the profound effect smoking has on birthweight, thereby diminishing the opportunity to identify a coffee effect. They give only lip service to the concept of residual confounding, which is perhaps the major limitation of this study.

C63. Fenster L., Hubbard A.E., Swan S.H., Windham G.C., Waller K., Hiatt R.A., Benowitz N.. Caffeinated

beverages, decaffeinated coffee and spontaneous abortion. Epidemiology 1997, 8, 515–523.

Five-thousand-one hundred and forty-four women enrolled in the Kaiser Permanente system in California were recruited in 1990 and 1991 when presenting for prenatal care before week 13 of gestation. 9.7% of these women had a spontaneous abortion before week 20. Among women who drank caffeinated coffee, about 75% reduced their intake by the time of the interview. Approximately 13% of all women consumed more than 300 mg of caffeine/day before pregnancy, but only 4% consumed such levels during the first trimester.

For first trimester beverage consumption, the adjusted OR of spontaneous abortion associated was 1.3 for consumption of 300 mg/day of caffeine, 0.8 for consumption of 3 or more cups of caffeinated coffee/day, 1.5 for consumption of 3 or more cans of caffeine-containing soda/day and 2.4 (95% CI = 1.2, 4.6) for consumption of 3 or more cups of decaffeinated coffee/day. Similar ORs were associated with consumption of these beverages before pregnancy.

The authors identify the four major strengths of this study: prospective design, almost complete follow-up of pregnancy outcomes, adjustment for potential confounders, and a sufficiently large sample to examine risk by individual beverages. In this sample, consumption of caffeine was not associated with spontaneous abortion, but consumption of decaffeinated coffee was. This intriguing finding surprised the author. They raise the possibility of random phenomena, but also wonder about how an early pregnancy signal might account for their findings. The authors appear to regret not having collected better data about nausea and other early symptoms of pregnancy.

C64. Curtis K.M., Savitz D.A., Arbuckle T.E., 1997, Effects of cigarette smoking, caffeine consumption, and alcohol intake on fecundability. American Journal of Epidemiology 146, 32–41.

This study, identified as the Ontario Farm Family Health Study, 1991–1992, was originally designed assess the contribution of pesticide exposure to reproduction hazards. The sample of this retrospective cohort study was restricted to couples in the childbearing years who lived on grain, fruit and vegetable farms in Ontario Canada and wanted to become pregnant.

The authors acknowledge the potential limitations of this study, including the retrospective nature of the design, the asking about coffee consumption at the time of the interview (and not at the time of trying to conceive), the sometimes long interval between interview and the pregnancy asked about (average interval was 9 years, but was as high as 26 years), the exclusion of sterile couples, and the exclusion of couples who had

not planned to conceive, and what they call the “want- edness bias” (the reporting of contraceptive failures as planned pregnancies).

Caffeine consumption by both men and women, even at levels greater than 500 mg/day was not associated with a statistically significant reduction in the fecundability ratio. Similar findings were seen with consumption of coffee, even at levels exceeding 6 cups per day. As this is the most recently published study about this topic, and its findings are consistent with those of other studies, consideration needs to be given to the possibility that caffeine consumption does not influence fecundity.

C65. Ford R.P.K., Schluter P.J., Mitchell E.A., Taylor B.J., Scragg R., Stewart A.W. and the New Zealand Cot Death Study Group. Heavy caffeine intake in pregnancy and sudden infant death syndrome Archives of Diseases in Childhood 1998, 78, 9–13.

This is a late report of additional analyses of a case-control study of what is called cot death in New Zealand and sudden infant death syndrome (SIDS) in the United States. The authors found that consumption of 400+ mg of caffeine during the third trimester was associated with an increased risk of cot death months after birth. Consumption of lower levels of caffeine were not associated with any increased risk of cot death.

This study has a number of limitations, one of which should be considered potentially fatal to the author’s findings/hypothesis.

1. Not a test of a previously generated hypothesis. The major finding of this paper should not be viewed as testing a hypothesis, because no-one previously suggested such a hypothesis. Rather, the authors generated a hypothesis never before considered.

The major paper from this group was published in 1992. The authors have been productive, but their publishing this hypothesis-generating report six years later raises issues of data dredging.

2. Retrospective design comparing non-comparables. Although controversy continues about the most appropriate referent group for mothers of infants with deforming, disabling or fatal disorder (Smith et al., 1988; Drews et al., 1993), the authors compared mothers of infants who died with mothers of infants who were doing well. Asking a grieving mother what she might have done to contribute to her infants disorder poses enormous burdens on her that are not placed on referent mothers (Drews et al., 1990). To get around this potential problem, some authors have selected referent mothers those who infant has another major disorder that would

prompt similar reporting bias. For example, some investigators have chosen mothers of infants with other anomalies when studying a specific malformation (Werler et al., 1989).

3. Residual confounding may be a fatal flaw.

(a) SIDS is a tobacco-associated disorder. The risk of sudden infant death syndrome (SIDS) has repeatedly been increased among infants exposed to parent's cigarette smoke, often in a dose response pattern. Thus, parents' smoking should be viewed as an important risk factor for SIDS.

(b) Tobacco consumption measured inadequately. The authors classified maternal smoking during pregnancy as a yes/no variable. Mother's smoking during the months preceding the interview was not assessed, nor was father's cigarette smoking.

Since the more cigarettes smoked, the higher the risk of SIDS (Golding, 1997; MacDorman et al., 1997), the authors should have measured parental (and not just mother's) cigarette smoking as a continuous variable (i.e. number of cigarettes smoked in the presence of the infant each day) or perhaps as a categorical variable providing a reasonable approximation of a continuous variable (e.g. 1–10, 11–20, etc.).

(c) Caffeine measured better than smoking. Caffeine consumption was assessed as a categorical variable, (small, light, moderate, heavy). Since those who smoke the most cigarettes drink the most coffee/caffeine, caffeine will carry the information about magnitude of cigarette smoking that was not, carried by the "maternal tobacco: yes/no" variable, but should have been carried by a "parental tobacco: small, light, moderate, heavy" variable.

The result of having better information about caffeine than about tobacco is that all the adjustment for tobacco will not eliminate tobacco confounding of the association between caffeine consumption and the risk of SIDS (Leon, 1993; Leviton, 1996). Indeed, the failure of the authors to find a dose-response relationship between caffeine consumption and SIDS is in keeping with this paper's hypothesis that their findings can all be explained by residual confounding. Consider the possibility that the caffeine consumption variable tends to carry tobacco consumption information at the highest levels of caffeine consumption. During the third trimester, when caffeine degradation is slowed, those who are able to consume 400 or more mg of caffeine per day tend to be those who smoke the most cigarettes. Thus, only those women who consume

the most caffeine, smoke the most cigarettes, and thereby put their infant at risk of SIDS.

The authors have committed an epidemiologic error that students of introductory epidemiology are taught to avoid. At a minimum, they should have been identified in the discussion section of their paper that their failure to adequately measure some of the most important variables is a major, potential limitation.

C66. Klebanoff M.A., Levine R.J., DerSimonian R., Clemens J.D., Wilkins D.G., 1999, Maternal serum paraxanthine, a caffeine metabolite, and the risk of spontaneous abortion. *New England Journal of Medicine* 341, 1639–1644.

Mark Klebanoff and his colleagues reviewed data collected as part of the Collaborative Perinatal Project and measured paraxanthine, a metabolite of caffeine, in sera that had been frozen since collected for more than 30 years. They compared paraxanthine levels in the sera of 591 women who had a spontaneous abortion before week 20 of gestation to the levels of 2558 matched women from the same clinic who gave birth to live infants during or after week 28 and who had serum drawn on the same day of gestation as the women who had abortions.

The authors concluded, "Only extremely high serum paraxanthine concentrations (i.e. above the 95th centile) are associated with spontaneous abortion. This suggests that moderate consumption of caffeine is unlikely to increase the risk of spontaneous abortion." The association of spontaneous abortion with very high levels of a metabolite of caffeine is entirely compatible with the pregnancy signal hypothesis that women who are destined to have a miscarriage tend to have less of a pregnancy signal than women who carry to term, and therefore less likely to want to decrease their coffee/caffeine consumption.

C67. Cnattingius S., Signorello L.B., Anneren G., Clausson B., Ekblom A., Ljunger E., Blot W.J., McLaughlin J.K., Petersson G., Rane A., Granath F., 2000, Caffeine intake and the risk of first-trimester spontaneous abortion. *New England Journal of Medicine* 343, 1839–1845.

In this population-based, case-control study of early spontaneous abortion in Uppsala County, Sweden, the authors compared 562 women who had a spontaneous abortion at 6–12 completed weeks of gestation (the case patients) to 953 women who did not have spontaneous abortion and were matched to the case patients according to the week of gestation (controls).

The major finding is that women who drank the most caffeine were at elevated risk of miscarriage. One can

interpret this finding to mean that caffeine increases the risk of miscarriage. On the other hand, the researchers report a number of findings that support an alternative interpretation.

Women who miscarried were half as likely as comparison women to have had an aversion to coffee (21% vs 41%). The women who miscarried also tended to decrease their coffee consumption later and to a much smaller extent than women who did not miscarry.

Because beverage aversion appears to be an early “pregnancy signal”, which predicts carrying to term, coffee consumption and aversion can be viewed as indicators of the well-being of the pregnancy and not necessarily as contributors to the pregnancy loss. Everything the investigators found can be explained by the “pregnancy signal hypothesis”.

C68. Grosso L.M., Rosenberg K.D., Belanger K., Saftlas A.F., Leaderer B., Bracken M.B., 2001, Maternal caffeine intake and intrauterine growth retardation. Epidemiology 12, 447–455.

In this study from Yale, 2714 women who delivered a liveborn infant between 1988 and 1991 were interviewed about many lifestyle characteristics, including coffee, tea and soda drinking during the first and third trimesters of pregnancy. Women who consumed more than 300 mg caffeine per day either during the first or seventh months of pregnancy did not tend to have smaller babies than women who consumed less caffeine. In the words of the authors, “This study provides evidence that antenatal caffeine consumption has no adverse effect on fetal growth.”

3. Discussion

The relationship between caffeine (or coffee) consumption and the risk of reproductive adversities in humans has been reviewed repeatedly. Differences in the papers reviewed, and in the reviewer’s perspective/expertise, appear to account for many of the differences in the inferences drawn.

Few of the reviews have addressed those methodologic issues that are so important to an appreciation of the limitations of many of these studies. Those that deserve consideration are:

1. *Ascertainment of exposure.* Some studies measured coffee consumption, whereas others evaluated caffeine. Often the exposure was estimated after the occurrence of the event evaluated. Few studies have assessed the quality of the data collected (e.g. Is a cup 5.5 oz. or 8 oz? Does the person always consume the entire contents of a cup/mug/container? Were the caffeine consumption

decreases that accompany pregnancy considered?).

2. *Sample studied.* Was the sample one of convenience? This is an especially important issue for coffee and caffeine consumption. Very few studies are funded to evaluate coffee consumption per se. Rather, coffee and caffeine are incidental issues, allowing investigators to “get another paper” from all their work. Were efforts made to minimize selection bias? Did the sample size provide the desired power?
3. *Analyses.* Was the hypothesis considered post hoc? Was the categorization of continuous variables post hoc? Was the possibility considered that caffeine/coffee consumption might be an indicator of exposures that place the fetus at risk (e.g. cigarette smoking, maternal age), and were multivariate models created to attempt to deal with potential confounders not adequately excluded in the sample selected?
4. *Biases.* The literature dealing with caffeine and reproductive hazards needs to deal with confounders. Cigarette smoking is probably the best example of an exposure that can confound our perception of the relationship between maternal caffeine consumption and fetal well-being. Many of the reports described here use multivariate analyses to minimize confounding, all too frequently suboptimally. Unfortunately, even the best statistical analyses cannot compensate for low quality data. Residual confounding is the name given to the bias that occurs when women underreport their cigarette consumption (presumably because it is socially undesirable), but accurately report their coffee consumption. Because women who smoke the most cigarettes drink the most caffeine, the coffee consumption variable then conveys supplemental information about the number of cigarettes smoked. When residual confounding occurs, the coffee consumption variable will be blamed for smoking effects.

3.1. Delayed conception

After the first report of a relationship between caffeine consumption and delayed conception (C21), claiming that consumption of as little as 100 mg of caffeine was associated with delayed conception, 11 additional studies were published. Some provide no support for this hypothesis, whereas others show an association in a subsample.

The key issue for this outcome is the repeated finding that cigarette smoking appears to place a woman at risk of subfecundity. Residual confounding might explain all the associations reported between coffee/caffeine consumption and subfecundity.

3.2. Spontaneous abortion

No study of the association between spontaneous abortion and caffeine consumption has adequately addressed the issue raised by Stein and Susser (1991). They postulate that a healthy placenta produces a surge of one or more hormones that in some women produce such minor toxicities as a reduced desire for aromatic and strongly flavored beverages. If their postulate is true, then unchanged (i.e. persistently “high”) caffeine consumption during the first trimester can indicate diminished placental hormone synthesis and a vulnerable implantation. Thus, all of the studies purporting to show a link between caffeine consumption and the risk of spontaneous abortion may reflect nothing more than the Stein–Susser postulate. In perhaps the most thoughtful study of its kind, Kline and colleagues found that neither chromosomally normal nor chromosomally aberrant abortions were more common among women who consumed the most caffeine (C33). The most recent report on the topic found an unexplained association between early fetal loss and consumption of decaffeinated coffee, but not with caffeine (C63).

3.3. Anomalies

As with low birthweight, so with congenital anomalies. The methodologically limited studies found an association with caffeine/coffee consumption (C4, C13), whereas large properly analyzed data sets show no association (C7, C8, C10, C33). No paper has been published on this topic during the last 12 years. Perhaps investigators of the antecedents of anomalies feel the matter is closed.

3.4. Prematurity

The largest studies have tended not to find any relationship between maternal caffeine consumption and the risk of delivery before week 37 of gestation. The same can be said when the outcomes of interest are delivery before week 34, and prematurity subtypes (i.e. premature onset of labor, premature rupture of membranes, and maternal medical indication).

3.5. Low birthweight

The studies that claim an association between caffeine consumption and low birthweight tend to have methodologic flaws. By and large, the larger the sample and the better the analyses, the more likely no association is seen between coffee/caffeine consumption and reduced birthweight. Of special note is the observation that data collected before smoking was socially undesirable tend not to find any relationship between coffee/caffeine consumption and any reduction in birthweight. This

raises the possibility that residual confounding really does plague this field since women have been admonished not to smoke if they are pregnant.

In conclusion, no reproductive adversity has been consistently associated with caffeine consumption.

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Weinsetel, Natalia

Subject: FW: Theobromine GRAS Notification Submission : RESPONSE TO REVIEWERS QUESTIONS ON GRN340

From: Stanley M Tarka Jr, PhD [mailto:tarkagroup@comcast.net]

Sent: Saturday, August 07, 2010 9:42 PM

To: Weinsetel, Natalia

Subject: RE: Theobromine GRAS Notification Submission : RESPONSE TO REVIEWERS QUESTIONS ON GRN340

Dear Dr. Weinsetel,

Thank you very much for your message. I certainly hope that you are feeling better since you returned from vacation and will look forward to your call next week after you have had a chance to review the information I sent you.

I hope that you also have a great weekend.

Best regards,

Stanley Tarka

Stanley M. Tarka, Jr., Ph.D.

President

The Tarka Group, Inc.

210 N. Old Stone House Road

Carlisle, PA 17015-8517

(Ph): 717-243-9216

(Fax): 702-993-5458

tarkagroup@comcast.net

website: www.tarkagroup.com

From: Weinsetel, Natalia [mailto:Natalia.Weinsetel@fda.hhs.gov]

Sent: Saturday, August 07, 2010 3:02 PM

To: 'Stanley M Tarka Jr, PhD'

Subject: RE: Theobromine GRAS Notification Submission : RESPONSE TO REVIEWERS QUESTIONS ON GRN340

Dear Dr. Tarka,

I apologize for not returning your call yesterday, I got sick during my trip back and was not able to make it to work. I will review the information you sent me and return your phone call next week. Just wanted to let you know that I had received it.

Thank you and have a great weekend,
Natalia

Natalia Weinsetel, Ph.D.

Consumer Safety Officer

Division of Biotechnology and GRAS Notice Review

Office of Food Additive Safety

Center for Food Safety and Applied Nutrition

U.S. Food and Drug Administration

Tel: (301) 436-2907/Fax: (301) 436-2965

E-mail: Natalia.Weinsetel@fda.hhs.gov

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Weinsetel, Natalia

Subject: FW: Theobromine GRAS Notification Submission : RESPONSE TO REVIEWERS QUESTIONS ON GRN340

From: Weinsetel, Natalia

Sent: Friday, August 13, 2010 4:52 PM

To: 'Stanley M Tarka Jr, PhD'

Subject: RE: Theobromine GRAS Notification Submission : RESPONSE TO REVIEWERS QUESTIONS ON GRN340

Dr. Tarka,

I am waiting for the reviewers to finish looking over your responses. As soon as I hear from them, I will let you know if we need additional information. You will not need to submit a revised version of the GRN. The answers to our questions, as you submitted them to me, is sufficient.

Thank you and have a great weekend.

Natalia

From: Stanley M Tarka Jr, PhD [mailto:tarkagroup@comcast.net]

Sent: Saturday, August 07, 2010 9:42 PM

To: Weinsetel, Natalia

Subject: RE: Theobromine GRAS Notification Submission : RESPONSE TO REVIEWERS QUESTIONS ON GRN340

Dear Dr. Weinsetel,

Thank you very much for your message. I certainly hope that you are feeling better since you returned from vacation and will look forward to your call next week after you have had a chance to review the information I sent you.

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Best regards,

Stanley Tarka

Stanley M. Tarka, Jr., Ph.D.

President

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tarkagroup@comcast.net

website: www.tarkagroup.com

From: Weinsetel, Natalia [mailto:Natalia.Weinsetel@fda.hhs.gov]

Sent: Saturday, August 07, 2010 3:02 PM

To: 'Stanley M Tarka Jr, PhD'

Subject: RE: Theobromine GRAS Notification Submission : RESPONSE TO REVIEWERS QUESTIONS ON GRN340

Dear Dr. Tarka,

I apologize for not returning your call yesterday, I got sick during my trip back and was not able to make it to work. I will review the information you sent me and return your phone call next week. Just wanted to let you know that I had received it.

Thank you and have a great weekend,

Natalia

Natalia Weinsetel, Ph.D.

Consumer Safety Officer

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FD

**Weinsetel, Natalia**

Subject: FW: Theobromine GRAS Notification Submission : RESPONSE TO REVIEWERS QUESTIONS ON GRN340

From: Weinsetel, Natalia

Sent: Friday, August 20, 2010 4:01 PM

To: 'Stanley M Tarka Jr, PhD'

Subject: RE: Theobromine GRAS Notification Submission : RESPONSE TO REVIEWERS QUESTIONS ON GRN340

Hi Stanley,

Based on our phone conversation this afternoon, I have scheduled our telephone conference for Monday, August 23rd, at 1:30pm. Please let me know if you are still available during this time and what phone number we should use to contact you (if more than one party will be joining you, please let me know and I will set up a conference number).

In terms of our discussion, we need you to please elaborate on answer 4(d) (see email below dated 07/30). We don't believe the question has been completely answered. Second, in response to question 8 (same email), end of first paragraph, we need to know if this reported theobromine intake is from the Cantox report or if there is an outside reference associated with the information and if there is, could you please provide it.

Please let me know if you need further clarification of these questions.

Have a great weekend.

Natalia

From: Stanley M Tarka Jr, PhD [mailto:tarkagroup@comcast.net]

Sent: Friday, August 13, 2010 5:35 PM

To: Weinsetel, Natalia

Subject: RE: Theobromine GRAS Notification Submission : RESPONSE TO REVIEWERS QUESTIONS ON GRN340

Dear Dr. Weinsetel,

Thank you very much for your message updating me on the status of the review. Thanks as well for letting me know that the responses to the questions are sufficient and no revision is needed for the GRN.

I will be out of the office from Sunday August 15 through Tuesday August 17 and will look forward to hearing from you on my return on any additional follow-up needs.

I hope that you also have a great weekend, are feeling much better, and that the weather cooperates for both of us.

Best regards,

Stanley Tarka

Stanley M. Tarka, Jr., Ph.D.

President

The Tarka Group, Inc.

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website: www.tarkagroup.com

Memorandum of Telephone Conversation

Date: August 23, 2010

Between: Ronald Chanderbhan, Ph.D. HFS-255
Karen Cheng, M.S. HFS-255 (ORISE fellow)
Rebecca P. Danam, Ph.D. HFS-255
Michael DiNovi, Ph.D. HFS-255

and

Stanley M. Tarka Jr., Ph.D. The Tarka Group, Inc. on behalf of
Theocorp Holding Co, LLC (Tel: 717-243-9216)

Subject: GRN 000340

At a pre-arranged time, we spoke with Dr. Tarka as a follow up on certain answers that he provided in an email of July 30, 2010. Specifically, these were answer 4(d) and answer 8.

In terms of the answer 4(d), Dr. Tarka clarified the intake values that they obtained for methylxanthine at the high dose level and confirmed that the following paragraph in the notification was redundant. Regarding answer 8, Dr. Tarka explained that the reported data of 61-147 mg/person/day representing the estimated daily background intake of theobromine from all dietary sources was obtained from the Cantox report and not from any particular experimental study, as it appeared to be in their response. We asked Dr. Tarka to formally document these responses electronically.

~~~~
Rebecca P. Danam, Ph.D.

(b) (5) 

AM

**Weinsetel, Natalia**

Subject: FW: Theobromine GRAS Notification Submission : RESPONSE TO REVIEWERS QUESTIONS ON GRN340: August 23 Conference Call

Importance: High

From: Stanley M Tarka Jr, PhD [mailto:tarkagroup@comcast.net]

Sent: Monday, August 23, 2010 2:15 PM

To: Weinsetel, Natalia

Subject: FW: Theobromine GRAS Notification Submission : RESPONSE TO REVIEWERS QUESTIONS ON GRN340: August 23 Conference Call

Importance: High

Dear Dr. Weinsetel,

This is a follow-up to the conference call held this afternoon with your associates (Drs. Mike DiNovi, Ron Chanderbhan, Karen Cheng and Rebecca Danam) who are part of the GRN340 Review Team. I provided responses to both of the questions raised in your August 20 email and these responses and clarifications resolved their questions to their satisfaction. I was also asked by Dr. Ron Chanderbhan to provide an email formally documenting these responses and it was agreed that the responses highlighted in green for questions #4 (d) and #8 (end of first paragraph) in this email would be satisfactory. These have been incorporated in the July 30 response below under the specific questions.

Thank you very much for facilitating this discussion. Would you kindly please confirm receipt of this message when you are in your office? I hope that you are feeling better.

Best regards,

Stanley Tarka

Stanley M. Tarka, Jr., Ph.D.

President

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(Fax): 702-993-5458

tarkagroup@comcast.net

website: www.tarkagroup.com

From: Weinsetel, Natalia [mailto:Natalia.Weinsetel@fda.hhs.gov]

Sent: Friday, August 20, 2010 4:01 PM

To: 'Stanley M Tarka Jr, PhD'

Subject: RE: Theobromine GRAS Notification Submission : RESPONSE TO REVIEWERS QUESTIONS ON GRN340

Hi Stanley,

Based on our phone conversation this afternoon, I have scheduled our telephone conference for Monday, August

23rd, at 1:30pm. Please let me know if you are still available during this time and what phone number we should use to contact you (if more than one party will be joining you, please let me know and I will set up a conference number).

In terms of our discussion, we need you to please elaborate on answer 4(d) (see email below dated 07/30). We don't believe the question has been completely answered. Second, in response to question 8 (same email), end of first paragraph, we need to know if this reported theobromine intake is from the Cantox report or if there is an outside reference associated with the information and if there is, could you please provide it.

Please let me know if you need further clarification of these questions.

Have a great weekend.

Natalia

**Gaynor, Paulette M**

From: Weinsetel, Natalia
Sent: Friday, August 27, 2010 11:23 AM
To: 'Stanley M Tarka Jr, PhD'
Cc: Gaynor, Paulette M
Subject: RE: Theobromine GRAS Notification Submission : RESPONSE TO REVIEWERS QUESTIONS ON GRN340: August 23 Conference Call

Dear Dr. Tarka,
I wanted to thank you for sending the responses to our questions following our conference call. I also wanted to remind you that I'll be leaving the agency, therefore, should you have any questions regarding this notice, please contact Dr. Paulette Gaynor (paulette.gaynor@fda.hhs.gov; 301-436-1192). Dr. Gaynor will be assigning a new CSO to this notice and they will contact you directly.
It was a pleasure working with you.
Sincerely,
Natalia

Natalia Weinsetel, Ph.D.
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Tel: (301) 436-2907/Fax: (301) 436-2965
E-mail: Natalia.Weinsetel@fda.hhs.gov

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From: Stanley M Tarka Jr, PhD [<mailto:tarkagroup@comcast.net>]
Sent: Monday, August 23, 2010 2:15 PM
To: Weinsetel, Natalia
Subject: FW: Theobromine GRAS Notification Submission : RESPONSE TO REVIEWERS QUESTIONS ON GRN340: August 23 Conference Call
Importance: High

Dear Dr. Weinsetel,
This is a follow-up to the conference call held this afternoon with your associates (Drs. Mike DiNovi, Ron Chanderbhan, Karen Cheng and Rebecca Danam) who are part of the GRN340 Review Team. I provided responses to both of the questions raised in your August 20 email and these responses and clarifications resolved their questions to their satisfaction. I was also asked by Dr. Ron Chanderbhan to provide an email formally documenting these responses and it was agreed that the responses highlighted in green for questions #4 (d) and #8 (end of first paragraph) in this email would be satisfactory. These have been incorporated in the July 30 response below under the specific questions.

Thank you very much for facilitating this discussion. Would you kindly please confirm receipt of this message when you are in your office? I hope that you are feeling better.

Best regards,

Stanley Tarka

Stanley M. Tarka, Jr., Ph.D.

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AM | **Gaynor, Paulette M**

From: Stanley M Tarka Jr, PhD [tarkagroup@comcast.net]
Sent: Thursday, September 02, 2010 9:19 AM
To: Gaynor, Paulette M
Cc: Harry, Molly *
Subject: RE: GRN 000340

Dear Dr. Gaynor,

Thanks for your follow-up note indicating that Molly Harry will be the new CSO assigned to GRN 000340. By separate email, I will forward the responses sent to Natalie documenting what was discussed and agreed upon in the August 23 conference call. These responses are highlighted in green in the list of responses to earlier questions.

Thank you very much and I will look forward to working with Ms. Harry.

Best regards,

Stanley Tarka

Stanley M. Tarka, Jr., Ph.D.

President

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tarkagroup@comcast.net

website: www.tarkagroup.com

From: Gaynor, Paulette M [mailto:Paulette.Gaynor@fda.hhs.gov]

Sent: Thursday, September 02, 2010 8:39 AM

To: 'tarkagroup@comcast.net'

Cc: Harry, Molly *

Subject: GRN 000340

Dr. Tarka,

As a follow up to Natalia Weinsetel's note of August 27, 2010, the new CSO assigned to this notice is Molly Harry (tel: 301-436-1075)

Also in Natalia's note, reference is made to responses provided following a conference call. Could you resend those responses to both Ms Harry and me?

Thank you,
Paulette

Paulette Gaynor, Ph.D.

FDA/CFSAN, HFS-255

Office of Food Additive Safety

5100 Paint Branch Pkwy
College Park, MD 20740

Tel: (301) 436-1192
Fax: (301) 436-2964

E-mail: paulette.gaynor@fda.hhs.gov

**Gaynor, Paulette M**

From: Stanley M Tarka Jr, PhD [tarkagroup@comcast.net]
Sent: Thursday, September 02, 2010 9:36 AM
To: Gaynor, Paulette M; Harry, Molly *
Subject: FW: Theobromine GRAS Notification Submission : RESPONSE TO REVIEWERS QUESTIONS ON GRN340: August 23 Conference Call
Importance: High

Here is the July 30 email that was updated on August 23 with the responses to the questions raised for clarification in item #4(d) and item #8 highlighted in green.

Thank you.

Stanley Tarka

Stanley M. Tarka, Jr., Ph.D.

President
The Tarka Group, Inc.
210 N. Old Stone House Road
Carlisle, PA 17015-8517
(Ph): 717-243-9216
(Fax): 702-993-5458
tarkagroup@comcast.net
website: www.tarkagroup.com

From: Stanley M Tarka Jr, PhD [mailto:tarkagroup@comcast.net]
Sent: Monday, August 23, 2010 2:15 PM
To: 'Weinsetel, Natalia'
Subject: FW: Theobromine GRAS Notification Submission : RESPONSE TO REVIEWERS QUESTIONS ON GRN 340: August 23 Conference Call
Importance: High

Dear Dr. Weinsetel,

This is a follow-up to the conference call held this afternoon with your associates (Drs. Mike DiNovi, Ron Chanderbhan, Karen Cheng and Rebecca Danam) who are part of the GRN340 Review Team. I provided responses to both of the questions raised in your August 20 email and these responses and clarifications resolved their questions to their satisfaction. I was also asked by Dr. Ron Chanderbhan to provide an email formally documenting these responses and it was agreed that the responses highlighted in green for questions #4 (d) and #8 (end of first paragraph) in this email would be satisfactory. These have been incorporated in the July 30 response below under the specific questions.

Thank you very much for facilitating this discussion. Would you kindly please confirm receipt of this message when you are in your office? I hope that you are feeling better.

Best regards,

Stanley Tarka

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(Fax): 702-993-5458

tarkagroup@comcast.netwebsite: www.tarkagroup.com**From:** Weinsetel, Natalia [<mailto:Natalia.Weinsetel@fda.hhs.gov>]**Sent:** Friday, August 20, 2010 4:01 PM**To:** 'Stanley M Tarka Jr, PhD'**Subject:** RE: Theobromine GRAS Notification Submission : RESPONSE TO REVIEWERS QUESTIONS ON GR N340

Hi Stanley,

Based on our phone conversation this afternoon, I have scheduled our telephone conference for Monday, August 23rd, at 1:30pm. Please let me know if you are still available during this time and what phone number we should use to contact you (if more than one party will be joining you, please let me know and I will set up a conference number).

In terms of our discussion, we need you to please elaborate on answer 4(d) (see email below dated 07/30). We don't believe the question has been completely answered. Second, in response to question 8 (same email), end of first paragraph, we need to know if this reported theobromine intake is from the Cantox report or if there is an outside reference associated with the information and if there is, could you please provide it.

Please let me know if you need further clarification of these questions.

Have a great weekend.

Natalia

From: Stanley M Tarka Jr, PhD [<mailto:tarkagroup@comcast.net>]**Sent:** Friday, July 30, 2010 11:31 AM**To:** Weinsetel, Natalia; Gaynor, Paulette M**Subject:** RE: Theobromine GRAS Notification Submission : RESPONSE TO REVIEWERS QUESTIONS ON GR N340**Importance:** High

Dear Dr. Weinsetel:

Thank you very much for your email of July 20 indicating that the review team has completed their preliminary assessment of the GRAS Notification for theobromine and requesting clarification on issues that they identified in the notice. I apologize for the slight delay in responding but had been out of town. The reviewers have been very thorough and I have prepared responses to all of the questions below. I also spoke with Dr. Gaynor yesterday and she asked me to also copy her on this response so that she can send it on to the reviewers in your absence.

RESPONSE TO FDA REVIEWERS OF GRN340

1. The notifier (Theocorp) mentions that: "TINOS LLC submitted a letter of intent to market theobromine as a New Dietary Ingredient and FDA filed this letter as an official filing on January 22, 2004." However, it also states that FDA issued a supplemental letter dated April 6, 1996. Please

confirm if this is an error or explain the date discrepancy (page 20).

RESPONSE: The reviewers are correct; this is an error and the correct date is January 22, 1996 not January 22, 2004.

2. Please spell out the acronym PADI (page 21).

RESPONSE: Possible average daily intake (PADI) is spelled out.

3. The percentage of unchanged theobromine (1-18%) found in the human urine as noted on page 36 is inconsistent with that presented in Table 5 (page 37).

RESPONSE: This is a typographical error and should be 10-18% on page 36 and not 1-18%. Table 5 is correct.

4. In the calculations provided for total methylxanthine intake from cocoa powder on page 60, there appears to be some miscalculations (see below).

- a. The notifier calculated that "cocoa powder provided 25.8 (mg/g) theobromine + 0.19 (mg/g) caffeine = 25.99 mg total methylxanthines/g cocoa powder". However, based on their previous statement that cocoa powder is comprised of 93% theobromine and 7% caffeine, the amount of caffeine would be 1.94 mg/g resulting in 27.74 mg total methylxanthines/g cocoa powder.

RESPONSE: The reviewer is correct; there is a miscalculation and the reviewer's calculated numbers are correct (27.74 mg total methylxanthines/g cocoa powder).

- b. The total methylxanthine intake (mg/day) would be 21.64 mg/d instead of 20.27 mg/day.

RESPONSE: The reviewer is correct; there is a miscalculation and the correct number is 21.64 mg/d.

- c. That translates to 161.49 mg/kg/day (instead of 151 mg/kg/day) of methylxanthines in rats consuming 5% cocoa powder.

Please re-calculate and correct or explain how you derived your numbers

RESPONSE: The reviewer is correct; there is a miscalculation and their calculated number (161.49 mg/kg/day) is correct.

- d. The notifier states that "The high dose level provided a mean methylxanthine intake of approximately 57 mg/kg bw/d for males and 74 mg/kg bw /d for females from wk 26- 104 of the study. Again, the next paragraph provides different numbers for the same period. Moreover, it is not specified what 2.1g/kg bw/d and 2.7g/kg bw/d represent.

The description of the same study on pages 58, 60, and 78 are not identical.

RESPONSE: The reviewer is correct; there is a miscalculation in the notification where 57 mg/kg bw/day should be 58 mg/kg bw/day where this occurs in the text. The 2.1g/kg bw/d and 2.7g/kg bw/d represent cocoa powder intake in the text on pages 58, 60, and 78.

August 23 Response to additional question: The confusion on pages 58, 60 and 78 resulted from the information being presented in loose approximations rather than exact

consistency in reporting. The cocoa powder used contained 2.58% theobromine and 0.19% caffeine with theobromine being 93% of total methylxanthines and caffeine being 6.85% .

To clarify this: 2.1 grams/kg body weight of cocoa powder for males in weeks 26-104 provided 54.18 mg/kg body weight/day of theobromine and 3.99 mg/kg body weight/day of caffeine for a total methylxanthine intake of 58.17 mg/kg body weight/day for males.

For females consuming 2.7 g cocoa powder/kg body weight/day during weeks 26-104, this translated to 69.66 mg/kg body weight/day of theobromine and 5.13 mg/kg bodyweight/day of caffeine for a total of 74.79 mg/kg body weight/day of total methylxanthines.

Therefore, on pages 58, 60 and 78, the correct numbers in reference to the chronic toxicity /carcinogenicity study for total methylxanthine intake during weeks 26-104 are 58.2 mg/kg body weight/day for males, and 74.79 mg/kg body weight/day for females.

Also, on page 58, there is an error in the original submission in the middle of the first paragraph. The sentence should read "Diets containing the highest concentration of cocoa powder (5.0%) provided mean **cocoa powder** intakes during weeks 26-104 of 2.1 g **cocoa powder**/kg body weight/day and 2.7 g **cocoa powder**/kg body weight/day respectively for male and female rats."

Also, on page 60, the next to last paragraph beginning with "Diets containing the highest concentration of Cocoa Powder (5.0%)....." is redundant in that the same information is presented in the paragraph that precedes this.

5. Please explain how you derived that the lowest theobromine dose tested was approximately 300 mg/kg b.w. per day in the goat study? (Aregheore, 2002), page 70.

RESPONSE: This is a good question as there were few specific details about theobromine content of the material fed and thus several assumptions were made relative to its concentration. Thus, the data provided should be considered at best an estimate. Aregheore (2002) evaluated the inclusion of cocoa shell or cocoa dust (a waste byproduct in the manufacture of chocolate) into goat feed. Goats 18-20 months of age and weighing ~20.5-21.3 kg b.w. were fed a diet containing up to 50% of a cocoa product (unknown theobromine content) for 56 days. Goats fed cocoa dust (from cocoa powder production) in particular, but also those fed cocoa shell had significantly reduced voluntary dry matter intake compared to the controls fed brewer's yeast which resulted in correspondingly reduced body weight gain. The author indicated that the effect could be due to occurrence of theobromine in the cocoa material but no concentration levels were provided in the publication. In order to derive an approximate exposure to theobromine, theoretical estimates of theobromine concentrations were used for these materials based on what is known from the literature for cocoa shell and cocoa dust waste to arrive at approximate exposure concentrations from intake. For cocoa shell, based on what is known from the literature, theobromine content of shell was estimated to be 13 g/kg and the theobromine content of cocoa dust was considered to be similar to that of cocoa bean meal, 20 g/kg. From these data and based on food intake estimates, the theobromine intake was calculated to be 6.9 and 9.7 g/animal per day for the cocoa shell and cocoa dust rations respectively, corresponding to roughly 323 and 465 mg/kg b.w. per day of theobromine. Since in goats consuming these diets there was both reduced dry matter intake and body weight gain at the lowest theobromine level tested (cocoa shell diet),

the amount of theobromine was estimated and rounded to 300 mg/kg b.w. per day.

6. We were unable to find the NOAEL and LOAEL for young chickens and older broiler chickens and laying hens in the references you have provided. Please clarify.

RESPONSE: I understand that it is difficult to determine the exact NOAEL and LOAEL from these studies. These studies were included for the sake of completeness even though data have limitations based on assumptions and calculated values. The European Food Safety Authority Expert Panel report (2008) examined the use of theobromine in animal feed and made a number of assumptions based on typical chicken weights at certain ages in order to arrive at an approximate numbers for the NOAEL and LOAELs in these poultry studies. EFSA's detailed analysis of these studies and derivations of estimated NOAELs and LOAELs are presented below.

Day and Dilworth (1984) fed broiler chickens starter diets with 0, 1, 2, 4, or 6% cocoa shell meal (at the expense of maize) from day 1 to 21 of age. By analysis, cocoa shell meal contained 13g theobromine per kg. The cocoa shell meal did not significantly affect 3 week body weights, but feed conversion at 3 weeks was significantly affected by feeding 6% cocoa shell meal. The investigators claimed that performance tended to be depressed over 1% cocoa shell meal. The addition of pure theobromine to four additional diets at levels identical to those provided by 1, 2, 4 and 6% cocoa shell meal depressed performance somewhat more than did cocoa shell meal and reached significance at the two highest doses. The highest dietary theobromine concentration without significant adverse effects (NOAEL) was estimated by EFSA to be 260 mg theobromine/kg diet (corresponding to 2% cocoa shell meal), and this was further estimated to correspond to a theobromine dose of 26-39 mg/kg b.w. per day.

Odunsi and Longe (1995a) fed six groups of day-old chickens (Isa Brown pullet type) isonitrogenous (but not isocaloric) diets with 0, 5, 10, 20, 30 or 40% cocoa bean cake for 9 weeks. As the theobromine content of the cocoa bean cake was 22.4 g/kg, the diets contained 1.1, 2.2, 4.5, 6.7, and 9.0 g theobromine per kg feed, respectively. At 4 weeks of age, 2 chickens were randomly selected for blood collection, and at 8 weeks of age, 2 chickens were sacrificed to evaluate the influence of the feed on the relative weights of liver, kidney and heart to the body weight. The experiment was ended after 9 weeks. Feed intake and weight gain were depressed at 20% inclusion of cocoa bean cake and above. As the metabolizable energy of the cocoa bean cake-containing feed was reduced, an increased feed consumption was expected. Feed intake was however reduced at 20% cocoa bean cake and above. The reduced weight gain was correlated to the reduced feed consumption (and reduced protein intake). Inclusion rates of 10% or more cocoa bean cake resulted in reduced kidney and heart weights and increased liver weights. No effects were observed at the 5% cocoa bean cake level, estimated to correspond to a theobromine dose of 110 mg/kg b.w. per day after the first week. Due to the relatively higher feed intake in the first week of life, the estimated theobromine dose during their first days was 165 mg/kg b.w. per day. Mortality was low and not related to treatment. Hematological parameters such as hemoglobin concentration, packed cell volume and red blood cell count were reduced with increases in dietary cocoa bean cake. The authors interpreted these findings as possibly a consequence of the reduced feed intake.

Odunsi and Longe (1998) fed 28-day old broiler chickens a standard maize-groundnut based diet, or diets with 15 or 30% cocoa bean meal (22.4 g theobromine/kg) for 14 days. Broiler chickens that received the cocoa meal had reduced feed intake and weight gain, and increased mortality with dose. The lowest inclusion of cocoa bean meal, 15%, was estimated to correspond to 3.4 g theobromine/kg diet (estimated to be 340 mg theobromine/kg b.w. per day). The experiment also included diets with cocoa bean meal that had been pretreated to reduce the content of theobromine. Hot water-extracted cocoa bean meal contained 9.8 g theobromine/kg and cocoa pod ash-treated cocoa bean meal

3.3-17 g theobromine/kg. However, the pretreatment also changed the nutritional composition of the meal. The theobromine-reduced cocoa bean meals were mixed in diets at 15, 30 or 45% levels and fed to the 28-day old broilers for 14 days. The pretreatment of the feeding material reduced the adverse effects but also reduced feed intake and weight gain was observed at the lowest theobromine concentration; 15% cocoa pod ash treated cocoa bean meal with a theobromine concentration of 0.95 g/kg diet, estimated to be 95 mg theobromine /kg b.w. per day. In another experiment, Odunsi et al. (1999) fed hot water pretreated cocoa pod ash, alkali treated and untreated cocoa bean meal to 28-days old Anak 180 broiler chickens for four weeks. The theobromine concentrations in the hot water extracted cocoa bean meal, the alkali treated cocoa bean meal, and the non-treated cocoa bean meal were 9.8, 6.3 and 22.4 g/kg. The three types of cocoa bean meal were included in separate diets at levels of 15% and 30%, respectively. Chickens receiving 15% of the untreated cocoa bean meal or more, corresponding to an intake of 3.4 g theobromine or more per kg diet, performed less well than chickens on the control diet. The most pronounced effects were reduced feed intake, reduced daily weight gain, reduced hemoglobin levels and increased creatinine levels. These negative effects were not observed in chickens given the hot-water or alkali-treated cocoa bean meal feeds at an inclusion rate of 15% of the diet, reducing theobromine exposure to 1.5 and 0.95 g theobromine per kg feed (estimated to be 150 and 95 mg/kg b.w. per day). However, the higher inclusion rate of pretreated cocoa bean meal, at 30%, resulted in those adverse effects.

In conclusion, EFSA estimated that the NOAEL of theobromine in young chickens was found to vary between 260 and 1100 mg/kg diet (approximately 26-110 mg theobromine/kg b.w. per day). In older broiler chickens, a LOAEL of 950 mg/kg (approximately 95 mg theobromine/kg b.w. per day) was calculated.

Laying hens

Fangauf and Haenfel (1938) reported that substituting 20% of laying Leghorn hens feed with cacao shell meal for four months resulted in a decreased feed consumption, reduced weight gain, reduced egg production and lower egg weight than in fowls given normal hen diets. Assuming the theobromine concentration in cocoa shell meal to be 13 g/kg, the diet contained 2.6 g theobromine/kg, corresponding to 160 mg theobromine/kg b.w. per day.

Black and Barron (1943) reported on a poisoning episode in laying hens. Among 300 hens that were fed a diet including 15% cacao shell 80 birds died suddenly in convulsions. The cocoa shell contained 17 g theobromine/kg. The only organ changes observed post mortem were a color change of the liver, and mottled appearance of kidneys that, coupled with histological changes, indicated subacute glomerulo-nephritis. During the feeding period of 15% cacao shell, egg-production was reduced by around 80%. When the cacao shell ration in the feed was reduced to 7.5%, egg production rose again. The cause of the poisoning episode mentioned above was tested in a small feeding experiment in which groups of three fowls were fed for 200 days diets with 0, 7.5, 15 or 30% cacao shell (contained 17 g theobromine/kg). All hens in the highest dose group died. In the 15% dose group two hens died. Hens in the 7.5% dose group (1.3 g theobromine/kg diet, estimated to be around 80 mg/kg b.w. per day) survived and consumed a normal amount of feed but the droppings were looser than normal.

Black and Barron (1943) concluded that feeding cocoa meal containing 15 g of theobromine per kg may be lethal to hens. The authors concluded that feeding 15% and upwards of cocoa meal to laying birds is extremely harmful; it decreased appetite and egg production, and caused scouring and high mortality.

Four groups of 20-week old layers (Isa Brown pullet type) were supplied isonitrogenous (but not isocaloric) diets with 0, 5, 10 or 20% cocoa bean meal for 25 weeks (Odunsi and Longe, 1995b).

Assuming the same feed was used as in the study of Odusi and Longe (1995a), the pullets were given diets containing 1.1, 2.2, 4.5, 6.7, and 9.0 g theobromine per kg feed. Egg production was followed carefully. Delayed start of egg production was observed in all groups fed cocoa bean meal. It was not known whether the effect was caused by theobromine. Otherwise, there were no adverse effects on layers and laying performance. During the second half of the laying period, no influence of the diets was observed. Thus, the diet with 5% cocoa bean meal assumed to contain 1.1 g theobromine/kg feed (estimated to correspond to 66 mg theobromine/kg b.w. per day), was the lowest observed effect level.

In conclusion, EFSA concluded that for laying hens the LOAEL is 1100 mg cocoa shell/kg diet (corresponding to 66 mg theobromine/kg b.w. per day). No NOAEL was identified.

ADDITIONAL REFERENCES CITED BY EFSA:

Black, D.J.G. and Barron, N.S. 1943. Observations on the feeding of a cacao waste product to poultry. *The Veterinary Record* 55: 166-167.

Fangauf, R. and Haenfel, A. 1938. Bericht über einen Fütterungsversuch mit Kakaoschalenmehl bei Legehühnern. *Archiv für Geflügelkunde* 12:183-187.

7. We noticed a typographical error in the reference. Hostetler et al. is listed as 1991 on page 77 while the same reference is listed as 1990 in the subsequent paragraph. Please confirm which is the correct one.

RESPONSE: The correct date is 1990.

8. The notifier states that the acceptable daily intake (ADI) of ~30 mg/person/day is significantly lower than the estimated daily intake (EDI) of 319 mg/person/day (90th percentile) from the intended uses of theobromine in the specified foods. The notifier discusses that the safety and exposure assessments of caffeine by Health Canada can be applied/considered to evaluate theobromine which is structurally similar to caffeine and is one of its metabolites. However, the notifier does not provide any comparative exposure estimate of theobromine derived from caffeine metabolism. Although the notifier notes that theobromine is consumed in large quantities by humans in various forms, the notifier does not clearly justify why the proposed 10x excess use on a daily basis can be considered as safe.

RESPONSE: The following explanation provides a clear justification for the safety of theobromine at the proposed use levels.

Various reports in the scientific literature have reported on the estimated contribution of theobromine from caffeine metabolism. Gu *et al.* 1992, Rodopoulos and Norman (1996), Lelo *et al.* 1986, all reported that the metabolic profile of caffeine biotransformation averaged 81.5% for paraxanthine, 10.8% for theobromine and 5.4% for theophylline formation. Assuming 10.8% theobromine is produced from caffeine metabolism and that caffeine consumption estimates range from a mean of approximately 200 mg/p/d up to 400 mg p/d at higher intake levels from various dietary sources, then it follows that 21.8 to 43.2 mg per person per day of theobromine could be generated endogenously as part of caffeine metabolism from various sources (coffee, tea, kola nut flavored beverages, guarana, mate and cocoa and chocolate products). Additionally, based on what is known about the metabolic pathways of caffeine, the same metabolic end products produced from consumption of theobromine when administered or consumed as the parent compound are also produced from these other sources of dietary caffeine when it is metabolized to theobromine. Toxicological evaluations of caffeine have consistently concluded that

there are no safety concerns with reasonable levels of consumption. Similarly, it has been shown that no safety concerns have been reported for theobromine from normal intakes (61-147 mg/p/d) regardless of dietary source.

August 23 response to question: the reported data of 61-147 mg/p/d was obtained from the Cantox report on page 8 and represents the estimated daily background intake of theobromine from all background sources in the diet and is reported for All Users and represents the mean and 90th percentiles for them.

Theobromine is recognized as being less toxic than caffeine. This is clearly demonstrated in the table below particularly in regard to reproductive or developmental toxicity of theobromine versus caffeine which experts agree is the primary concern with regard to the safety of caffeine and where much research has been focused. Any conclusions regarding caffeine's safety can be equally applied to conclusions regarding theobromine's safety while taking into account its lower order of toxicity.

Comparison of Caffeine and Theobromine in Rats

Methylxanthine	Oral LD50 mg/kg	Developmental NOEL mg/kg/day	Reproductive NOEL mg/kg/day
Caffeine	200	30	80-120
Theobromine (as theobromine sodium acetate)-oral gavage	950	50	
Theobromine (oral gavage) via diet		~100	250

Christian and Brent (2001) evaluated the developmental and reproductive effects of caffeine and reported that the developmental NOEL for caffeine in rodents is approximately 30 mg/kg/day, the teratogenic NOEL is 80 to 100 mg/kg/day, and the reproductive NOEL approximately 80 to 120 mg/kg/day. They noted that the probable blood level of caffeine required to produce teratogenic effects in rats is in excess of 60 µg/mL, which can only be reached in rodents by administration of large bolus dosages achieving peak short-term exposure. As shown in the table for theobromine, a higher dose is required to achieve the same observation.

Neither rodents nor humans can attain a 60 µg/mL peak exposure by consuming solutions of caffeine over several hours, the usual mode of human caffeine consumption. Christian and Brent (2001) hypothesized that this blood peak plasma concentration of 60 µg/mL might be achieved in the rodent by an 800 mg/kg/day dosage of caffeine in the drinking water; however, this is a dose equivalent to a 60 kg human consuming an enormous amount of caffeine. Again, the same analogy would hold true for theobromine consumption and additionally, this would not be achievable based on the excessive and unrealistic caloric intake required from foods naturally containing theobromine or combined with the specified food uses identified in this Notification.

Under normal conditions of oral consumption, humans cannot achieve blood levels of caffeine that are within the range of those that affect reproductive performance or development of offspring in the most sensitive animal species (Christian and Brent, 2001). The same would hold true for theobromine consumption.

While critical reviews of available animal studies demonstrate that caffeine can produce adverse effects in some species when given at a sufficiently high dose by gavage or injection, Christian and Brent (2001) also demonstrate that caffeine does not affect reproductive performance or development of the offspring of any animal species, unless given at a maternally toxic dosage that exceeds normal levels of human

dietary consumption. They conclude that the usual range of human exposures to caffeine from food and beverages is well below the threshold dose that would result in developmental/teratogenic or reproductive effects in experimental animals. Klebanoff et al. (1999) cited in the Notification is the only study that dealt with actual blood levels of caffeine metabolites in humans and they reached the conclusion with regard to caffeine consumption and spontaneous abortion: "that moderate consumption of caffeine is unlikely to increase the risk of spontaneous abortion." These observations and conclusions are equally applicable to theobromine whether as a metabolite of caffeine or from natural background sources in the diet or combined with the proposed food uses as specified in the Notification.

Recent support for the analysis and conclusions of Christian and Brent (2001) regarding the safety of caffeine which are summarized above can be found in the critical analysis by Peck *et al.* (in press and available online) who have published a critical review of the literature of the epidemiologic evidence concerning the consumption of caffeine-containing products and any association with potential reproductive effects in humans. Humans must be regarded as the most sensitive target species in the safety evaluation of caffeine and theobromine. This review is an update of the comprehensive critical report previously published by Leviton and Cowan (2002). As such, this review is restricted to human studies of caffeine and reproductive health published in English between January 2000 and December 2009. From their review, the authors concluded that the evidence for an effect of caffeine on reproductive health and fetal development is limited by the inability to rule out plausible alternative explanations for the observed associations, namely, confounding by pregnancy symptoms and smoking, and by exposure measurement error. Further, because of these limitations, the weight of evidence does not support a positive relationship between caffeine consumption and adverse reproductive or perinatal outcomes. The authors concluded that the studies available from January 2000 through December 2009 do not provide convincing evidence that caffeine consumption increases risk of any reproductive adversity. Future studies addressing the methodological limitations of current research may alter this conclusion. This is consistent with the earlier conclusions of Leviton and Cowan (2002) regarding caffeine and reproductive and perinatal outcomes in humans who also concluded from their review that no convincing evidence had been presented to show that caffeine consumption increases the risk of any reproductive adversity. Additionally, as discussed above, this is also applicable to theobromine which is a metabolite of caffeine but with a lower order of toxicity. The Expert Panel critically reviewed information and data on the safety of theobromine and caffeine and concluded that the proposed use of theobromine as an ingredient in certain selected foods and beverages as described in the Notification is Generally Recognized as Safe (GRAS) based on scientific procedures.

Health Canada (referenced in the Notification) indicated that humans can safely tolerate (no adverse effects reported) caffeine at a level of up to 400 mg/day. Since theobromine has been shown to be less toxic than caffeine, this conclusion of safety derived from the evaluation of human studies on caffeine should be equally applicable to theobromine consumption at intake levels up to 400 mg/day which is below the 90th percentile of the combined natural dietary background sources and proposed food use levels in the Notification.

Finally, it should also be noted that the intake estimates for theobromine in this Notification assume a 100 percent market penetration of the proposed uses of theobromine listed in Table 4 combined with background dietary intake estimates. Because 100 percent market penetration of the specified theobromine use in these products is highly unlikely, this estimate almost certainly overstates actual intake, which is likely to be much lower.

REFERENCES FOR THIS QUESTION:

Christian MS and Brent RL. (2001). Teratogen Update: Evaluation of the reproductive and developmental risks of caffeine. *Teratology* 64(1): 51-78. (Cited in the Notification.)

Gu L, Gonzalez FJ, Kalow W, Tang BK. (1992). Biotransformation of caffeine, paraxanthine, theobromine and theophylline by cDNA-expressed human CYP1A2 and CYP2E1. *Pharmacogenetics*; 2 (2): 73-77.

Klebanoff MA, Levine RJ, DerSimonian R, Clemens JD, Wilkins DG (1999). Maternal serum paraxanthine, a caffeine metabolite, and spontaneous abortion. *New England Journal of Medicine* 34(22): 1639-1644.

Lelo A, Birkett DJ, Robson RA and Miners JO. (1986) Comparative pharmacokinetics of caffeine and its primary demethylated metabolites paraxanthine, theobromine and theophylline in man. *Br J Clin Pharmacol*; 22: 177-182. (Cited in the Notification.)

Leviton A and Cowan L. (2002). A review of the literature relating caffeine consumption by women to their risk of reproductive hazards. Review. *Food and Chemical Toxicology*; 40:1271-1310.

Peck JD, Leviton A, Cowan LD. (in press). A review of the epidemiologic evidence concerning the reproductive health effects of caffeine consumption: A 2000–2009 update. *Food and Chemical Toxicology* doi:10.1016/j.fct.2010.06.019.

Rodopoulos, N, Hojvall, L and Norman, A. (1996). Elimination of theobromine metabolites in healthy adults. *Scandinavian Journal of Clinical and Laboratory Investigation* 56(4):373-383. (Cited in the Notification.)

9. Using data from NHANES 2003-2006 and the food codes reported, FDA calculated that 261 mg/p/d of theobromine is consumed at the mean and 367 mg/p/d at the 90th percentile for users. This is twice as high as 150 mg/person/day at the mean and 319 mg/person/day at the 90th percentile that was reported on page 19. In addition, FDA calculated a higher number of users (89.6%) vs. the 65.1% found in the Cantox assessment report (page 8 of Appendix 1). Please explain this discrepancy.

RESPONSE: It is difficult to explain this discrepancy without more information concerning the intake assessment conducted by the FDA and due to some confusion as to what values specifically the FDA is calling into question. The 65.1% users identified by Cantox refers only to the background intake of theobromine, and this was associated with intake of 61 and 147 mg/person/day at the mean and 90th percentile. This was calculated using all foodcodes in the NHANES database and by employing the USDA measured levels of theobromine. When the proposed food uses were added to the background levels, the intake was reported to be 150 and 319 mg/person/day, the numbers listed in the FDA question; however, this assessment was associated with 94.6% users, a number higher than that identified by the FDA. The FDA question does not detail whether the numbers referenced refer to calculations completed with the proposed food uses only or the proposed food uses and the background levels. It would be extremely helpful if this information could be provided and if the discrepancy between the scenarios being employed for comparison could be explained. In any case, the discrepancy in the levels of intake reported (150 and 319 mg/person/day as compared to the FDA derived 261 and 367 mg/person/day) likely result from differing approaches in food code selection with the powdered fruit flavored drinks being the most likely food use impacting the different estimates. Cantox limited the foodcodes chosen to represent this category to those identified as being produced from

powders. If the FDA included all foodcodes from this category, which are widely consumed, this could explain the difference in the intake estimates.

10. The 90th percentile of consumption of powdered fruit-flavored drinks is nearly 11x larger than the reported mean on page 26. Typically, the amount consumed at the 90th percentile is 2x greater than the mean. Please explain this difference.

RESPONSE: The mean and 90th percentile all-person intakes of powdered fruit flavored drinks are reported on page 12 as being 12 and 135 mg/person/day, respectively. This is a typographical error as this is an error in the insertion of the values from table A-7. The mean intake of powdered fruit flavored drinks is indeed equivalent to 12 mg/person/day; however, there is no 90th percentile value for this intake as insufficient data were available and so there is no value for the 90th percentile intake. Instead the mean all-user intake of 135 mg/person/day was mistakenly inserted in place of that information. The correct values were present in Table A-7; however, please find attached a version of the report with the correct values also reported on page 12.

11. The description of the manufacturing process on page 15 states that sodium bicarbonate is added to 3-methylxanthine in acetone and water before the addition of dimethyl sulfate. On page 16, the figure and written description shows that 3-methylxanthine is converted to the potassium salt before the addition of dimethylsulfate. Are there two methods for preparation of theobromine, or is one method preferred over the other?

RESPONSE: The method that is preferred and ascribed to the theobromine material that is the subject of the Notification requires 3-methylxanthine to be converted to the potassium salt before the addition of dimethylsulfate.

In addition to the response to the questions raised, I am attaching an electronic copy of the revised CanTox intake exposure document as referenced above as well as the four publications cited in the response to Question #8 for completeness. A hard copy of this information will also be sent to your attention by regular mail. We trust that these responses have addressed the questions raised by the review team.

Should you have any additional questions regarding this GRAS Notice, please contact me at 717-243-9216 or by email. We look forward to FDA's completion of their review on this submission.

Sincerely,

Stanley M. Tarka, Jr., Ph.D.

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From: Weinsetel, Natalia [<mailto:Natalia.Weinsetel@fda.hhs.gov>]

Sent: Tuesday, July 20, 2010 1:22 PM

To: 'tarkagroup@comcast.net'

Subject: RE: Theobromine GRAS Notification Submission

Dear Dr. Tarka,

The review team for GRN340 has completed their preliminary assessment, and the reviewers would like clarification on issues that they identified on the notice. I have provided the reviewers' questions for you below. So that we can complete our review and respond to your notice in a timely manner, we would appreciate receiving your responses to the reviewers' comments by close of business on Friday, August 6, 2010. If you will be unable to respond by this date, or if you have questions regarding the reviewers' comments, please feel free to contact me. Best regards,

Natalia

1. The notifier (Theocorp) mentions that: "TINOS LLC submitted a letter of intent to market theobromine as a New Dietary Ingredient and FDA filed this letter as an official filing on January 22, 2004." However, it also states that FDA issued a supplemental letter dated April 6, 1996. Please confirm if this is an error or explain the date discrepancy (page 20).
2. Please spell out the acronym PADI (page 21).
3. The percentage of unchanged theobromine (1-18%) found in the human urine as noted on page 36 is inconsistent with that presented in Table 5 (page 37).
4. In the calculations provided for total methylxanthine intake from cocoa powder on page 60, there appears to be some miscalculations (see below).
 - a. The notifier calculated that "cocoa powder provided 25.8 (mg/g) theobromine + 0.19 (mg/g) caffeine = 25.99 mg total methylxanthines/g cocoa powder". However, based on their previous statement that cocoa powder is comprised of 93% theobromine and 7% caffeine, the amount of caffeine would be 1.94 mg/g resulting in 27.74 mg total methylxanthines/g cocoa powder.
 - b. The total methylxanthine intake (mg/day) would be 21.64 mg/d instead of 20.27 mg/day.
 - c. That translates to 161.49 mg/kg/day (instead of 151 mg/kg/day) of methylxanthines in rats consuming 5% cocoa powder.

Please re-calculate and correct or explain how you derived your numbers.

- d. The notifier states that "The high dose level provided a mean methylxanthine intake of approximately 57 mg/kg bw/d for males and 74 mg/kg bw /d for females from wk 26-104 of the study. Again, the next paragraph provides different numbers for the same period. Moreover, it is not specified what 2.1g/kg bw/d and 2.7g/kg bw/d represent.

The description of the same study on pages 58, 60, and 78 are not identical.

5. Please explain how you derived that the lowest theobromine dose tested was approximately 300 mg/kg b.w. per day in the goat study? (Aregheore, 2002), page 70.
6. We were unable to find the NOAEL and LOAEL for young chickens and older broiler chickens and laying hens in the references you have provided. Please clarify.
7. We noticed a typographical error in the reference. Hostetler et al. is listed as 1991 on page 77 while the same reference is listed as 1990 in the subsequent paragraph. Please confirm which is the correct one.
8. The notifier states that the acceptable daily intake (ADI) of ~30 mg/person/day is significantly lower than the estimated daily intake (EDI) of 319 mg/person/day (90th percentile) from the intended uses of theobromine in the specified foods. The notifier discusses that the safety and exposure assessments of caffeine by Health Canada can be applied/considered to evaluate theobromine which is structurally similar to caffeine and is one of its metabolites. However, the notifier does not provide any comparative exposure estimate of theobromine derived from caffeine metabolism. Although the notifier notes that theobromine is consumed in large quantities by humans in various forms, the notifier does not clearly justify why the proposed 10x excess use on a

daily basis can be considered as safe.

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10. The 90th percentile of consumption of powdered fruit-flavored drinks is nearly 11x larger than the reported mean on page 26. Typically, the amount consumed at the 90th percentile is 2x greater than the mean. Please explain this difference.
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 E-mail: Natalia.Weinsetel@fda.hhs.gov

CONFIDENTIALITY NOTE: This message is intended only for the named recipient(s) and may contain confidential, proprietary, or legally privileged information. Unauthorized individuals or entities are not permitted access to this information. Any unauthorized dissemination, distribution, or copying of this information is strictly prohibited. If you have received this message in error, please advise the sender by reply email, and delete this message and any attachments. Thank you.

From: Weinsetel, Natalia
Sent: Tuesday, June 22, 2010 5:10 PM
To: 'Stanley M Tarka Jr, PhD'
Subject: RE: Theobromine GRAS Notification Submission

Hello Dr. Tarka,
 Just wanted to let you know that I placed the acknowledgement letter for GRN340 on the mail for you this afternoon.
 Best regards,
 Natalia

Natalia Weinsetel, Ph.D.
 Consumer Safety Officer
 Division of Biotechnology and GRAS Notice Review
 Office of Food Additive Safety

Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Tel: (301) 436-2907/Fax: (301) 436-2965
E-mail: Natalia.Weinsetel@fda.hhs.gov

From: Stanley M Tarka Jr, PhD [mailto:tarkagroup@comcast.net]
Sent: Friday, June 18, 2010 2:31 PM
To: Weinsetel, Natalia
Subject: RE: Theobromine GRAS Notification Submission

Dear Dr. Weinsetel,
Thank you very much for your quick response. I greatly appreciate this information and look forward to working with you. Please do not hesitate to contact me at any time with any questions that may arise during FDA's evaluation of this GRAS Notification.

Sincerely,

Stanley Tarka

Stanley M. Tarka, Jr., Ph.D.
President
The Tarka Group, Inc.
210 N. Old Stone House Road
Carlisle, PA 17015-8517
(Ph): 717-243-9216
(Fax): 702-993-5458
tarkagroup@comcast.net
website: www.tarkagroup.com

From: Weinsetel, Natalia [mailto:Natalia.Weinsetel@fda.hhs.gov]
Sent: Friday, June 18, 2010 9:54 AM
To: tarkagroup@comcast.net
Subject: RE: Theobromine GRAS Notification Submission

Dr. Tarka,
I am the CSO assigned to this notice. The acknowledgement letter is under internal review. You will be receiving a copy of it within a week or so.
Thank you,

Natalia Weinsetel, Ph.D.
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Tel: (301) 436-2907/Fax: (301) 436-2965
E-mail: Natalia.Weinsetel@fda.hhs.gov

From: Stanley M Tarka Jr, PhD [mailto:tarkagroup@comcast.net]
Sent: Thursday, June 17, 2010 2:25 PM
To: Martin, Robert L
Subject: RE: Theobromine GRAS Notification Submission

Dear Dr. Martin,

I am writing to follow-up with you regarding the Theobromine GRAS Notification that I submitted on behalf of Theocorp Holding Co and which was filed by FDA on May 17 as GRN 340. I had not received any official letter indicating that this was filed but do appreciate knowing that it has been filed based on FDA's website for pending GRAS Notices and is under review.

Could you please let me know who has been assigned as the official Consumer Safety Officer with the responsibility for this filing?

Thanks in advance.

Best regards,

Stanley Tarka

Stanley M. Tarka, Jr., Ph.D.

President

The Tarka Group, Inc.

210 N. Old Stone House Road

Carlisle, PA 17015-8517

(Ph): 717-243-9216

(Fax): 702-993-5458

tarkagroup@comcast.net

website: www.tarkagroup.com

From: Martin, Robert L [<mailto:Robert.Martin@fda.hhs.gov>]

Sent: Monday, May 17, 2010 7:20 AM

To: Stanley M Tarka Jr, PhD

Subject: RE: Confirmation of Receipt of Theobromine GRAS Notification Submission

Dr. Tarka, this is to confirm that we have received your submission. You can expect to hear from us soon.

Thanks.

Robert L. Martin

301-436-1219

From: Stanley M Tarka Jr, PhD [<mailto:tarkagroup@comcast.net>]

Sent: Friday, May 14, 2010 8:12 AM

To: Martin, Robert L; Gaynor, Paulette M

Subject: RE: Confirmation of Receipt of Theobromine GRAS Notification Submission

Dear Dr. Martin,

I am writing you to confirm that you did indeed receive the theobromine GRAS Notification submission that I sent by overnight delivery on behalf of Theocorp Holding Co, LLC. It was sent to your attention and delivered on Tuesday morning, May 10 and signed for by S. Johnson.

Thanks in advance and I look forward to hearing from you that the package was indeed received. After it is reviewed for completeness, I will look forward to the next step of in the process of receiving a written confirmation that is has been accepted for filing.

Sincerely,

Stanley Tarka

Stanley M. Tarka, Jr., Ph.D.

President

The Tarka Group, Inc.

210 N. Old Stone House Road

Carlisle, PA 17015-8517

(Ph): 717-243-9216

(Fax): 702-993-5458

tarkagroup@comcast.net

website: www.tarkagroup.com

From: Martin, Robert L [mailto:Robert.Martin@fda.hhs.gov]

Sent: Friday, March 19, 2010 6:48 AM

To: Stanley M Tarka Jr, PhD; Gaynor, Paulette M

Subject: RE: Request to Schedule Pre-GRAS Consultation Meeting

Dr. Tarka, 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: Stanley M Tarka Jr, PhD [mailto:tarkagroup@comcast.net]

Sent: Thursday, March 18, 2010 12:21 PM

To: Martin, Robert L

Subject: Request to Schedule Pre-GRAS Consultation Meeting

Importance: High

Dear Dr. Martin,

As a follow-up to the voice message I left you this morning, we are formally requesting on behalf of our client Theocorp Holding Company, LLC, a pre-GRAS consultation meeting with the FDA for a proposed food additive, theobromine (3,7-dimethylxanthine). The intended use of theobromine is as a nutrient to improve dentition. We would like to request a one hour meeting with the FDA with the primary objective of providing a review of our completed safety assessment and conclusions regarding the safety of the intended uses of theobromine in specified foods prior to submitting a GRAS Notification.

Company Name:

Theocorp Holding Company, LLC

3512 8th Street

Metairie, LA 70002

Date Preferred by Requestor:

April 28-PM

Alternatively, April 27 or 29-PM.

NOTE: Due to travel logistics of participants, it would be greatly appreciated if this meeting could be scheduled in the early PM if at all possible.

Names of Theocorp Holding Company Attendees at Meeting:

Dr. Arman Sadeghpour, President & CEO, Joseph Fuselier, Dr. Tetsuo Nakamoto

Names of Consultant Attendees at Meeting:

Dr. Stanley M. Tarka, Jr., The Tarka Group, Inc. Carlisle, PA
 Professor Joseph F. Borzelleca, Virginia Commonwealth University School of Medicine
 Professor John A. Thomas, Indiana University School of Medicine

Name of substance:

Theobromine (3,7-dimethylxanthine)

IUPAC Nomenclature: 3,7-dimethyl-2,3,6,7-tetrahydro-1*H*-purine-2,6-dione

EINECS number: 201-494-2

CAS No: 83-67-0

FEMA Number 3591

Objectives of the Meeting:

The primary objective of the meeting is to review the safety assessment work completed on theobromine, and our conclusions regarding the safety of its intended use in specified foods prior to submitting a GRAS Notification. The secondary objective is to solicit FDA's comments and advice on this or any other matters that may need to be addressed.

Proposed Agenda

1. Brief introduction of Attendees-All
2. Overview of Briefing Information- Dr. Tarka
3. Power Point presentation overview of comprehensive safety evaluation of theobromine for its intended uses- Dr. Tarka
4. FDA comments and recommendations- FDA
5. Any other matters

Thank you for the opportunity to consult with the Agency and OFAS on this matter. I look forward to hearing from you regarding confirmation of a date and time for this meeting. Please feel free to e-mail at tarkagroup@comcast.net or call me at 717-243-9216 if you have any questions. I will be traveling from March 20-25 and will have limited email or telephone access.

Thanks in advance.

Sincerely,

Stanley Tarka

Stanley M. Tarka, Jr., Ph.D.

President

The Tarka Group, Inc.

210 N. Old Stone House Road

Carlisle, PA 17015-8517

(Ph): 717-243-9216

(Fax): 702-993-5458

tarkagroup@comcast.net

website: www.tarkagroup.com

Gaynor, Paulette M

From: Gaynor, Paulette M
Sent: Thursday, September 02, 2010 8:39 AM
To: 'tarkagroup@comcast.net'
Cc: Harry, Molly *
Subject: GRN 000340

Dr. Tarka,

As a follow up to Natalia Weinsetel's note of August 27, 2010, the new CSO assigned to this notice is Molly Harry (tel: 301-436-1075)
Also in Natalia's note, reference is made to responses provided following a conference call.
Could you resend those responses to both Ms Harry and me?

Thank you,
Paulette

Paulette Gaynor, Ph.D.
FDA/CFSAN, HFS-255
Office of Food Additive Safety
5100 Paint Branch Pkwy
College Park, MD 20740

Tel: (301) 436-1192
Fax: (301) 436-2964

E-mail: paulette.gaynor@fda.hhs.gov



Memorandum of Telephone Conversation

Date: October 29, 2010

Subject: Theobromine (GRN 000340)

Participants:

Industry: Stanley M. Tarka Jr., Ph.D. The Tarka Group, Inc. (Agent for Theocorp Holding Company, LLC)


FDA: Ronald Chanderbhan, Ph.D. HFS-255
Michael DiNovi, Ph.D. HFS-255
Molly Harry, M.S. HFS-255(ORISE Fellow)

Drs. DiNovi and Chanderbhan and Ms. Harry called Dr. Tarka on October 29, 2010 to discuss the issue of the estimated daily intake (EDI) and the acceptable daily intake (ADI) values in GRN 000340 which Theocorp Holding Company, LLC submitted to FDA. Dr. DiNovi recommended that Theocorp re-visit their exposure data and exclude infants 0-2 years old from their exposure calculation and also state that their products will not be marketed for consumption to infants in this age group. FDA also explained that the review team is having difficulty getting past the fact that the EDI is higher than the ADI.

Dr. Tarka promised getting in touch with Theocorp immediately on the issues we discussed, and he will get back to us early the following week (week of November 1, 2010).

~~Michael DiNovi, Ph.D.~~
Michael DiNovi, Ph.D.

(b) (5)



From: Stanley M Tarka Jr, PhD [mailto:tarkagroup@comcast.net]
Sent: Friday, October 29, 2010 11:23 PM
To: Martin, Robert L
Cc: Harry, Molly *
Subject: RE: GRN 340 Clarification on Population Exposure Estimates
Importance: High

Dear Dr. Martin,

Please find attached a signed copy of a letter (pages 1 and 2) clarifying the exposure estimates in GRN 340 for infants. This is a follow-up to a conference call held on October 29 with Molly Harry, Dr. Michael DiNovi and Dr. Ron Chanderbhan pertaining to an exposure intake assessment question in the Theocorp Holding Company, LLC GRAS notification for theobromine. The specific question posed dealt with the level of exposure in the infant population (0-2 years) in the mathematical model used that shows exposure estimates for various segments of the population.

As Theocorp's Agent for GRN340, the attached letter is provided to confirm that Theocorp does not intend to market products to the infant population (0-2 years), and this population was not considered as part of their safety assessment for the intended uses of theobromine. Consequently, by removing and discounting this population to accurately reflect the basis for this GRAS determination, the Estimated Daily Intake (EDI) for the total population is slightly reduced on a mg/kg bw basis as reflected in the tables provided in this letter. . You will note that removing the infants only had a minor impact on the consumption estimates. The actual amounts of consumption did not change whereas the mg/kg bw went down somewhat and likely as a result of the smaller body weights of the infants. The small changes result from the fact that the number of infants is relatively small compared to other population groups originally considered.

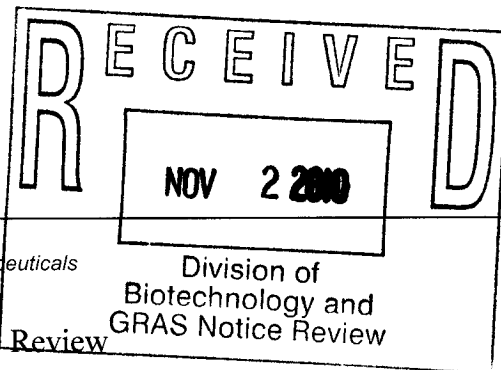
The original letter is being sent by FedEx to your attention on Monday, November 1, and should arrive on Tuesday.

Thank you very much for your attention to this matter and we look forward to FDA's completion of their assessment.

Sincerely,

Stanley Tarka

Stanley M. Tarka, Jr., Ph.D.
President
The Tarka Group, Inc.
210 N. Old Stone House Road
Carlisle, PA 17015-8517
(Ph): 717-243-9216
(Fax): 702-993-5458
tarkagroup@comcast.net
website: www.tarkagroup.com



October 29, 2010

Scientific and Regulatory Solutions for Food and Pharmaceuticals

Robert L. Martin, Ph.D.

Deputy Director, Division of Biotechnology and GRAS Notice Review

Office of Food Additive Safety (HFS-255)

Center for Food Safety and Applied Nutrition

U.S. Food and Drug Administration

5100 Paint Branch Parkway

College Park, MD 20740-3835

RE: GRN340: Notification of GRAS Determination for Theobromine (3,7-Dimethylxanthine) Clarification on Population Exposure Estimates

Dear Dr. Martin:

This letter is in response to a conference call held on October 29 with Molly Harry, Dr. Michael DiNovi and Dr. Ron Chanderbhan pertaining to an exposure intake assessment question in the Theocorp Holding Company, LLC GRAS notification for theobromine. The specific question posed dealt with the level of exposure in the infant population (0-2 years) in the mathematical model used that shows exposure estimates for various segments of the population.

As Theocorp's Agent for GRN340, this letter is to confirm that Theocorp does not intend to market products to the infant population (0-2 years), and this population was not considered as part of their safety assessment for the intended uses of theobromine. Consequently, by removing and discounting this population to accurately reflect the basis for this GRAS determination, the Estimated Daily Intake (EDI) for the total population is slightly reduced on a mg/kg bw basis as shown in the tables below.

Table 4.2-5 Summary of the Estimated Daily Intake of Theobromine from All Background Levels and Proposed Food Uses in the U.S. by Population Group (excluding infants) (2003-2004, 2005-2006 NHANES Data)							
Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption (mg)		All-User Consumption (mg)	
				Mean	90 th Percentile	Mean	90 th Percentile
Children	3 to 11	99.3	2,713	148	285	149	285
Female Teenagers	12 to 19	97.2	1,931	133	275	137	280
Male Teenagers	12 to 19	96.8	1,877	157	329	161	335
Female Adults	20 and Up	96.7	4,142	144	318	148	321
Male Adults	20 and Up	96.1	3,693	155	339	160	341
Total Population	All Ages	97.1	14,355	147	317	151	319

Table 4.2-6 Summary of the Estimated Daily per Kilogram Body Weight Intake of Theobromine from All Background Levels and Proposed Food Uses in the U.S. by Population Group (excluding infants) (2003-2004, 2005-2006 NHANES Data)

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption (mg/kg bw)		All-User Consumption (mg/kg bw)	
				Mean	90 th Percentile	Mean	90 th Percentile
Children	3 to 11	99.3	2,713	5.6	10.9	5.7	10.9
Female Teenagers	12 to 19	97.2	1,931	2.3	4.7	2.3	4.7
Male Teenagers	12 to 19	96.8	1,877	2.5	5.5	2.5	5.5
Female Adults	20 and Up	96.7	4,142	2.0	4.4	2.1	4.5
Male Adults	20 and Up	96.1	3,693	1.8	4.0	1.9	4.1
Total Population	All Ages	97.1	14,295	2.4	5.5	2.5	5.5

Should you have any additional questions regarding this GRAS Notice, please contact me at 717-243-9216.

Sincerely,



Stanley M. Tarka, Jr., Ph.D.
President
The Tarka Group, Inc.

cc: Arman Sadeghpour, Ph.D., Theocorp Holding Company, LLC, Metairie, LA

Memorandum of Telephone Conversation

Date: November 9, 2010

Between: Ronald Chanderbhan, Ph.D. HFS-255
Rebecca Danam, Ph.D. HFS-255
Paulette Gaynor, Ph.D. HFS-255
Molly Harry, M.S. HFS-255(ORISE Fellow)
Robert Martin, Ph.D. HFS-255

and

Stanley M. Tarka Jr., Ph.D. The Tarka Group, Inc. on behalf of Theocorp Holding Company, LLC (Tel: 717-243-9216)

Subject: Theobromine (GRN 000340)

At a prearranged time, FDA called Dr. Tarka to discuss a way forward for GRN 000340. FDA explained that we have come across some stumbling blocks in our review and would like to discuss the options at this stage. The main issue is that the cumulative estimated daily intake (EDI) [2.5 milligrams per kilogram body weight per day (mg/kg bw/d) at the mean, and 5.5 mg/kg bw/d at the 90th percentile level] is higher than the acceptable daily intake (ADI) [0.5 mg/kg bw/d] calculated by Theocorp Holding Company, LLC in their notice. Secondly, there were other issues with some of the studies summarized in the notice that Theocorp did not successfully explain why these adverse effects would not be of concern (e.g, the issue of testicular toxicity and its relevance/non-relevance was not put to rest in the notice). We informed Dr. Tarka that we cannot agree that the notice provides a basis for a GRAS determination given the submission we have.

FDA explained that the options would be for FDA to send a "bad day" letter or for Theocorp to withdraw the notice. FDA advised that if Theocorp decides to withdraw the notice that they should come for a pre-submission discussion with FDA prior to making another submission.

Dr. Tarka inquired about the food categories that are a problem. In response, FDA focused Dr. Tarka on Theocorp's ADI, reminding Dr. Tarka that the EDI is five times higher than the ADI calculated by Theocorp. Regarding Dr. Tarka's inquiry about studies, FDA explained that the notice looks to have all the necessary elements and that if put together in a cohesive discussion, could get to a conclusion that theobromine is generally recognized as safe for the intended uses. Additionally, FDA explained that some studies summarized in the notice show reproductive and developmental toxicity in animals. Some data show testicular atrophy in male rats and rabbits administered theobromine, the effects occur at 300 mg/kg bw in male rats, with the NOAEL for reproductive toxicity of 150 mg/kg bw/d. Other studies show delayed ossification in rats with a NOAEL of 50 mg/kg bw/d that Theocorp used to calculate the

(b) (5)			OFFICE	SURNAME	DATE	OFFICE	SURNAME	DATE	OFFICE	SURNAME	DATE


ADI with a safety factor of 100. FDA also stated that the Office of Nutrition Labeling and Dietary Supplements expressed concern in 1996 regarding the adverse effects observed in animal consuming theobromine, and that these concerns are currently unresolved. Theocorp did not successfully explain why these adverse effects would not be of concern, and overall, why in light of the totality of the data, would the intended uses be GRAS.

FDA advised that in their new submission Theocorp should consider explaining why some of the adverse effects resulting from consumption of theobromine may or may not occur in humans using data on metabolism of theobromine in humans and rodents; decrease their reliance on caffeine data, and also consider reducing the safety factor when calculating the ADI.

Dr. Tarka agreed to discuss these issues with Theocorp.

Paulette Gaynor, Ph.D.

(b) (5)





Harry, Molly *

From: Stanley M Tarka Jr, PhD [tarkagroup@comcast.net]
Sent: Wednesday, November 10, 2010 5:01 PM
To: Martin, Robert L; Harry, Molly *
Cc: 'Arman Sadeghpour PhD'
Subject: GRN 340 Theocorp Letter Notification to Cease Evaluation and Withdrawal
Importance: High
Attachments: GRN 340 Theocorp Letter Notification of Withdrawal 11 Nov 2010.pdf

Dear Dr. Martin and Molly,
After discussion with Theocorp Holding Company, LLC, please find attached the letter of withdrawal for Theocorp's GRN 340-theobromine notification. As discussed, Theocorp Holding Company requests that FDA cease to evaluate their GRAS Notification submission GRN 000340 for theobromine. Following the voluntary withdrawal of this notification, Theocorp intends to provide a new notification submission after discussions with the agency in the near future. At that time, they will be contacting you to arrange a pre-meeting with the original reviewers so that they can fully understand and address their unresolved questions in a subsequent submission.

Sincerely,

Stanley Tarka

Stanley M. Tarka, Jr., Ph.D.
President
The Tarka Group, Inc.
210 N. Old Stone House Road
Carlisle, PA 17015-8517
(Ph): 717-243-9216
(Fax): 702-993-5458
tarkagroup@comcast.net
website: www.tarkagroup.com



RECEIVED
NOV 10 2010
BY e-Mail (b) (6)

Scientific and Regulatory Solutions for Food and Pharmaceuticals

November 10, 2010

Robert L. Martin, Ph.D.
Deputy Director, Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

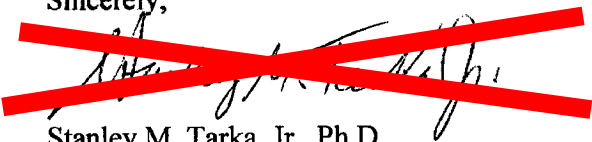
RE: GRN 000340: Notification of GRAS Determination for Theobromine (3,7-Dimethylxanthine)-Request to Cease Evaluation

Dear Dr. Martin:

On behalf of Theocorp Holding Company, LLC, I am writing as their agent to formally request that FDA cease to evaluate their GRAS Notification submission GRN 000340 for theobromine. As the notifier, Theocorp is also requesting to voluntarily withdraw their submission GRN 000340 with the intention of providing a new submission after discussions with the agency.

Should you have any additional questions regarding this GRAS Notice, please contact me at 717-243-9216.

Sincerely,


Stanley M. Tarka, Jr., Ph.D.
President
The Tarka Group, Inc.

cc: Arman Sadeghpour, Ph.D., Theocorp Holding Company, LLC, Metairie, LA

Memorandum of Telephone Conversation

Date: November 9, 2010

Between: Ronald Chanderbhan, Ph.D. HFS-255
Rebecca Danam, Ph.D. HFS-255
Paulette Gaynor, Ph.D. HFS-255
Molly Harry, M.S. HFS-255(ORISE Fellow)
Robert Martin, Ph.D. HFS-255

and

Stanley M. Tarka Jr., Ph.D. The Tarka Group, Inc. on behalf of Theocorp Holding Company, LLC (Tel: 717-243-9216)

Subject: Theobromine (GRN 000340)

At a prearranged time, FDA called Dr. Tarka to discuss a way forward for GRN 000340. FDA explained that we have come across some stumbling blocks in our review and would like to discuss the options at this stage. The main issue is that the cumulative estimated daily intake (EDI) [2.5 milligrams per kilogram body weight per day (mg/kg bw/d) at the mean, and 5.5 mg/kg bw/d at the 90th percentile level] is higher than the acceptable daily intake (ADI) [0.5 mg/kg bw/d] calculated by Theocorp Holding Company, LLC in their notice. Secondly, there were other issues with some of the studies summarized in the notice that Theocorp did not successfully explain why these adverse effects would not be of concern (e.g, the issue of testicular toxicity and its relevance/non-relevance was not put to rest in the notice). We informed Dr. Tarka that we cannot agree that the notice provides a basis for a GRAS determination given the submission we have.

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Dr. Tarka inquired about the food categories that are a problem. In response, FDA focused Dr. Tarka on Theocorp's ADI, reminding Dr. Tarka that the EDI is five times higher than the ADI calculated by Theocorp. Regarding Dr. Tarka's inquiry about studies, FDA explained that the notice looks to have all the necessary elements and that if put together in a cohesive discussion, could get to a conclusion that theobromine is generally recognized as safe for the intended uses. Additionally, FDA explained that some studies summarized in the notice show reproductive and developmental toxicity in animals. Some data show testicular atrophy in male rats and rabbits administered theobromine, the effects occur at 300 mg/kg bw in male rats, with the NOAEL for reproductive toxicity of 150 mg/kg bw/d. Other studies show delayed ossification in rats with a NOAEL of 50 mg/kg bw/d that Theocorp used to calculate the

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ADI with a safety factor of 100. FDA also stated that the Office of Nutrition Labeling and Dietary Supplements expressed concern in 1996 regarding the adverse effects observed in animal consuming theobromine, and that these concerns are currently unresolved. Theocorp did not successfully explain why these adverse effects would not be of concern, and overall, why in light of the totality of the data, would the intended uses be GRAS.

FDA advised that in their new submission Theocorp should consider explaining why some of the adverse effects resulting from consumption of theobromine may or may not occur in humans using data on metabolism of theobromine in humans and rodents; decrease their reliance on caffeine data, and also consider reducing the safety factor when calculating the ADI.

Dr. Tarka agreed to discuss these issues with Theocorp.

Paulette Gaynor, Ph.D.

(b) (5)



TRANSMISSION VERIFICATION REPORT

TIME : 11/17/2010 14:20
NAME : FDA CFSAN OFAS
FAX : 3014362965
TEL :
SER.# : BROF6J488319

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Page 1 of

FACSIMILE



Office of Food Additive Safety

Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
HFS -255
College Park, MD 20740

TO: Stanley M. Tarka, Jr., Ph.D

The Tarka Group Inc

210 N. Old Stonehouse Road

Carlisle, PA 17015-8517

FAX NO: 702-993-5458

FROM: Molly Harry

DATE Nov 17, 2010

PHONE NO: 301-436-1075

FAX NO (301) 436- 2965

MESSAGE

Response letter for GRN 000340 (theobromine), original copy will be sent in the mail.
Index to FDA Response to NRDC's FOIA Request No. 2013-8042 for GRN-340

Page 229 of 229