

**Summary Minutes**  
**US Environmental Protection Agency Science Advisory Board**  
**Meeting**

**Public Teleconference Meeting**  
**October 28, 2008**  
**8:30 am – 3:00 pm (Eastern Time)**  
**Meeting Location: Via Telephone**

**Purpose of the Meeting:** The Meeting was held to allow for the Chartered SAB to conduct a quality review of two draft SAB reports. The meeting agenda is in Attachment A. The list of SAB and other participants follows.

**Meeting Participants:**

**Members Participating in the Meeting:**

Dr. Deborah L. Swackhamer, Chair	Dr. David Allen
Dr. Greg Biddinger	Dr. Tim Buckley
Dr. Thomas Burke	Dr. Deborah Cory-Slechta
Dr. Terry Daniel	Dr. David Dzombak
Dr. Rogene Henderson	Dr. James Johnson
Dr. Cathy Kling	Dr. George Lambert
Dr. Jill Lipoti	Dr. L.D. McMullen
Dr. Judith Meyer	Dr. Jana Milford
Dr. Christine Moe	Dr. Duncan Patten
Mr. Steve Roberts	Dr. Joan Rose
Dr. James Sanders	Dr. Jerry Schnoor
Dr. Thomas Theis	Dr. Valerie Thomas
Dr. Thomas Wallsten	Dr. Daniel Watts (Liaison NACEPT)
Dr. Lauren Zeise	

**MEETING SUMMARY**

**Tuesday, October 28, 2008**

This meeting was announced in the *Federal Register* (see 73 FR p 70344 of November 20, 2008 - Attachment B). The SAB Roster is in Attachment C.

**1. Convene the Meeting:** The DFO convened the meeting noting that it was a federal advisory committee meeting and that the Board's deliberations are held as "public meetings" pursuant to the Federal Advisory Committee Act (FACA), its regulations, and the policies of the US EPA for advisory activities. Mr. Miller noted that several members of the public had requested time and some had provided written input for the Board's consideration.

Mr. Miller noted that SAB members must comply with Federal ethics and conflict-of-interest laws and that SAB ethics officials review relevant information to ensure that SAB panels reflect

appropriate balance and that COI and bias issues are addressed and that the SAB members participating in this meeting had submitted information on whether they knew of any potential appearance of impartiality issues that could link them with the topics on the agenda. As a result of this process one Board Member (i.e., Dr. James Bus) asked to be recused from participating in the acrylamide advisory quality review because of a legacy issue related to potential employer liability. The SAB Ethics Official agreed that this was an appropriate recusal and also determined that other Members participating in the day's issues on the call did not have any such issues within the meaning of the relevant ethics and conflict of interest requirements that apply to the advisory activities.

Mr. Miller then turned the meeting over to the SAB Chair, Dr. Deborah L. Swackhamer, to carry out the agenda. Dr. Swackhamer welcomed those participating in the review, noted the purpose of the meeting, and explained the nature of an SAB quality review.

**2. Discussion of Future Directions for EPA's Research Program:** Dr. Swackhamer introduced the session noting that the intent is now to think of how the information presented to the Board during the October 27 meeting that focused on *Looking to the Future* (see Attachment D for the notes from that meeting) might provide lessons that should be integrated into the Board's ongoing consideration of EPA's *Strategic Research Directions*. The session included summaries of the October 27 presentations with follow up Board discussions and an interaction with Dr. Kevin Teichman, EPA ORD Deputy Assistant Administrator for Science.

- a) To initiate the SAB's discussion **of the biofuels issue**, Dr. David Dzombak focused on several important messages that he took from the presentations at the October 27 seminar. He noted that:
- i) Dr. Bruce Dale emphasized of "sustainable paths to a biofuel-powered transportation sector" highlighted the significant opportunity for innovation and invention in cellulosic biofuels and the need for complete life-cycle analyses in this area;
  - ii) Dr. Kenneth Cassman discussed "biofuels and environmental sustainability" and noted the population stress associated with increased food and fuel conflicts and the importance for EPA leadership in research planning for biofuels;
  - iii) Dr. David Tilman discussed the "environmental impacts of food versus cellulose based biofuels" and called for EPA progress in doing a thorough and well-documented life cycle analysis for biofuels;
  - iv) Dr. Christopher Field discussed climate change relative to biofuels production and use and the challenge involved in a coordinated effort to ensure the environmentally friendly development of biofuels.

SAB Members mentioned a number of issues that were thought to be of importance to EPA as the biofuels issues moves forward, including:

- i) The need for decision making at the watershed level;
- ii) The tasks that EPA must accomplish under EISA;
- iii) How to deal with the need for greenhouse gas foot-printing for biofuels;
- iv) The new EISA requirements provide an opportunity for conducting high quality life cycle assessments for biofuels as contrasted with limited LCAs of the past—the life-

cycle assessments should not be confined to biofuels alone – the SAB could be a part of doing a broad LCA;

- v) There must be an appreciation for land use challenges that are associated with biofuels;
- vi) Implications of nitrogen fertilization that are associated with biofuels are enormous and uncontrolled;
- vii) Cost-benefit and cost-effectiveness analyses will be important in “control” decisions for nitrogen;
- viii) SAB could produce nutrient management guidance for communities in the biofuels area if there was a desire for our assistance;
- ix) EPA is well-positioned in environmental measurement and it should help define the measurement requirements associated with biofuels monitoring;
- x) Water scarcity is a major international issue and the implications of biofuels on water availability should be assessed – water use in biofuels is large and wastewater reuse should be a part of the issue;
- xi) NACEPT is looking at regulatory structures for biofuels;
- xii) Matching the right crop to the right lands is an important component of this issue;
- xiii) The coordination need across government and non-government groups is large in the alternative fuels area.

b) Dr. Deborah Cory-Slechta initiated **the epigenomics discussion**. She noted that:

- i) The context of the seminar discussions was health assessment;
- ii) The need is for assessments that consider more than just one agent at a time;
- iii) Better models are needed, for example the way the air program is focusing on one-air is a good example of how to meet the need;
- iv) Yesterday’s seminar pointed out the importance of under-nutrition early in life causing major problems later in life;
- v) Assays like current rodent model systems for toxicity do not help to get at these issues.

Members commented on a number of issues, including:

- i) Some members noted that one at least needs to evaluate how the rat models relate to actual human physiology;
- ii) New approaches are needed or we will find ourselves in the same place for health assessment in 50 years as we now are in – upstream indicators of human disease would be helpful;
- iii) EPA might be well advised to focus more on prevention than mechanism which seems to be well staked out by NIH;
- iv) EPA should look closely at susceptible populations;
- v) There are both chemical and non-chemical risk factors to be considered;
- vi) The recently released NRC report on improving risk assessment at EPA also asks the question about the effectiveness of continuing to do single chemical risk assessment or to focus on individual components. The focus advised there is to think of cumulative exposures and to recognize that humans are exposed to many things concurrently.

- vii) Upstream markers of multiple exposures are also recommended in another NAS report on toxicologic testing. This should also be coupled with consideration of what this means on the risk management side.
- viii) EPA must also come to grips with new and much broader conceptions of risk that go beyond the old narrow ways of thinking – they need to consider transgenerational issues as well as issues that play out on a massive scale (e.g., as the land use issues, etc. that are associated with biofuels). We must also help the public understand what this new “meta” way of looking at risk involves.

**3. Public Comments:** Mr. Mark Greenwood, presented comments on behalf of the Coalition for Effective Environmental Information (see [Attachment E](#)). Their comments supported the SAB’s emphasis on the need for risk communication research that was raised in the SAB’s report on the EPA Strategic Research vision. The coalition sees risk communication as essential and the need to go beyond mere news releases is evident. EPA should consider establishing a center of excellence for risk communication.

**4. Discussion with Dr. Kevin Teichman, DAA for Science, US EPA ORD:** The Board continued its running discussions with Dr. Teichman on the EPA research program. Dr. Teichman thanked the Board for its initial 2008 report on EPA’s strategic research directions and commented on several issues, including:

- a) His opinion that the current approach of separating the budget discussion from the strategic research vision seems to be allowing more focus on science and that is helpful;
- b) A reminder that science occurs throughout EPA and that just discussing ORD’s program misses many things that occur in the program offices;
- c) Information on the ORD National Program Directors initiative to identify the three most critical environmental issues facing the nation and ORD’s unique role – its niche – in responding to the issue. Issues identified included:
  - i) Global Climate Change
  - ii) Toxicology Testing “Revolution”
  - iii) Water and Energy with an eye toward foot-printing for water sustainability as well as carbon foot-printing)
- d) And the “elevator speech” for ORD’s uniqueness: that focuses on
  - i) contributions to cutting edge technologies
  - ii) development of techniques ranging from risk assessment through risk management
  - iii) its ability to focus on EPA’s unique needs to support its specific mission
  - iv) its role as a key player in interagency discussions and activities on major cross-cutting environmental problems (e.g., energy and biofuels; nanotechnology)
- e) In regard to the October 27 seminars, ORD shares the view of the importance of the alternative fuels issue and the role and development of emerging epigenomic technologies for use in policy analysis, nanotechnology assessment, ecosystem service valuation, and the need to think outside the box when it comes to how science and technology might help policy making and implementation to get better outcomes.
- f) ORD appreciates the need for economic research (noting that the research is the lead area for the National Center for Environmental Economics).

- g) ORD also agrees with the need to look at more integrated ways for integrated consideration of issues but also recognizes that there continue to be more narrow short-term needs in EPA's programs.

Members thanked Dr. Teichman for his reflections and mentioned a number of issues:

- a) The needs and concerns that face EPA at the regional level differ from one geographic area to the next. These needs are important for ORD to address with its research and technology programs. – Dr. Teichman agreed and noted the ORD initiatives that place technical liaisons in each regional office and the RARE program that provides some research funds to regional scientists for use in Region-specific science efforts.
- b) The need for non-point source control initiatives for EPA (legislation, research).
- c) The importance for ORD to continue to inform decision makers of the unique role played by the STAR program and the cost associated with its decrease over the years.
- d) The importance of risk communications research

Members noted the importance of the “elevator” speech on ORD's uniqueness including statements that make it clear that the future of EPA depends on full utilization of ORD. Lack of resources invested in research and development causes conditions that lead those on the outside to question EPA's fitness and future.

**5. Quality Review of the Draft SAB Advisory on Acrylamide:** The Board conducted its quality review of the draft SAB advisory on *SAB Advisory on Acrylamide* (see Attachment F). At the Chair's request, Dr. Deborah Cory-Slechta summarized the issue and the primary conclusions of the Committee's draft report. SAB Member comments are in Attachment G. Dr. Swackhamer asked Members if they wanted to highlight any of their written comments, or if they had other comments to raise in regard to the draft report. Several members highlighted comments (i.e., Dr. Lambert's and Dr. Buckley's comment on reference dose and the need to bring that forward to the executive summary and Dr. Henderson's suggestions on toxicokinetics vs. pharmacokinetics. Dr. Cory-Slechta stated that the Members' comments will all be able to be accommodated in revisions and edits to the existing draft.

- a) **Public Comments:** The Chair noted that several people from the public had requested time to make an oral statement and that many had also sent written comments which had been distributed to the Board for consideration in regards to the draft. She called upon these persons to make their statements.
  - i) Mr. Robert Fensterheim, Dr. Al Wiedow, and Dr. Marvin Friedman spoke on behalf of the North American Polyelectrolyte Producers Association (see Attachment H – physical file only - and I). Mr. Fensterheim spoke to the perceived rarity of having an SAB review of an IRIS chemical; the need for the SAB report to give greater attention to ongoing TVM studies at NCTR, and noted that his colleagues suggest that the SAB Panel did not recognize this in the draft report. Dr. Friedman's statement focused on a number of issues including “alleged” brain tumors in rats after acrylamide chronic drinking water studies and the Panel's finding fault with the protocol used in the study (see Attachment J – physical file only). He stated that the NCTR study should be used to resolve the issue of whether acrylamide was a CNS carcinogen. He also stated that

at worst acrylamide was only a very weak mutagen. He also criticized the Panel for not using human data that is available.

- ii) Dr. Robert Tardiff presented comments on the draft EPA report on behalf of the Grocery Manufacturer's Association. He agrees that the PBTK model is key to the issue and stated that EPA's draft toxicity review of acrylamide missed essential validation; missed 3 years of relevant data, and erred in the MoA involved in detoxification. He referenced a major carcinogenicity study at NTP that should provide key insights to the issue. He stated that the ARP panel report should recommend as robust a toxicological review as possible by incorporating updated and validated PBTK models, by incorporating the NTP data, and by expanding the report to recommend fixes to current limitations and review during EPA's next draft of the toxicological report. He noted the importance of considering the context of dietary intake for acrylamide (see Attachment K).

Dr. Cory-Slechta was asked to respond to the SAB and public comments. She noted that the NCTR study was discussed by the Panel, that the study's "author" was a panel member, and that in the interest of getting the SAB report completed in a timely fashion that they did not delay the panel report for its completion. They were assured by EPA staff that the results of that study would be considered as they continued to revise the EPA draft toxicological review. She noted that some other clarifying information that was provided was from unpublished data and that it would not be considered by the Panel. It will be up to EPA to decide on how to consider unpublished data in their continued work. She noted that the report will clarify that EPA needs to consider newly arriving data as it goes forward.

Dr. Swackhamer asked members for a motion. A motion was offered that the draft advisory report be approved subject to revisions noted by members and agreed to otherwise in the quality review and that the draft be provided to SAB vectors Drs. Karol and Lambert for a final look at the revisions. If they do not object to the revisions, the report shall be transmitted to the EPA Administrator. The motion was seconded.

In the ensuing discussion, a member reacted to the statement by the public commenters that indicated they are unclear about how their earlier interactions with the Panel (i.e., their written and oral comments) were considered. How the SAB considers such comments is not prescribed, but it was thought that the issue should be taken up by the Board and that some further guidance issued to clarify how the public can be shown that their comments have been considered. The Board will consider such guidance at a future meeting.

The Chair called for a vote on the motion. All members voted for the motion. There were no abstentions or no votes.

**ACTION:** Dr. Cory-Slechta, and the Panel DFO, will edit the advisory to reflect the comments provided by SAB Members. The final draft will be vetted by Drs. Karol and Lambert and once they have approved the revisions the report will be transmitted to the EPA Administrator.

## **5. Quality Review of the Draft SAB Advisory on Aquatic Life Criteria for Contaminants of**

**Emerging Concern**: The Board conducted its quality review of the draft *SAB Advisory on Aquatic Life Criteria for Contaminants of Emerging Concern* (see [Attachment L](#)). At the Chair's request, Dr. Judith Meyer summarized the issue and the primary conclusions of the Committee's draft report. SAB Member comments are in [Attachment M](#). The DFO noted that a written comment had been provided to Board members on this issue by Dr. Amanda Palumbo (see [Attachment N](#)).

Dr. Swackhamer asked Members if they wanted to highlight any of their written comments, or if they had other comments to raise in regard to the draft report. Several members highlighted comments they had made and Dr. Meyer referred to her written response to the member comments (see [Attachment O](#)) and noted that the Board member concerns would be handled in the way proposed therein unless objections were heard to that approach. No objections were offered.

Dr. Swackhamer asked for a motion on the draft report. A motion was made and seconded to Approve the report subject to revisions being made as proposed in the response document from Dr. Meyer. The Chair asked for a vote and all present voted for approval with no abstentions and no member voting no.

**ACTION**: Dr. Meyer, and the Panel DFO, will edit the advisory to reflect the comments provided by SAB Members. The final draft will not need to be vetted and it can be sent as a final report to the EPA Administrator once the revisions are made.

## **6. Quality Review of the Draft SAB Advisory on EPA's Draft Third Drinking Water Contaminant Candidate List (CCL 3).**

The Board conducted its quality review of the draft *SAB Advisory on EPA's Draft Third Drinking Water Contaminant Candidate List (CCL 3)* (see [Attachment P](#)). At the Chair's request, Dr. Joan Rose summarized the issue and the primary conclusions of the Committee's draft report. SAB Member comments are in [Attachment Q](#). Dr. Rose noted that in its work, though the DWC generally supported the approach used, it was not possible for the DWC to reproduce the agency's work on the assessments because in many cases, key issues were resolved using professional judgment that was not easily discerned. In essence, implementation of the Agency process could be made more transparent.

Dr. Swackhamer asked Members if they wanted to highlight any of their written comments, or if they had other comments to raise in regard to the draft report. Several members highlighted comments they had made. Dr. McMullen noted that the draft DWC report was not even, in that the responses to questions were not all equal in detail (e.g., 1 included a greater amount detail than did the response to question 2). Clarification is needed in several areas. Dr. Moe supported Dr. Thomas' comments and noted that the response to questions 3 and 4 were not easily located in the draft. She also noted that some of the terminology is not accurate. Drs. Johnson and Thomas also indicated the need for clarification of the draft. In response to a question on use of biomonitoring data, EPA representatives noted that there were no biomonitoring data used. Dr. Burke noted the enormous body of body burden data that is available now, e.g., on contaminants like perchlorate and that these could be usefully employed in the CCL process. The CCL process is also one in which the SAB can provide

valuable advice early in EPA's consideration of the need for regulation instead of the end when a proposal for a regulation is being advanced outside EPA.

Dr. Rose noted that it would be possible to reorganize the report and to make some additional clarification to both make the DWC advice more to the point and to ensure that the Board clarifications are also a part of the advice. In regard to the last 2 charge questions, Dr. Rose noted that they asked the DWC to essentially do the work that the Agency should be doing in providing data to support additions or deletions to the draft CCL and that partially explains the lack of detail on those questions. This can be made more direct.

Dr. Swackhamer asked for a motion on the draft report. A motion was made and seconded that the draft report be returned to the DWC for major revision per the comments received from the Board in writing and during this discussion.

During the ensuing discussion, Dr. Rose noted that this would be accomplished in time for the draft to be circulated to the Board in time for a completion of the quality review during the December 16 teleconference.

Dr. Swackhamer called the motion for a vote and the members voted to approve the motion. There were no abstentions nor no votes.

**ACTION:** The report will be returned to the DWC Chair for revision as noted in the motion with the intention that the quality review be completed at the December 16, 2008 SAB teleconference.

**7. Discussion of a Collateral Issue Raised During the CCL 3 Review:** Dr. Rose noted that during the review of the draft CCL 3 that one candidate contaminant that was included in the draft list was perchlorate. EPA had in a separate Federal Register notice indicated that it intended to do a preliminary determination on whether or not it should move forward to regulate perchlorate with a drinking water regulation. In a recent Federal Register notice (73 FR 60262-60282 dated October 10, 2008), EPA has made a preliminary determination not to regulate perchlorate with a drinking water regulation (i.e., an MCL – maximum contaminant level) stating that "...a national primary drinking water regulation (NPDWR) for perchlorate would not present 'a meaningful opportunity for health risk reduction for persons served by public water systems'". The notice of preliminary determination is now out for comment and the comment period ends on November 10 thus there is not sufficient time for the DWC to reconvene to develop advice on the Agency's preliminary determination – which EPA intends to make final in December 2008. Dr. Rose noted concern with the transparency of the process used by EPA in arriving at its preliminary determination on perchlorate. Though the DWC was actively considering the CCL 3 during this time, the Agency did not raise the perchlorate issue to the DWC during this time and did not update the DWC on its intentions on the issue nor where it was in the analysis. It is not clear how the Agency reached its conclusion on perchlorate. The issue clearly falls within the intent of EPA's Charge Questions 3 and 4 to the DWC which ask about contaminants on the draft CCL 3 that are listed which should not be on the list (question 3) or contaminants which are not on the list which should be (question 4). By not making it clear during the DWC's consideration of the draft CCL 3 what the status of perchlorate was in the Agency's analysis, it made it difficult for the DWC to clearly advise EPA on whether the Committee believed the perchlorate should continue on the list or whether it was to be removed from the list for reasons that were or were not scientifically sound. Dr. Rose also noted that there was lack of clarity about the

model used by EPA to on the key body burden question for perchlorate as well as how that model was peer reviewed.

Dr. Swackhamer asked if the EPA representatives in attendance cared to respond to Dr. Rose's concern? Ms. Barr noted that EPA sees the CCL process and the preliminary determination processes to be moving on separated tracks and that EPA relied on the May 2007 Federal Register notice on EPA's intention to move to a preliminary determination on perchlorate as adequate to register its intention to all that this was happening. In addition, the model in question is now undergoing peer-review,

The Board discussed whether it would be appropriate for it to send a letter from the SAB to the Administrator indicating the Board's concern. During the discussion, several members indicated a desire to receive more detailed information on EPA's analysis and how the issue was pursued prior to preparing a letter from the full SAB. Because this was not possible before the comment period was scheduled to close, it was left to the Chair to decide if she wished to inform the Administrator directly of her concern and to request additional time for due consideration by the SAB so that it could provide its own analysis on the issue.

With this concluded, the meeting was adjourned by the DFO, Mr. Miller.

Respectfully Submitted:

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Mr. Thomas O. Miller  
Designated Federal Officer, Acting  
US EPA Science Advisory Board

Certified as True:

*/ Signed /*

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Dr. Deborah L. Swackhamer  
Chair, EPA Science Advisory Board

#### ATTACHMENTS

- A Meeting Agenda
- B FR Notice
- C Roster
- D Looking to the Future – SAB Seminar Notes
- E Comments of Mr. Mark Greenwood
- F Draft Advisory on Acrylamide

- G SAB Member Comments on Acrylamide Draft
- H Robert Fensterheim comments on acrylamide – physical file only
- I Robert Fensterheim comment part 2 acrylamide
- J Dr Friedman comment on acrylamide – physical file only
- K Dr. tardiff's comment on acrylamide
- L Draft Advisory on Aquatic Life Criteria
- M SAB Member comments on Aquatic Life Criteria Draft
- N Dr. Palumbo's comment on Aquatic Life Criteria
- O Dr. Meyer's response to SAB Member comments
- P Draft Advisory on CCL3
- Q SAB Member comments on CCL3 Draft

**U.S. Environmental Protection Agency  
Science Advisory Board**

**Agenda  
Renaissance Mayflower, 1127 Connecticut Ave., NW  
October 28, 2008**

(For call-in information, please call the Staff Office at 202-343-9999)

*Purpose of the Meeting: The Board will meet to discuss new issues that might be recommended for inclusion within EPA's research program vision, with special emphasis on those topics discussed at the Board's October 27, 2008 seminar on biofuels and epigenomics. The Board will also conduct up to three quality reviews of draft SAB Panel reports.*

**Tuesday October 28, 2008**

8:30 a.m.	<b>Convene the Meeting</b>	<b>Thomas O. Miller</b> <i>Designated Federal Officer, EPA SAB</i>
8:40 a.m.	<b>Chair's Welcome and Introductions and Purpose and Approach for the Meeting</b>	<b>Dr. Deborah Swackhamer</b> <i>Chair EPA Science Advisory Board</i>
9:00 a.m.	<b>Discussion of Future Directions for EPA's Research Program:</b> <ul style="list-style-type: none"><li>- <b>Biofuels (Dr. Dzombak to lead the discussion)</b></li><li>- <b>Epigenomics (Dr. Cory-Slechta to lead the discussion)</b></li><li>- <b>Other Topics (TBD)</b></li></ul>	<b>Dr. Deborah Swackhamer and The Board</b> <b>Dr. Kevin Teichman,</b> <i>Deputy Assistant Administrator for Science US EPA ORD</i>
10:15 a.m.	<b>Break</b>	
10:30 a.m.	<b>Public Comments on Strategic Research Directions</b>	<b>TBA</b>
10:40 a.m.	<b>Continued Discussion of Future Directions for EPA Research</b>	<b>Dr. Deborah Swackhamer and The Board</b> <b>Dr. Kevin Teichman</b>
11:30 a.m.	<b>Quality Review of the Draft SAB <i>Aquatic Life Criteria Review</i> (Committee Lead: <b>Dr. Judith Meyer, Chair SAB Environmental Processes &amp; Effects Committee</b>)</b>	<b>Dr. Deborah Swackhamer and The Board</b>
	<b>Public Comments on Draft Aquatic Life Criteria Report</b>	<b>TBA</b>

12:00 p.m.	<b>Lunch</b>	
1:30 p.m.	<b>Quality Review of the Draft SAB Advisory on Contaminant Candidate List 3</b> (Committee Lead: <b>Dr. Joan Rose</b> , <i>Chair SAB Drinking Water Committee</i> )	<b>Dr. Deborah Swackhamer and The Board</b>
	<b>Public Comments on the Draft Report</b>	<b>TBA</b>
2:00 p.m.	<b>Quality Review of the Draft SAB Advisory on Acrylamide</b> (Committee Lead: <b>Dr. Deborah Cory-Slechta</b> , <i>Chair, SAB Acrylamide Review Panel</i> )	<b>Dr. Deborah Swackhamer and The Board</b>
	<b>Public Comments on the Draft Report</b>	<b>TBA</b>
3:00 p.m.	<b>Adjourn the Meeting</b>	<b>The DFO</b>

(October 23, 2008)

## Attachment B

### Science Advisory Board Staff Office Notification of a Meeting of the Science Advisory Board

[PDF Version](#) (2 pp, 72K, [About PDF](#))

[Federal Register: September 25, 2008 (Volume 73, Number 187)]  
[Notices]  
[Page 55512-55513]  
From the Federal Register Online via GPO Access [wais.access.gpo.gov]  
[DOCID:fr25se08-43]

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ENVIRONMENTAL PROTECTION AGENCY  
[FRL-8721-1]

Science Advisory Board Staff Office Notification of a Meeting of  
the Science Advisory Board

AGENCY: Environmental Protection Agency (EPA).  
ACTION: Notice.

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SUMMARY: The EPA Science Advisory Board (SAB) Staff Office announces a public face-to-face meeting of the chartered SAB to: discuss future environmental science issues within the context of EPA's research directions and priorities, and conduct quality reviews of up to three Draft SAB reports.

DATES: The meeting dates are Monday, October 27, 2008, from 8:30 a.m. to 5 p.m. through Tuesday, October 28, 2008, from 8:30 a.m. to 2:30 p.m. (Eastern Time).

ADDRESSES: The meeting will be held at the Mayflower Hotel, 1127 Connecticut Avenue, NW., Washington, DC; phone (202) 347-4430.

FOR FURTHER INFORMATION CONTACT: Members of the public who wish to obtain further information about this meeting may contact Mr. Thomas O. Miller, Designated Federal Officer (DFO), by mail at EPA SAB Staff Office, (1400F), U.S. EPA, 1200 Pennsylvania Avenue, NW., Washington, DC 20460; by telephone at (202) 343-9982; by fax at (202) 233-0643; or by e-mail at [miller.tom@epa.gov](mailto:miller.tom@epa.gov). The SAB mailing address is U.S. EPA, Science Advisory Board (1400F), 1200 Pennsylvania Avenue, NW., Washington, DC 20460. General information about the SAB, as well as any updates concerning the meeting announced in this notice, may be found on the SAB Web site at <http://www.epa.gov/sab>.

SUPPLEMENTARY INFORMATION: The SAB was established by 42 U.S.C. 4365 to provide independent scientific and technical advice, consultation, and recommendations to the EPA Administrator on the technical basis for Agency positions and regulations. The SAB is a Federal advisory committee chartered under the Federal Advisory Committee Act (FACA), as

amended, 5 U.S.C., App. The SAB will comply with the provisions of FACA and all appropriate SAB Staff Office procedural policies.

Background: 1. Future Science and Research. On October 27, 2008, the EPA Science Advisory Board will hold a one day meeting entitled Looking to the Future. During this meeting, the SAB will hear from, and interact with, outside experts on: (i) The environmental implications of biofuels, and (ii) the implications for environmental health sciences and human health risk assessment of epigenomics research. Exploration of biofuels and epigenomics research is intended to provide the chartered SAB with an inter-disciplinary introduction to these topics, and to stimulate their thinking generally about future advice to strengthen EPA's response to emerging science issues, especially how EPA might implement inter-disciplinary approaches that incorporate significant emerging research.

In 2007, the chartered SAB committed to provide ongoing advice on strategic research directions for EPA and how they can be implemented. This activity complements the SAB's traditional review of EPA's annual research budget. The first day's seminar-style meeting will be followed by a half-day advisory meeting on October 28, when the chartered SAB will discuss possible implications of the October 27 meeting for ongoing SAB advice on EPA research directions.

2. Review of Draft SAB Reports: (a) Quality Review of the Draft SAB Advisory on Aquatic Life Criteria. EPA's Office of Water asked the Science Advisory Board for advice on the scientific merits of a white paper that identifies and addresses technical issues in deriving aquatic life criteria for emerging contaminants such as pharmaceuticals and personal care products exhibiting endocrine disrupting activity or other toxic mechanisms. The EPA SAB Ecological Processes and Effects Committee (EPEC) augmented with additional experts conducted this review. Additional information on this review can be obtained on the EPA SAB Web site at:

[http://yosemite.epa.gov/sab/sabproduct.nsf/fedrgstr\\_activites/MOA%20criteria%20methodology](http://yosemite.epa.gov/sab/sabproduct.nsf/fedrgstr_activites/MOA%20criteria%20methodology).

(b) Quality Review of the Draft Advisory on the Drinking Water Contaminant Candidate List 3. EPA's Office of Water asked the SAB to review EPA's draft Drinking Water Contaminant Candidate List 3 (CCL 3). The 1996 Safe Drinking Water Act Amendments (SDWA) require EPA to (1) publish every five years a list of currently unregulated contaminants in drinking water that may pose risks and (2) make determinations on whether or not to regulate at least five contaminants from that list on a staggered five year cycle. The list must be published after consultation with the scientific community, including the SAB, after notice and opportunity for public comment, and after consideration of the occurrence database established under section 1445(g) of the SDWA. The unregulated contaminants considered for the list must include, but are not limited to, substances referred to in section 101(14) of the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA), and substances registered under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Additional information on this

[[Page 55513]]

review can be obtained on the EPA SAB Web site at: [http://yosemite.epa.gov/sab/sabproduct.nsf/fedrgstr\\_activites/CCL3](http://yosemite.epa.gov/sab/sabproduct.nsf/fedrgstr_activites/CCL3).

(c) Quality Review of the Draft SAB Advisory on Acrylamide. EPA's National Center for Environmental Assessment, within the Office of

Research and Development, has been updating the human health hazard and dose-response assessment for Acrylamide. EPA's Office of Research and Development requested that the Science Advisory Board review its draft assessment entitled ``Toxicological Review of Acrylamide,' ' a polymer used primarily in waste water treatment, paper and pulp processing, and mineral processing. The EPA SAB established the Acrylamide Review Panel to conduct this review. Additional information on this review can be obtained on the EPA SAB Web site at [http://yosemite.epa.gov/sab/sabproduct.nsf/fedrgstr\\_activites/Acrylamide-IRIS-Asst](http://yosemite.epa.gov/sab/sabproduct.nsf/fedrgstr_activites/Acrylamide-IRIS-Asst).

Availability of Meeting Materials: Materials in support of this meeting will be placed on the SAB Web site at <http://www.epa.gov/sab> in advance of this meeting.

Procedures for Providing Public Input: Interested members of the public may submit relevant written or oral information for the SAB to consider. Oral Statements: The total time available for public comments for topics to be discussed at this October 28, 2008 meeting of the SAB will be one hour. Fifteen minutes will be allocated for each of the quality reviews to be conducted and for the general discussion session on strategic research directions. Individuals or groups requesting an oral presentation at a public meeting on October 28, 2008 will be limited to three minutes per speaker. Each person making an oral statement should consider providing written comments as well as their oral statement so that the points presented orally can be expanded upon in writing. Interested parties should contact Mr. Miller, DFO, at the contact information provided above, by October 17, 2008, to be placed on the public speaker list for the October 28, 2008 meeting. Written Statements: Written statements should be received in the SAB Staff Office by October 20, 2008, so that the information may be made available to the SAB for their consideration prior to this meeting. Written statements should be supplied to the DFO via e-mail to [miller.tom@epa.gov](mailto:miller.tom@epa.gov) (acceptable file format: Adobe Acrobat PDF, WordPerfect, MS Word, MS PowerPoint, or Rich Text files in IBM-PC/Windows 98/2000/XP format).

Meeting Accommodations: For information on access or services for individuals with disabilities, please contact Mr. Thomas Miller at (202) 343-9982, or via e-mail at [miller.tom@epa.gov](mailto:miller.tom@epa.gov). To request accommodation of a disability, please contact Mr. Miller, preferably at least 10 days prior to the meeting, to give EPA as much time as possible to process your request.

Dated: September 18, 2008.  
Anthony F. Maciorowski,  
Deputy Director, EPA Science Advisory Board Staff Office.  
[FR Doc. E8-22539 Filed 9-24-08; 8:45 am]  
BILLING CODE 6560-50-P

## Attachment C

### U.S. Environmental Protection Agency Science Advisory Board October 27, 2008

#### CHAIR

**Dr. Deborah Swackhamer**, Professor of Environmental Health Sciences and Co-Director Water Resources Center, Water Resources Center, University of Minnesota, St. Paul, MN

#### SAB MEMBERS

**Dr. David T. Allen**, Professor, Department of Chemical Engineering, University of Texas, Austin, TX

**Dr. Gregory Biddinger**, Coordinator, Natural Land Management Programs, Toxicology and Environmental Sciences, ExxonMobil Biomedical Sciences, Inc., Houston, TX

**Dr. Timothy Buckley**, Associate Professor and Chair, Division of Environmental Health Sciences, School of Public Health, The Ohio State University, Columbus, OH

**Dr. Thomas Burke**, Professor, Department of Health Policy and Management, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD

**Dr. Deborah Cory-Slechta**, Professor, Department of Environmental Medicine, School of Medicine and Dentistry, University of Rochester, Rochester, NY

**Dr. Terry Daniel**, Professor of Psychology and Natural Resources, Department of Psychology, Environmental Perception Laboratory, University of Arizona, Tucson, AZ

**Dr. David A. Dzombak**, Walter J. Blenko Sr. Professor of Environmental Engineering, Department of Civil and Environmental Engineering, College of Engineering, Carnegie Mellon University, Pittsburgh, PA

**Dr. Rogene Henderson**, Senior Scientist Emeritus, Lovelace Respiratory Research Institute, Albuquerque, NM

**Dr. James H. Johnson**, Professor and Dean, College of Engineering, Architecture & Computer Sciences, Howard University, Washington, DC

**Dr. Catherine Kling**, Professor, Department of Economics, Iowa State University, Ames, IA

**Dr. George Lambert**, Associate Professor of Pediatrics, Director, Center for Childhood

Neurotoxicology, Robert Wood Johnson Medical School-UMDNJ, Belle Mead, NJ

**Dr. Jill Lipoti**, Director, Division of Environmental Safety and Health, New Jersey Department of Environmental Protection, Trenton, NJ

**Dr. Lee D. McMullen**, Water Resources Practice Leader, Snyder & Associates, Inc., Ankeny, IA

**Dr. Judith L. Meyer**, Distinguished Research Professor Emeritus, Odum School of Ecology, University of Georgia, Athens, GA

**Dr. Jana Milford**, Professor, Department of Mechanical Engineering, University of Colorado, Boulder, CO

**Dr. Christine Moe**, Eugene J. Gangarosa Professor, Hubert Department of Global Health, Rollins School of Public Health, Emory University, Atlanta, GA

**Dr. Duncan Patten**, Research Professor, Department of Land Resources and Environmental Sciences, Montana State University, Bozeman, MT, USA

**Dr. Stephen M. Roberts**, Professor, Department of Physiological Sciences, Director, Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL

**Dr. Joan B. Rose**, Professor and Homer Nowlin Chair for Water Research, Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI

**Dr. James Sanders**, Director and Professor, Skidaway Institute of Oceanography, Savannah, GA

**Dr. Jerald Schnoor**, Allen S. Henry Chair Professor, Department of Civil and Environmental Engineering, Co-Director, Center for Global and Regional Environmental Research, University of Iowa, Iowa City, IA

**Dr. Thomas L. Theis**, Director, Institute for Environmental Science and Policy, University of Illinois at Chicago, Chicago, IL

**Dr. Valerie Thomas**, Anderson Interface Associate Professor, School of Industrial and Systems Engineering, Georgia Institute of Technology, Atlanta, GA

**Dr. Thomas S. Wallsten**, Professor, Department of Psychology, University of Maryland, College Park, MD

**Dr. Lauren Zeise**, Chief, Reproductive and Cancer Hazard Assessment Branch, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Oakland, CA

## **LIAISON MEMBERS**

### **NACEPT:**

**Dr. Daniel J. Watts**, Executive Director, Otto H. York Center for Environmental Engineering and Science and the Panasonic Professor of Sustainability at New Jersey Institute of Technology, Monmouth Jct., NJ

### **SCIENCE ADVISORY BOARD STAFF**

**Mr. Thomas Miller**, Designated Federal Officer, 1200 Pennsylvania Avenue, NW 1400F, Washington, DC, Phone: 202-343-9982, Fax: 202-233-0643, (miller.tom@epa.gov)

**U.S. Environmental Protection Agency**  
**Science Advisory Board**  
*Looking to the Future*  
**Renaissance Mayflower, 1127 Connecticut Avenue NW**  
**Washington DC 20036**  
**October 27, 2008**

**Meeting Summary**

## Table of Contents

<b>Background and purpose of meeting .....</b>	<b>3</b>
<b>Biofuels: What are the net environmental implications?.....</b>	<b>4</b>
<b>Epigenomics research: What are the implications for environmental health sciences and human health risk assessment? .....</b>	<b>9</b>
<b>Attachment 1 – Agenda .....</b>	<b>11</b>
<b>Attachment 2 – Biofuel Speakers’ Biosketches, Abstracts, and Handouts</b>	
<b>Attachment 3 – Epigenomic Speakers’ Biosketches, Abstracts, and Handouts</b>	

## **Background and purpose of meeting**

On October 27-28, 2008, the EPA chartered Science Advisory Board held a one-and-a-half-day public meeting entitled *Looking to the Future*. The meeting focused on two questions:

- Biofuels: What are the net environmental implications?
- Epigenomic research: What are the implications for environmental health sciences and human health risk assessment?

The seminar-style meeting was followed by a half-day advisory meeting on October 28, 2008. At that meeting, the chartered SAB discussed possible implications of the October 27, 2008 discussions for ongoing SAB advice on EPA research.

Exploration of the biofuels and epigenomic topics was intended to provide the chartered SAB with an interdisciplinary introduction to these topics. It was also intended to stimulate SAB thinking generally about future advice to strengthen EPA's response to emerging science issues, especially how EPA might implement interdisciplinary approaches that incorporate important emerging research.

In 2007, the chartered SAB committed to provide ongoing advice on strategic research directions for EPA and how they can be implemented. This advice on strategic directions complemented the SAB's traditional review of EPA's annual research budget. Exploration of emerging science related to biofuels and genomics at the October 27, 2008 meeting had the goal of further stimulating SAB advice. Focus on these two significant topics was designed to highlight the need to address inherent complexities and interconnections among human and ecological systems through integrated, multi-disciplinary science and research.

Dr. M. Granger Morgan, past chair of the chartered SAB, introduced the workshop and facilitated the discussion of biofuels. Dr. Deborah Cory-Slechta facilitated the discussion of epigenomics. Dr. Deborah Swackhamer, Chair of the chartered SAB, provided concluding remarks. She thanked the speakers and Drs. Morgan and Cory-Slechta for planning the program and noted the significance of the two topics discussed.

This summary document briefly describes the discussions following the speakers' presentations. The agenda for October 27, 2008 appears in Attachment 1. Attachment 2 contains the speakers' abstracts, biosketches and the handouts that speakers made electronically available for distribution.

## **Biofuels: What are the net environmental implications?**

Dr. Granger Morgan introduced the four speakers: Dr. Bruce Dale (Michigan State University), who gave a presentation developed in collaboration with Dr. Lee Lynd (Dartmouth College) on *Sustainable Paths to a Biofuel-Powered Transportation Sector: The Role of Innovation and Invention*; Dr. Kenneth Cassman (University of Nebraska), whose presentation was entitled *Ensuring Sustainability of Biofuel Systems*; Dr. G. David Tilman (University of Minnesota), who presented on *Environmental Impacts of Food versus Cellulose-Based Biofuels*; and Dr. Christopher Field (Carnegie Institution), who provided a presentation on *Biofuels potential: The climate protective domain*. After the speakers' presentations (see Attachment 2), Dr. Morgan asked the speakers to lead the discussion with their initial questions or comments.

In that initial discussion, speakers focused on the relationship between intensive agriculture and carbon release. Dr. Cassman described the concept of indirect land use change and its effects on greenhouse gas emissions. For example, any changes in U.S. crop area that results in higher soybean prices theoretically results in the expansion of agriculture into the Brazilian rainforest. Because cutting down the rainforest and burning its trees results in a tremendous amount of greenhouse gas emissions, this "GHG debt" must be credited to the reason for the change in crop area in the U.S. that caused the higher soybean prices. Thus, the expansion of U.S. corn area to meet demand from the rapid increase in ethanol production capacity came largely at the expense of soybean area, which in turn resulted in higher soybean prices. This caused Brazilian farmers to clear more rainforest and plant soybeans. Because the loss of carbon from clearing rainforest is many times greater than the GHG emissions reduction from use of ethanol to replace gasoline, there would be a large negative GHG debt due to indirect land use change. Likewise, putting marginal land that produces corn and soybeans into the conservation reserve program (CRP) to reduce environmental degradation and erosion associated with farming such marginal land, would also have a large GHG debt. This debt occurs because retiring land from production would result in higher crop prices and trigger indirect land use change in the rainforest, and the GHG loss from clearing rainforest is many times greater than the GHG savings from retiring crop land to the CRP. But CRP land is good for the environment in the U.S. so in effect, consistent application of the indirect land use change concept can have negative impacts on local environmental quality in the U.S. in order to reduce GHG emissions on a global scale. Given the expected increase in demand for human food, livestock feed, and biofuel, there is an urgent need to invest on research with the explicit goal of achieving a large crop yield increases on existing farm land while at the same time reducing negative environmental impacts from the higher yields—a process called ecological intensification.

Dr. Field noted that EPA should not only look at carbon release, but also consider water quality and quantity impacts, use of pesticides and release of PM 2.5 in analyzing possible costs and benefits. EPA should consider indirect land use in analyzing the multiple impacts of biofuels in an effort to minimize negative impacts. Dr. Field agreed that intensive agriculture imposes a carbon debt. In his view, when lands were cleared for bioenergy purposes, society should look at the implications of deforestation. Dr. Tilman noted a long-term (150-year) study comparing cultivation practices in England, where traditional intensive agricultural practices using manure have proved as productive than modern chemical fertilizers. Dr. Dale emphasized the importance of analyzing direct land use changes occurring as a result of increased biofuel production. He emphasized, however, that lifecycle planning tools did not yet exist for analyzing indirect land uses on an international scale. The Congressional requirement for such analysis was a radical innovation, for which reliable models and data do not yet exist.

Dr. Morgan then asked SAB members for their comments and questions. The first question concerned science and research needs to address water quality and water quantity impacts of biofuels, given projected increases in human and animal population. Dr. Dale responded that there was great potential to substitute capital investments for water in processing corn and cellulosic ethanol. He estimated that corn and cellulosic ethanol could be processed with half the water used in producing gasoline, due to the lower temperatures associated with biofuel production leading to lower heat transfer losses of water. Water quantity issues could be reduced by growing biofuel stock in the right locations using efficient agricultural methods. Local impacts could be reduced if perennial grasses were grown for biofuel stock. Dr. Cassman then noted that water quality and water scarcity issues existed because of world population growth, regardless of the development and promotion of biofuels. Projected population growth and economic development will increase demand for water; cultivation of corn for biofuels only accelerates the issue. He noted that biofuel cultivation will raise the cost of water. These rising costs may foster exploration of expensive irrigation technologies that promise efficiencies and reduced environmental impacts. Dr. Tilman addressed the water use question by emphasizing that negative impacts of biofuels could best be managed by wise decisions about how and where to grow feed stocks for biofuels. He emphasized the needs for price structure and incentives to motivate farmers and other decision makers to make environmentally sound decisions. Policy makers should examine the ecological impacts of using ground water and waters pumped from low-lying wetlands to grow corn in dry, unproductive soil. Dr. Field noted the importance of recovering nutrients and improving the efficiency of fertilizer use to reduce nutrient runoff.

The second question concerned current models for assessing the impacts of crops grown for biofuels. Speakers agreed that models were limited and not sufficiently validated by monitoring results. Speakers noted the need for models and data to predict the impact of temperature on crop yields, the significance of the color of different crops, and impacts on regional weather patterns.

The next question concerned the impact of prices and subsidies for corn-based ethanol. Dr. Tilman expressed concern about increased corn production on land unsuitable for corn, which increases the need for irrigation and fertilizers. He called for research on alternatives to ethanol-based biofuels. Dr. Cassman took a different perspective. He called for research to increase agricultural output to meet both food and fuel needs because of the sharp increase projected for world population.

Dr. Morgan then asked groups of SAB members for clusters of questions for speakers to address. In the first cluster of questions, SAB members asked about: 1) recommendations for incentives to encourage efficient production of biofuel crops; 2) investments in transportation and processing to support development of environmentally-friendly biofuels; and 3) logistical factors that affect environmental impacts of biofuels. In response, Dr. Dale noted the importance of developing regional biomass processing centers that can densify and pretreat biofuel stocks. Some byproducts could be used locally as animal feed and others could be sent further away for use as fuels. Dr. Tilman emphasized the importance of determining the right crop for the right location. He called for agronomy field trials for biofuels and increased research in the application of municipal solid waste and corn stover for fuel. He called for incentives for best management practices that would increase over time, resulting in efficiencies in using nitrogen, phosphorus, and irrigated water. Dr. Field advocated an analysis of land use potential to

maximize sequestration of carbon. He envisioned “tremendous opportunities” for biomass combustion of wastes for production to enhance rural development.

An SAB member then asked for speakers’ predictions of the fraction of total energy needs could be met by biofuels in the future. Dr. Dale responded that over the next few decades, with needed innovations and inventions, biofuels could replace all needs for liquid transportation fuels for the whole world and thereby benefit the rural poor internationally. He did not envision the use of battery-operated vehicles outside North America and Europe due to the relatively high costs of such vehicles, compared to subcompact vehicles like the Tata Motors Nanocar (\$2,500), which use liquid fuels. The 2007 Energy Independence and Security Act mandates 57 billion liters of ethanol production from starch-based crops like corn. Dr. Cassman estimated that this amount of corn-ethanol would replace 18% of current imported oil, and if the United States could double the efficiency of its motor vehicle fleet, it would replace 36%. Dr. Tilman predicted that approximately 20% of current liquid fuels for transportation could be globally produced in a sustainable manner. This would represent less than 7% of total global fossil energy demand.. Dr. Field estimated that biofuels might meet 7-8% of total global energy needs, given current levels of technology. He agreed with Dr. Tilman that biofuels might meet approximately 20% of current liquid fuels needs for transportation.<sup>1</sup>

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<sup>1</sup> Dr. Lee Lynd, who co-authored the presentation on *Sustainable Paths to a Biofuel-Powered Transportation Sector: The Role of Innovation and Invention* with Dr. Dale, was unable to participate in the meeting. However, on reviewing this summary he asked to provide a response to this question about predictions of the fraction of total energy needs could be met by biofuels in the future:

"I have made, and continue to make, a study of this important question and the widely misunderstood answers to it. In the enclosed book chapter ("Energy Myth Three – High Land Requirements and an Unfavorable Energy Balance Preclude Biomass Ethanol From Playing a Large Role In Providing Energy Services"), my colleagues and I point out that there are a large number of studies projecting very large contributions for biomass-based energy, and also a large number of studies projecting that such a large contribution is either impossible or undesirable. Curiously, the distribution of studies is bimodal rather than peaking at a mean value. This brings up two questions: 1) Who is right?, and 2) How can reasonable people with access to the same information reach such different conclusions? Since the many studies that have taken a crack at the first question and obtained disparate answers, the second question is probably the more fruitful one to think about. All seem to agree that the issue is not the analytical framework, but rather the assumptions made about the future. The chapter closes with the following observations which I believe are relevant to the question asked by the SAB member and the answers offered:

'Ultimately, questions related to the availability of land for biomass energy production and the feasibility of large-scale provision of energy services are determined as much by world view as by hard physical constraints. If the question is: "In a world motivated to solve sustainability and security challenges, assuming that innovation and change responsive to this objective are possible, could biomass make a large contribution to provision of energy services?" We think that the answer is unequivocally "Yes". On the other hand, biomass can make a much more limited contribution to energy supply in a world based on current or extrapolated realities with respect to important technical and behavioral variables determining biomass requirements and availability. To a substantial degree, the starkly different conclusions reached by different analysts on the biomass supply issue reflect different expectations with respect to the world's willingness or capacity to innovate and change. However, change is our only option if we are to achieve a sustainable and secure future, whether we are talking about biomass or all renewable energy sources.

Rejecting energy service supply options because they require innovation and change decreases the set of alternatives that can make a meaningful contribution markedly, and perhaps to zero. Such rejection also denies the essence of our current situation: that we cannot extrapolate the current unsustainable and insecure present and get to a sustainable and future. The scenarios most conducive to biomass playing a significant energy service supply role involve complimentary combinations of several changes, with the largest contributions made possible by a combination of technical advances and behavioral changes. We suspect that this is not limited to biomass and indeed is true of most if not all paths to a sustainable future. Studies that project a small role for biomass generally change only the source of fuel and leave other variables constant. This, however, amounts to projecting that

In the second cluster of questions, SAB members asked speakers about: 1) the most significant questions that could be addressed through sensitivity analysis and provide the most fruitful focus for research; 2) opportunities presented by the biofuel issue to focus EPA research on life-cycle assessment, rather than EPA's traditional pollutant by pollutant approach to risk assessment; 3) the potential for "intervention-based research" to influence current agricultural practices in the United States and world-wide, so that agricultural practices recognized to minimize adverse environmental effects were encouraged; and 4) the need for a new science and environmental management paradigm to address the complex environmental issues associated with biofuels.

Dr. Field identified the need for a research portfolio that would address biofuels from a broad perspective. He also spoke of the need for a legislative framework to address the full range of biofuel issues. Dr. Tilman emphasized that the environmental concerns associated with biofuels are multi-dimensional and that current approaches to life-cycle analysis have been too narrow in temporal and spatial scope to capture all dimensions of the problem. Dr. Cassman spoke of the need for EPA to play a major role in research strategy planning among federal agencies, including the U.S. Department of Energy (DOE) and U.S. Department of Agriculture (USDA). He called for research on carbon sequestration and carbon impacts related to different cultivation strategies for corn and cellulosic feed stocks. Dr. Dale agreed that EPA should increase its research coordination with DOE and USDA. He noted needs to improve models of agricultural impacts, life-cycle assessment tools, models to help allocate land for critical food, fuel, and animal feed needs. He called for greater rigor in reporting research results, showing the range of statistical results.

In the third cluster of questions, SAB members asked speakers about: 1) whether and how EPA should regulate agricultural activities to minimize the adverse environmental impacts of alternative energy strategies; 2) how to integrate their research with economic models, research, and systems; and 3) how to assess the impacts of potential fuels, such as palm biodiesel in the tropics, where development may pose risks to endangered species. Dr. Dale responded that economic factors will stimulate adoption of biofuels. New technologies will reduce the costs of feed stocks and processing costs. Economic incentives to encourage environmental management practices would be useful. Dr. Cassman agreed that economics should be part of the discussion. He agreed that agricultural polluters need to "to come under environmental regulations—it will be painful but has to be done." He noted the forthcoming work of the SAB's Integrated Nitrogen Committee, which held a workshop October 20-21, 2008 to discuss strategies for nitrogen management. He cautioned against the use of subsidies, which are hard to withdraw, once awarded. Dr. Tilman agreed for the need for interdisciplinary collaboration with economists to develop analyses for policy makers. There is a need for decision makers and consumers to see the "whole true price," including the production and ecological price, of different policy options.

Dr. Field cautioned against the use of price signals to help set policy. He noted that, "while we are calorie secure, the result of the world is not. " He expressed concern that economic pressures may pull food calories away from people who are not secure and that "price signals don't protect them." Dr. Field also noted that economic analysis cannot help address rare

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technologies and behaviors that arose in a world largely unconstrained by energy availability will continue in the future. This is unlikely if one believes that energy sustainability and security challenges will become yet more pressing as we move forward - a proposition for which more support is accumulating daily."

and endangered ecological resources. He called on the United States to define more clearly what it wishes biomass energy to accomplish and then develop the appropriate policies, based on those priorities. If the goal is to reduce the net burden on climate change, then the United States can identify the full set of climate-alternatives and appropriately set incentives. He expressed frustration that biofuels were originally viewed as a strategy aligning climate, energy independence, and rural development, but that the current science and current development of biofuels indicate that biofuels may no longer meet all those all these needs easily or equally.

Dr. Morgan closed the panel discussion by asking each speaker to comment briefly on the most pressing research priorities and policy directions for EPA. Dr. Field called for a clear priority to be set for biofuels that would make biomass energy production climate protective. Once this priority was established then research and policy efforts could help determine the most effective incentive structure. In his view, research is needed to address the overall biofuel system, including the costs and benefits of indirect land conversion, major conservation issues, food security issues, and technological development to improve agricultural efficiency on existing agricultural lands so that production will be sufficient to feed the world.

Dr. Tilman noted that EPA must build on past research on nutrient loading, sewage treatment, and criteria air pollutants to meet huge future challenges associated with energy and food production. EPA must be involved in critical biofuel decisions affecting the environment. There are risks posed by huge fertilizer impacts and increasingly intensive agricultural practices. EPA should invest in full lifecycle-analysis addressing greenhouse gas impacts and a wide range of other environmental impacts including direct and indirect land use. EPA should invest in research and foster policies that encourage environmentally friendly agricultural practices.

Dr. Cassman noted that EPA needs to provide leadership to develop appropriate models, monitoring, and measurement methods to quantify the environmental impacts of biofuels. He called for collaboration and coordination with DOE, USDA, the U.S. Geological Survey, the National Oceanic and Atmospheric Administration, and the National Science Foundation. He noted the need for improved models to better predict greenhouse gas impacts and nitrogen impacts of different biofuel policies. The priority is for research to crop raise yields and reduce ecological impacts. Such research requires collaboration between agronomists and ecologists.

Dr. Dale called for EPA to invest resources to improve lifecycle analysis, sensitivity analysis, analysis of land use partitioning, and indirect land use. He urged EPA to support and study the potential for cellulosic ethanol, including the use of grasses for ethanol.

## **Epigenomics research: What are the implications for environmental health sciences and human health risk assessment?**

Dr. Deborah Cory-Slechta introduced the four speakers and spoke of the potential implications of their research for hazard identification and human health risk assessment at EPA. Dr. Mark Hanson (University of Southampton) provided a presentation on the *Developmental Origins of Health and Disease - the Role of Epigenetic Mechanisms*: Dr. Randy Jirtle (Duke University) spoke on *Epigenetics: The new genetics of disease susceptibility*. Dr. Michael Skinner (Washington State University) spoke *Epigenetic transgenerational activity of endocrine disruptors on reproduction and disease; the ghosts in your genes*. After the speakers' presentations (see Attachment 2), Dr. Cory-Slechta took questions for the speakers from SAB members.

An SAB member asked about the implications for chemical companies of research showing potential epigenetic impacts of stressors. Dr. Hanson responded that the current state of science does not allow prediction of epigenetic effects from chemical structure. Dr. Jirtle suggested that it may be useful to identify areas of the genome that are labile and that risk assessors should not assume that "something is safe because does not cause modifications to the genome." Dr. Hanson agreed and suggested that EPA should identify biomarkers of risk. One possible biomarker might be the promoter regions for steroid receptor genes that can be methylated. Any stressor that affects them is of potential interest.

Another SAB member asked whether risk assessment for epigenetic effects was "condemned to agent-by-agent analysis" and whether there were opportunities to be anticipatory in designing research to protect against environmental risks. Dr. Jirtle suggested focusing on susceptibilities at early stages of life, especially fetal exposures through pregnant mothers. Dr. Skinner predicted that scientists will be able to map the epigenome within three years. They will then be able to study exposures related to people in different cohorts. Dr. Jirtle noted that the National Children's Study offered many targets for exposure analysis (e.g., placenta and cord blood samples, mothers' exposures) to complement the study of epigenetic effects. Researchers may be able to determine environmental epigenetic effects linked to cardio vascular disease and schizophrenia.

An SAB member enquired about human epigenetic variability. Dr. Skinner responded that research reporting the first genome-wide epigenome matching will be available in the spring of 2009. Baseline data will likely be available in a few years. Speakers noted that every different cell type has a different epigenome. Epigenetics presents a complex biological problem. Dr. Jirtle noted that it will be possible to track individuals with imprinted epigenomes.

The next question related to research support for epigenetics and epigenomics. Dr. Skinner reported that the National Institutes of Health has recently invested \$100 million in epigenetics. To his knowledge, EPA has not been involved in the award of this funding. Dr. Hanson spoke of the need for funding centralized facilities for bioinformatics technology. Speakers noted the possibility for identifying the biomarkers for nutrition and other environmental impacts as part of the mapping of the epigenome. Dr. Hanson noted the rich data available in China, Malaya, and India for linking epigenetics and toxicology.

An SAB member asked about potential epigenetic effects from environmental stressors in other animals. Dr. Jirtle responded that many animals would not have imprinted genes but would likely have epigenetic phenomena.

An SAB member asked how researchers would make connections between diet and environmental factors with epigenetic impacts. He asked “How would you know what exposures were? How would you establish dose-response?” Dr. Hanson responded that in many countries (e.g., Sweden, Denmark, Holland) cohorts were well identified and exposures understood. He also observed that researchers would need to coordinate animal and human studies closely to fully understand exposures and dose response.

Several SAB members asked about using epigenetic information to provide protection against environmental stressors. Dr. Jirtle noted that additional research is necessary to fully understand dose and timing. Folic acid, for example, is a big benefit in reducing neurotube defects, but “what could be helpful early in development could be detrimental later in life.”

An SAB member enquired about the potential of epigenetic research to address environmental justice communities that face low birth weights, multiple environmental exposures, and poor diet. Dr. Hanson stated his belief that “epigenetic basis for risk of cardiovascular and other chronic disease and noted that this research highlights the importance of multiple environmental factors, many associated with socioeconomic conditions, in affecting such epigenetic factors” He cited research on the epigenetic basis for risks to cardiac factors in diseases and noted that the research responded to people’s repeated questions about the impacts of multiple exposures.

The panelists then discussed research showing the relationship between multiple, different kinds of stressors and disease. They noted research linking prenatal stress to health consequences and research by Dr. Michael Meaney showing that behavior such as mothers’ licking and grooming behavior affected methylation and health impacts in their pups. Dr. Cory-Slechta noted that EPA uses uncertainty factors in risk assessment to account for vulnerability and susceptibility. These uncertainty factors are not empirically determined but do recognize variability among individuals. Epigenetics may offer a stronger scientific basis for addressing the different bases for variability.

An SAB member asked panelists to identify the health endpoints that may be most likely related to epigenetic effects. Dr. Jirtle suggested that EPA should focus on neurological effects, schizophrenia, autism, and neuro-degenerative disease. Dr. Hanson suggested focusing on childhood obesity, diabetes, and childhood diseases. Drs. Hanson and Skinner suggested focusing on endocrine disruptors. Dr. Jirtle noted that when environment presents organisms with new, challenging exposures for which they were not prepared, the epigenome can be adversely affected.

**Attachment 1 – Agenda**  
**U.S. Environmental Protection Agency**  
**Science Advisory Board**  
*Looking to the Future*  
**Renaissance Mayflower, 1127 Connecticut Avenue NW**  
**Washington DC 20036**  
**October 27, 2008**

**Purpose:** Is to stimulate SAB thinking about priorities for meeting critical environmental problems with an integrated approach to interdisciplinary science and research.

**Preliminary Agenda**

8:00 - 8:10 am	Welcome Remarks	Dr. M. Granger Morgan, SAB
<b>Biofuels: What are the net environmental implications?</b>		
8:10- 8:15 am	Introduction	Dr. M. Granger Morgan, SAB
8:15- 8:45 am	Sustainable paths to a biofuel-powered transportation sector; the role of innovation and invention	Dr. Bruce Dale, Michigan State University Dr. Lee Lynd, Dartmouth College
8:45- 9:15 am	Ensuring environmental sustainability of biofuel systems	Dr. Kenneth Cassman, University of Nebraska
9:15- 9:45 am	Lifecycle environmental and health costs and benefits of fossil and renewable fuels	Dr. G. David Tilman, University of Minnesota
9:45-10:15 am	Biofuels potential: The climate protective domain	Dr. Christopher Field, Carnegie Institution
10:15-10:30 am	Break	
10:30-12:00 pm	SAB discussion with invited speakers	
12:00-1:15 pm	Lunch	
<b>Epigenomics research: What are the implications for environmental health sciences and human health risk assessment?</b>		
1:15- 1:20 pm	Introduction	Dr. Deborah Cory-Slechta, SAB
1:20- 1:50 pm	Developmental Origins of Health and Disease - the Role of Epigenetic Mechanisms	Dr. Mark Hanson, University of Southampton

1:50- 2:20 pm	Epigenetics: The new genetics of disease susceptibility	Dr. Randy Jirtle, Duke University
2:20- 2:50 pm	Epigenetic transgenerational activity of endocrine disruptors on reproduction and disease; the ghosts in your genes	Dr. Michael Skinner, Washington State University
2:50 -3:15 pm	Break	
3:15- 4:45 pm	SAB discussion with invited speakers	
4:45- 5:00 pm	Concluding remarks	Dr. Deborah Swackhamer, SAB Chair
5:00 pm	Adjourn	

**Attachment 2 – Biofuel Speakers’ Biosketches, Abstracts, and Handouts**

## **Dr. Bruce Dale**

### **Michigan State University**

Professor Dale is Professor of Chemical Engineering and former Chair of the Department of Chemical Engineering and Materials Science at Michigan State University. He received his bachelors degree (summa cum laude) in chemical engineering from the University of Arizona (Tucson) in 1976 and the masters degree from that same university in 1976. Dr. Dale then studied under Professor George T. Tsao at Purdue University, receiving his Ph. D. degree in 1979. Dr. Dale's first academic position was in the Department of Agricultural and Chemical Engineering at Colorado State University, where he rose to the rank of Professor in 1988. In that same year he joined Texas A&M University where he became Professor of Chemical Engineering and Professor of Agricultural Engineering. Dr. Dale also directed two large interdisciplinary research centers at Texas A&M: the Engineering Biosciences Research Center and the Food Protein Research and Development Center. In 1996 Dr. Dale became Professor and Chair of the Department of Chemical Engineering at Michigan State University, where he also holds an appointment in the Michigan Agricultural Experiment Station. Also in 1996 he won the Charles D. Scott Award for contributions to the use of biotechnology to produce fuels, chemical and other industrial products from renewable plant resources. In 2001 he stepped down as Chair to return to full time research and teaching. Professor Dale's research and professional interests lie at the intersection of chemical engineering and the life sciences. Specifically, he is interested in the environmentally sustainable conversion of plant matter to industrial products- fuels, chemicals and materials- while meeting human and animal needs for food and feed. He led a National Research Council report entitled "Biobased Industrial Products: Research and Commercialization Priorities" which was published in May 2000.

## **Dr. Lee Lynd**

### **Dartmouth**

Dr. Lee Rybeck Lynd is a Professor of Engineering and an Adjunct Professor of Biology at Dartmouth College, Professor Extraordinary of Microbiology at the University of Stellenbosch, South Africa, and cofounder, Director and Chief Scientific Officer of Mascoma Corporation, a biomass energy start-up. He has been a member of the Dartmouth Faculty since 1987. Dr. Lynd holds a B.S. degree in biology from Bates College, an M.S. degree in bacteriology from the University of Wisconsin, and masters and doctoral degrees in engineering from Dartmouth. Professor Lynd is an expert on utilization of plant biomass for production of energy. His contributions span the science, technology, and policy domains and include leading research on fundamental and biotechnological aspects of microbial cellulose utilization. He has led an active research group addressing these issues over the last two decades, authoring over 75 archival papers, book chapters, and reviews as well as 11 patents and patent applications. A frequently invited presenter on technical and strategic aspects of biomass energy, Professor Lynd has three times testified before the United States Senate and was a speaker at the 2007 Nobel Conference. In 2007 Dr. Lynd was the inaugural recipient of the Lemelson-MIT Sustainability prize for inventions and innovations that enhance economic opportunity and community well-being while protecting and restoring the natural environment. In 2005 he received the Charles D. Scott Award for distinguished contributions to the field of biotechnology for fuels and chemicals. Professional activities include: co-leader, the Role of Biomass in America's Energy Future project; Focus Area Leader for Biomass Deconstruction and Conversion, DOE Bioenergy Science Center; Biofuels industry representative, committee advisory to the Executive Office of President Clinton on Reducing Greenhouse Gas Emissions from Personal Vehicles; Editorial Board Member, Biotechnology and Bioengineering; and Manager, Link Energy Fellowship Program.

*Sustainable Paths to a Biofuel-Powered Transportation Sector: The Role of Innovation and Invention*

Bruce Dale and Lee Lynd

Prior to the first industrial revolution, people were scarce and resources were plentiful. Now confronted with the opposite circumstance, humanity must mount a second industrial revolution featuring population stabilization, increased energy utilization efficiency, and adoption of new renewable and sustainable energy supply technologies. At present there are widely disparate evaluations of the potential of biofuels to play an important role in the transition to a sustainable world, and there is a pressing need to resolve this disparity. This presentation will address key issues associated with the feasibility and desirability of cellulosic biofuels used on a large scale - including energy balance, economic feasibility, land competition, carbon debts, and resource availability - with a focus on two questions: 1) Understanding the reasons underlying the different conclusions reached by different analysts, 2) identifying paths by which large-scale biofuels use would be feasible and desirable. Innovation and invention will play key roles in the development of a large scale biofuel industry, as they have in the development of the petroleum refining industry. The talk will close by commenting on the general applicability of lessons learned from the biofuel example.

Background Reading

Bruce E. Dale. 2008. Biofuels: Thinking Clearly about the Issues. *Journal of Agricultural & Food Chemistry* 56:3885–3891.

Joseph E. Carolan, Satish V. Joshi, and Bruce E. Dale. 2007. Technical and Financial Feasibility Analysis of Distributed Bioprocessing Using Regional Biomass Pre-Processing Centers. *Journal of Agricultural & Food Industrial Organization* 5 (SPECIAL ISSUE: Explorations in Biofuels Economics, Policy, and History):Article 10, pp 1-27.

Seungdo Kim, Bruce E. Dale. 2005. Life cycle assessment of various cropping systems utilized for producing biofuels: Bioethanol and biodiesel. *Biomass and Bioenergy* 29:426–439.

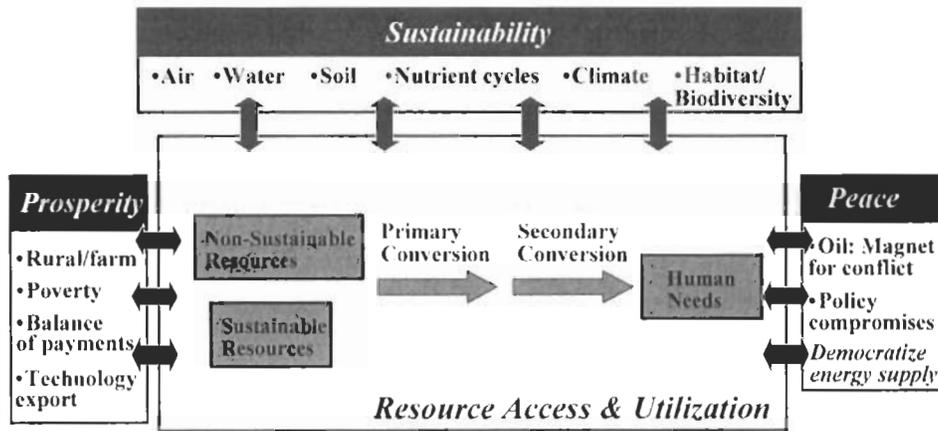
***Sustainable Paths to a Biofuel-Powered  
Transportation Sector: The Role of  
Innovation and Invention***

Lee R. Lynd and Bruce E. Dale  
Dartmouth College & Michigan State University

Presented at:  
U. S. Environmental Protection Agency  
Science Advisory Board Meeting  
Washington, DC  
October 27, 2008

1. Preliminary considerations.

*Dimensions of well being for human society...*

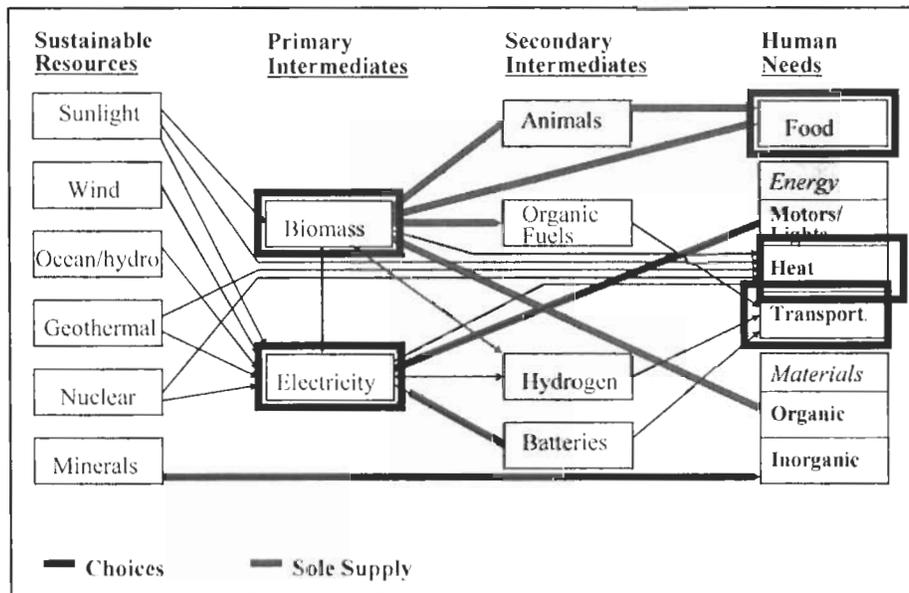


*... are dominated by resource access and utilization, particularly energy*

*Today & always*

*A convergence of factors makes this critical now - defining challenge of our time- we will invent and innovate to meet the challenge as we have done before*

**Imagining a Sustainable World**



Energy Carrier	Price	
	Common Units	\$/GJ
<b>Fossil</b>		
Petroleum	\$100/bbl	17.4
Natural gas	\$10/kscf	11.0
Coal	\$55/ton	2.5
w/ carbon capture @ \$150/ton C		6.5
<b>Electricity</b>	\$0.045/kWh	11.3
<b>Biomass</b>		
Soy oil	\$0.50/lb	30.0
Corn kernels	\$5/bu	14.4
Cellulosic crops <sup>a</sup>	\$50/tonne	3.0
Cellulosic residues		Some < 0

<sup>a</sup> e.g. switchgrass, short rotation poplar  
 Modified from Lynd et al., Nature Biotech., 2008

At \$3/GJ, cellulosic biomass purchase price competitive with oil at \$17/bbl.  
 Cellulosic biomass: The cheapest GJ in a carbon-constrained world.

### Different Plant Feedstocks are Responsive to Different Objectives

	Large Scale Production		Rural Economic Development		Petroleum Displacement (Security)		Fossil Fuel Displacement/ GHG Reductions		Soil Fertility & Ag-Ecology	Low Cost Fuels (feedstock & conversion)	
	Per unit	Total	Now	Future	Per unit	Total	Per unit	Total		Now	Future
Cellulosic	excellent	excellent	excellent	excellent	excellent	excellent	excellent	excellent	excellent	excellent	excellent
Starch-rich	poor	poor	poor	poor	poor	poor	poor	poor	poor	poor	poor
Sugar-rich	poor	poor	poor	poor	poor	poor	poor	poor	poor	poor	poor
Oil seed	poor	poor	poor	poor	poor	poor	poor	poor	poor	poor	poor

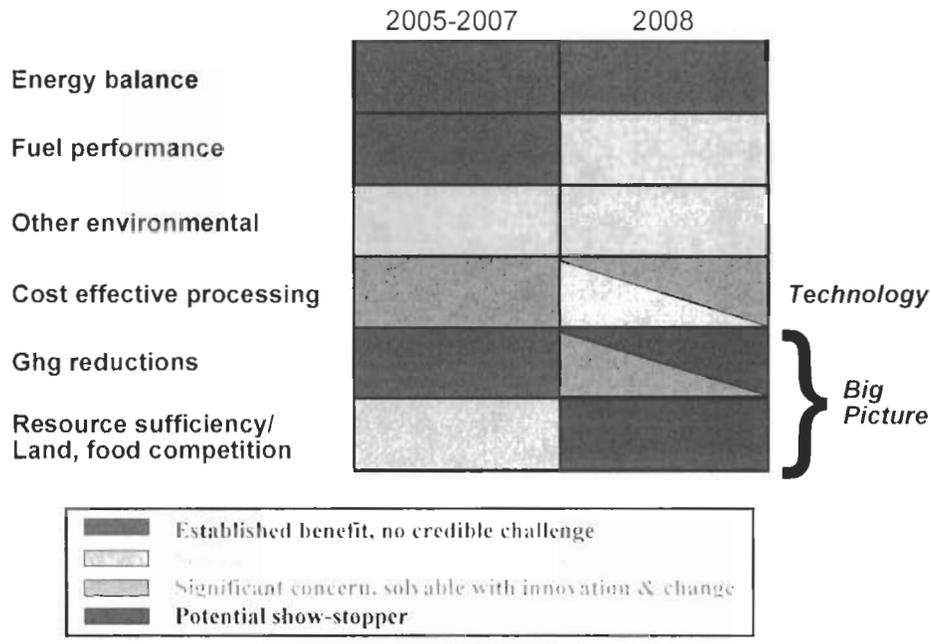
**Ratings:**

excellent	excellent
poor	poor

Cellulosic biofuels are the focus of all studies foreseeing (very) large-scale widespread fuel obtained from plants.

- Environmentally benign/beneficial production
- Low purchase cost
- Large potential scale of production

## Cellulosic Biofuels: Changing Perceptions of Challenge



### Why persist in considering biofuels if they have such large challenges?

**Because other transportation alternatives have large challenges too.**

**Hydrogen** - Should be in the running in light of efficiency and low pollution potential, but is about the worst way to move and store energy imaginable

Where will it come from?

Distribution & storage forecast to be 2x cost of fuel generation.

#### Electricity (EVs, renewable power --> H<sub>2</sub>, plug in hybrids)

Even with 2.5 higher efficiency than current fleet, providing for today's transportation energy consumption would require doubling U.S. power generation.

Plug in hybrids make good use of off-peak generating capacity, but will only achieve ghg emissions if power comes from low carbon sources.

Whereas cellulosic biomass is ~\$3/GJ, electricity is currently ~\$11/GJ

- Expected efficiency of biomass --> liquid fuels, electricity --> H<sub>2</sub> both ~ 70%
- Fuel cell efficiency is high, but efficiency losses in H<sub>2</sub> storage and distribution are much larger than for liquid fuels

There will be increasing pressure on power generation - some forecast  $\geq 2x$  price increase in the coming decade - without new transportation demand.

## 2. GHG accounting for cellulosic biofuels.

### Cellulosic Biofuels & Greenhouse Gas Emissions

**Through 2007, analysis focused on fuel production & utilization cycle**

- a) *Removal* of CO<sub>2</sub> via photosynthesis
- b) Agricultural energy inputs (typically small, e.g.  $\leq 7\%$  of feedstock heating value)
- c) Processing energy inputs (typically zero)
- d) Return of CO<sub>2</sub> in amount equal to a) when biomass-derived fuel is burned

***Picture generally very positive (e.g. ~10% of gas base case), widely accepted***

**Potentially large additional factors beyond fuel production & utilization cycle**

**Decreases ghg benefits (much recent attention)**

Land conversion prior to energy crop production.

These land conversion analyses **neglected**

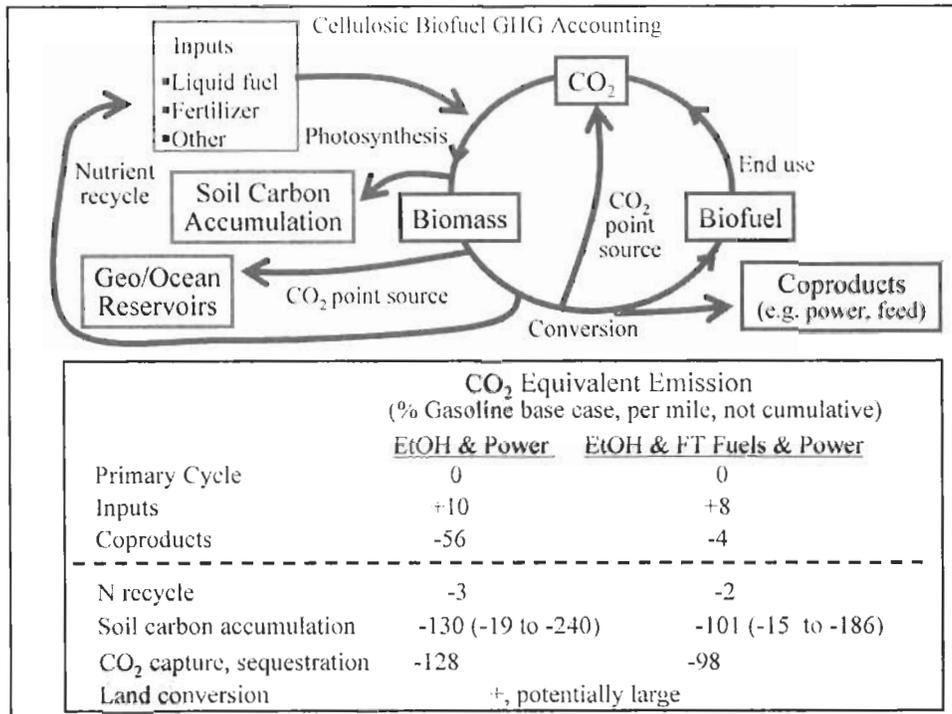
- 1) Use of standing biomass & 2) Land management options post land use change

**Increases ghg benefits (not much attention thus far)**

Soil organic matter accumulation

Carbon capture and sequestration (required for many coal scenarios to be low C)

Coproduce animal feeds along with cellulosic biofuels– large potential **reduction** in land requirements for food/feed/fuel



**3. Minimizing cellulosic biofuel land conversion carbon debts & Innovating and inventing to minimize land use for cellulosic biofuels**

**Or: "Going from Mega Acres to Nega Acres"**

**Innovating:** Make use of existing technology to change the game, eg:

1. Harvest & use standing biomass during land conversion
2. Improve land management post conversion using cover crops & reduced tillage (Searchinger & Fargione both assumed worst case: plow tillage)

**End result of these two relatively simple innovations is that  
“carbon debt” from forest conversion is greatly reduced if not  
entirely eliminated**

**Inventing:** Create new technology & approaches to meet needs

A viable cellulosic ethanol industry will require inventions including:

- Pretreatment to make available calories in structural carbohydrates
- Use of all components of plant material, including protein

Net result of these two inventions will be to completely change how we feed animals, particularly ruminant animals, resulting in much less land required to feed our livestock and provide fuel...“nega-acres”

## Land Conversion GHG Emissions

Recent papers of Searchinger et al. and Fargione et al. highlighted potentially large carbon emissions from land conversion

### Fargione et al.

"Biofuels are a potential low-carbon energy source, but whether biofuels offer carbon savings depends on how they are produced."

Carbon debt accompanying conversion of various unmanaged lands to established biofuels (corn ethanol, biodiesel from soy, palm) is large and requires a long time (17 to 429 years) to repay.

Production of biofuel from prairie grass on abandoned or marginal cropland repays the conversion carbon debt in less than a year with large carbon savings thereafter.

### Searchinger et al.

Focuses on converting existing US corn land to biofuel production.

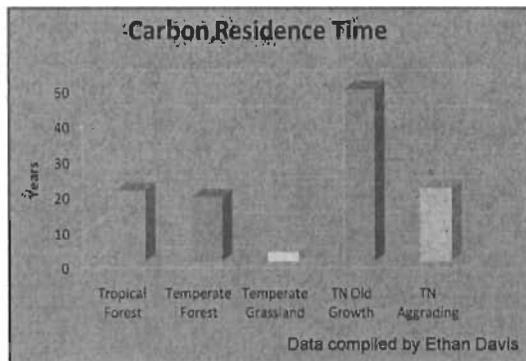
Use a global model to calculate *indirect land conversion impacts* - changes elsewhere to compensate for decreased grain production in the US.

Payback period for the carbon debt calculated for indirect land conversion:

- Corn EtOH: 42 to 640 yr
- Switchgrass EtOH: 52
- Cane EtOH: 4 to 42 yr

## Land Conversion GHG Emissions

Carbon residence time:  $C \text{ inventory} / \text{rate of } C \text{ accumulation}$



For ecosystems with a large carbon inventory, e.g. forests, land conversion may be accompanied by a large carbon debt unless:  
1) standing biomass is used to displace ghg emissions and/or  
2) forest land is managed after conversion to minimize ghg emissions

Grassland conversion **does not** generate any significant carbon debt

**Consider conversion of a temperate forest (Tennessee, aggrading) to switchgrass and biofuel production - Davis, Laser & Lynd**

Chosen to illustrate range of outcomes and key sensitivities, not necessarily because it is the most desirable large scale option

**Fate of standing biomass**

- Burn
- Biofuels
- Paper

**Additional management options**

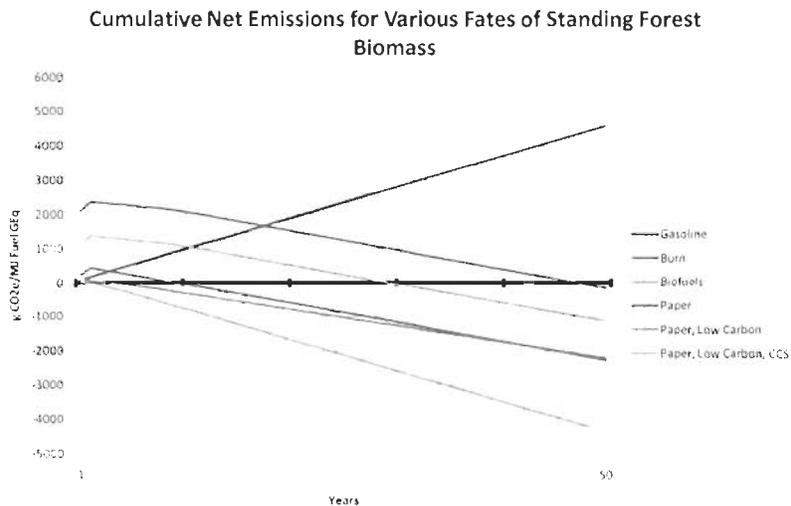
- Default - tilling, no carbon capture and sequestration
- Low carbon conversion - (no tilling, but lower biomass productivity)
- Carbon capture & sequestration

**Accounting**

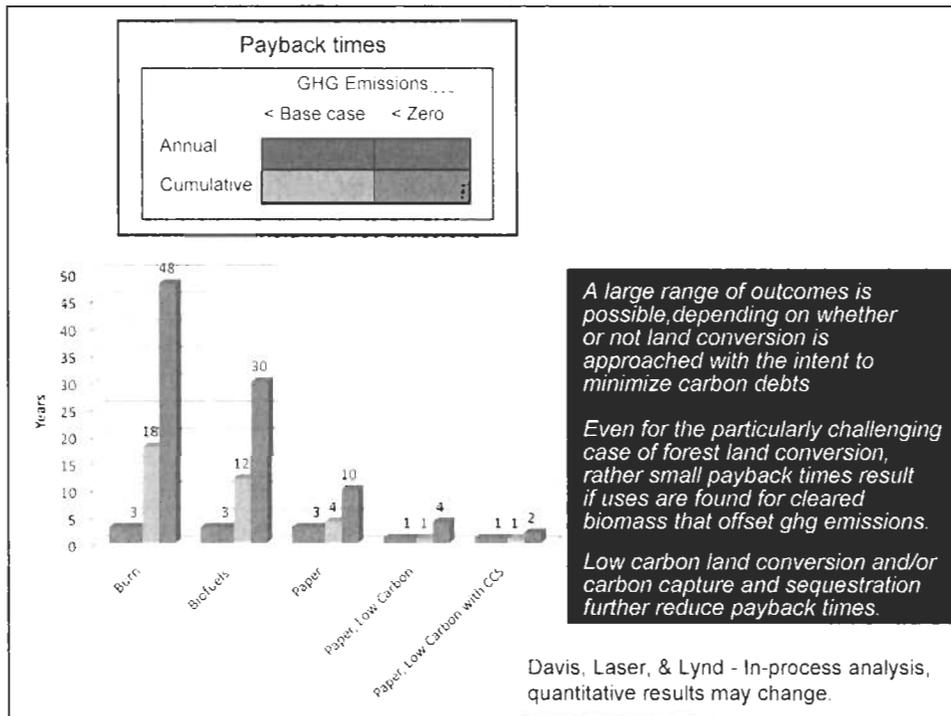
Life-cycle approach - based on changes relative to what would happen in the absence of land conversion and utilization of standing biomass

**Conversion technology**

Mature (on a per unit fuel basis avoided emission benefits *higher* than current technology, soil carbon and sequestration benefits *lower* than current technology)



Davis, Laser, & Lynd - In-process analysis, quantitative results may change.



## Land Management Post Land Use Change

1. Ethanol demand to corn price
2. Corn price to corn or soybean supply
3. Corn or soybean supply to land use change
4. Land use change to greenhouse gas consequences
5. Land management post land use change- assumed worst case of plow tillage

Very different predictions result from different models (FAPRI, GTAP, FASOMGHG) ... we do not discuss these issues here, but they are serious and deserve careful attention

Land doesn't cease to be managed once the land use change is executed.

What are the GHG consequences of different post land change management options?

Specifically, investigate cover crops & reduced tillage for temperate zone forests and grassland conversion. combine with corn stover utilization as fuel in the biorefinery

## Indirect Land Use Change Scenarios

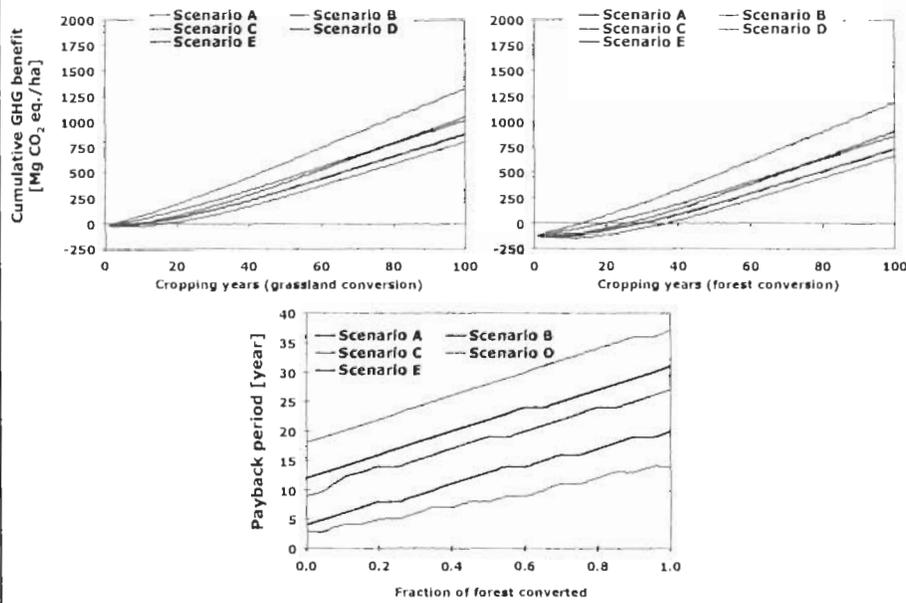
- Divert existing cornfield to ethanol production, and then convert grassland (or forest) to cornfield dedicated to animal feed production—harvest and use some corn stover as fuel for biorefinery

Scenario	Description
A	Cropping management: current tillage practice
B	Cropping management: no tillage practice
C	Cropping management: no tillage practice combined with winter cover crop
D	Cropping management: plow tillage
E	Scenario A with an assumption that ethanol would displace marginal gasoline fuel (from Athabasca oil sands)

\* Data for DAYCENT from 8 U. S. corn producing counties, different climates, etc.

Paper in review for publication in *Environmental Science & Technology*

## Cumulative GHG Benefit



## A Large Variable Space only Starting to be Explored

Biomass Source (8)	Biomass Fate (7)	Other Variables (9)
1. Sustainable wastes S, F	A. Burning S	Conversion technology i. Current S, F
2. Excess/degraded cropland F	B. Biofuel	ii. Mature
3. Integration into new agriculture	C. Power	iii. CCS
4. Forests, no land conversion	D. Lumber F	Accounting
5. Grassland --> HPCB	E. Paper	iv. Direct F
6. Forestland --> HPCB	F. Chipping	v. Indirect (LCA) S
7. Nonsustainable ag. land --> HPCB	G. Low carbon land conversion	Food production efficiency S, F vi. Current/extrapolated
8. Sustainable ag. land --> S HPCB		vii. Increased
		Mobility efficiency S, F viii. Current/extrapolated
		ix. Increased

Factorial combinations 8x7x9 = 504

S: Considered by Searchinger et al.

F: Considered by Fargione et al.

Cellulosic Biomass Source	Large at-risk C inventory	Food Competition	Observations
Sustainable wastes	No	No	Widespread agreement, sustainability must be verified
Excess/degraded cropland	No	No	Widespread agreement not problematic
Integration into new agriculture	No	Little or none	Potentially very large, Seldom considered
Forests, no land conversion	No	No	Widespread agreement broad needs served by increased "weed" harvest
Grassland → HPCB	No	None to some	Relatively low carbon inventory; Lots of abandoned pasture in NE, drainage-limited
Forestland → HPCB	Yes	No	<b>Mean age of C ~ 20 years → large potential debt</b>
Nonsustainable ag. land → HPCB	No	Only transiently	Land in ag. now, will not be for long - could beneficially support feedstock production
Sustainable ag. land → HPCB	No	Yes	<b>Problematic in a food-limited world—if in fact food is limited</b>

\*HPCB = High productivity cellulosic biomass

***For most but not all sources of cellulosic biomass, large land conversion carbon debts and food competition are either not a problem or readily avoided.***

**4. Inventing:** Create new technology & approaches to meet needs

A viable cellulosic ethanol industry will require inventions including:

- Pretreatment to make available calories in structural carbohydrates
- Use of all components of plant material, including protein

Net result of these two inventions will be to completely change how we feed animals, particularly ruminant animals, resulting in much less land required to feed our livestock... "nega-acres"

## Two Technical Advances Required for Cellulosic Biofuels

1. Key enabling advance: Effective, economical pretreatment to increase accessibility/digestibility of cellulose and hemicellulose (60-80% of forages)
2. Later advances: Complete utilization of all biomass components: carbohydrates, lignin, protein, lipids, minerals, pigments, pectin, organic acids, etc.
3. Taken together, these advances will significantly alter how we provide calories & protein to feed animals, particularly ruminant animals.

## Will People Go Hungry Because of Biofuels?

- Three major U.S. crops *alone* (corn, soy, wheat) produce 1300 trillion kcal & 51 trillion grams protein/yr
- Could meet U.S. human demand for protein & calories with 25 million acres of corn (~5% of our cropland)
- *Most U. S. agricultural production (inc. exports) is fed to animals-- i.e., we are meeting their protein/calorie needs from our land resources. Their needs are:*
  - 1040 trillion kcal/yr ( 6 times human demand)
  - 56.6 trillion gm protein/yr (10 times human demand)
- Thus we can address perceived “food vs. fuel” conflict by providing animal feeds more efficiently, on less land
- Dairy & beef cattle consume more than 70% of all calories and protein fed to livestock
- As nations grow richer, they want more protein, especially more meat....

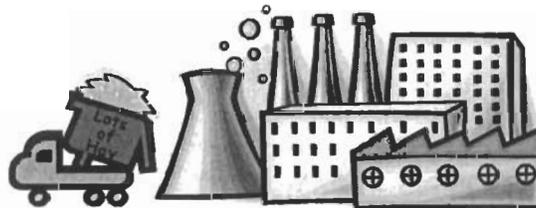
## Tale of Two Biorefineries

**Mobile Cellulose  
Biorefinery**



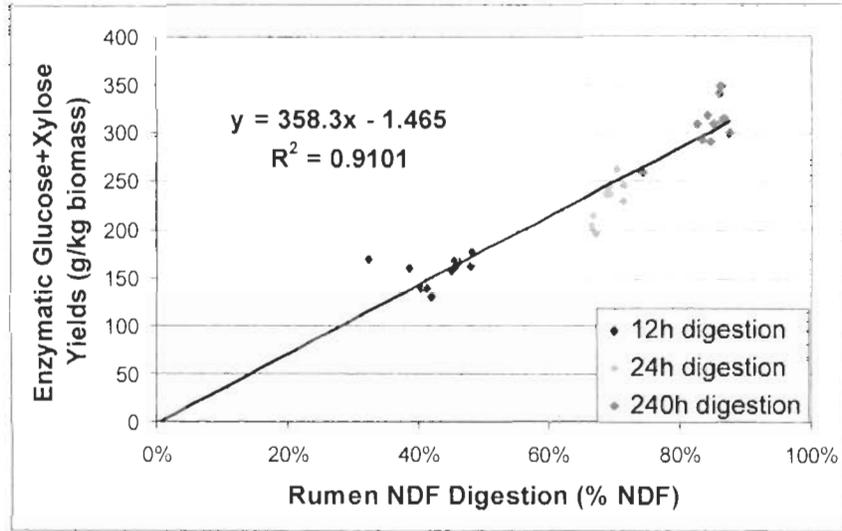
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**Stationary Cellulose  
Biorefinery**



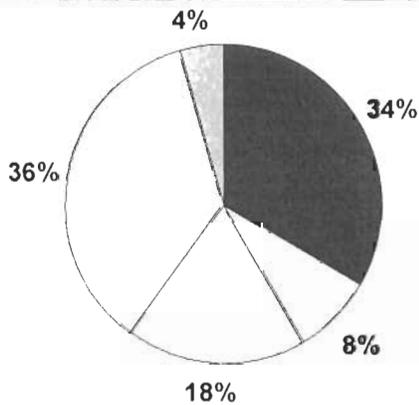
*Improve Cellulose Conversion for Biorefinery  
= Improve Cellulose Digestibility for Cows*

## Enzymatic and Rumen Fluid Digestion of AFEX-Treated Grass

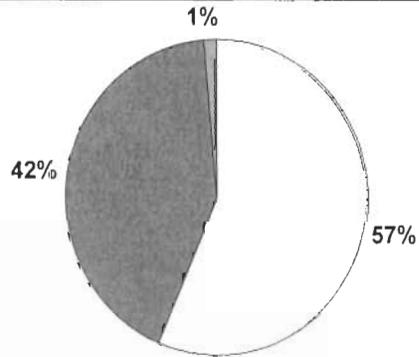


## Dairy Diet- Black Hawk County Iowa Farm

■ Alfalfa Silage    □ Alfalfa Hay    □ Grain Silage    □ Dry Grain    ◼ Soybean Meal, 44%  
 ■ AFEX Treated Switchgrass    ◼ Protein Supplement



**\$150,242/yr**  
**265 acres/yr**

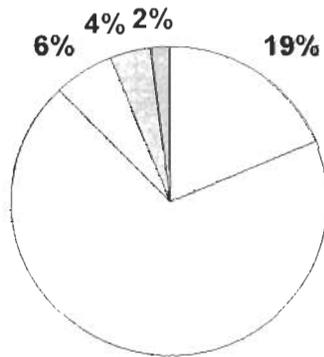


**\$92,388/yr**  
**167 acres/yr**

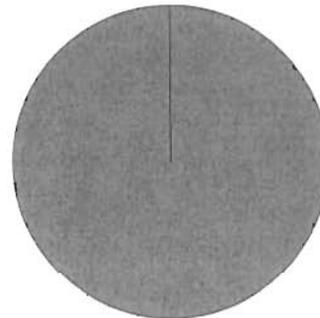
Using high digestibility grass feeds reduces land requirements by 1/3 and GHG due to removal of corn from the animal diet—assumes 6 ton/acre switchgrass

## Beef Diet- Aberdeen South Dakota Ranch

Grain Silage   
  High Moisture Grain   
  Dry Grain   
  Soybean Meal, 44%  
 Meat and Bone Meal   
  AFEX Treated Switchgrass



**69%**  
**\$248,381/yr**  
**436 acres/yr**



**100%**  
**\$134,897/yr**  
**227 acres/yr**

High digestibility grasses reduce land needed for animal feeds by almost 50% & reduces GHG by replacing corn in diet.

### Some early conclusions:

Innovating on the biofuels supply chain (eg, using standing biomass instead of just burning it, and/or managing the land appropriately after the conversion) can greatly reduce or eliminate the "carbon debt"

- Harvesting standing biomass for biofuel production reduces payback time by 20 years (from about 50 to about 30 years)
- Applying best management practices reduces the payback time by about 25 years (from 40 to about 15 years)
- These two approaches would be additive: thus the total savings could be as large as 20 + 25 years = 45 years, *paying back the entire carbon debt for forest conversion in the first year...*
- Grassland conversion "debt" is essentially zero in all scenarios we have studied
- Land use conversion will involve a mix of forest and grassland, therefore the carbon debt *may well be zero for real systems... it is far too early to be making regulations based on our current level of scientific understanding*

## Minimizing cellulosic biofuel land requirements & food competition by invention:

Invention will follow defined and knowable paths, even if the specific invention that is generated is unknown.

For cellulosic biofuels, invention will take place in:

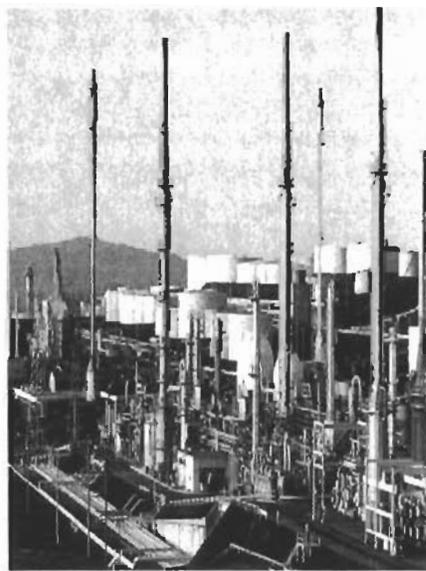
- 1) pretreatment (making cellulosic biomass calories more available for animal feed) and
- 2) improving feedstock use efficiency (making biomass protein more available for animal feed)

Since over 80% of crop and pasture land is used to produce feed (not food for direct human consumption) there is every reason to believe we can produce lots of cellulosic biofuels and lots of animal feed using much less land if we can ever get to large scale cellulosic biofuels

Please don't blow up the (corn ethanol) bridge to the future by ill-founded and premature regulations on indirect land use change, technology generally improves if we give it a chance.

Technological Improvement Takes us from This

To This

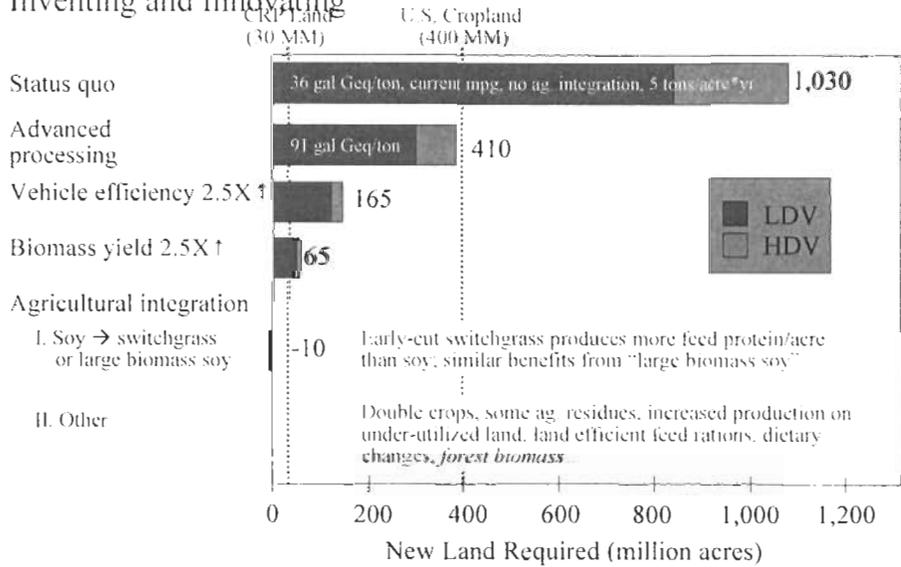


Or From This "Cell Phone"

To this One



New Land Required to Satisfy Current U.S. Mobility Demand:  
Inventing and Innovating



When new land requirement = 0, the land conversion carbon debt = 0, displaced food production = 0

Questions ??



## **Dr. Kenneth G. Cassman**

### **University of Nebraska**

Dr. Kenneth G. Cassman currently serves as Director of the Nebraska Center for Energy Sciences, and is the B. Keith and Norma F. Heuermann Professor of Agronomy at the University of Nebraska. He received a BSc degree in biology from the University of California--San Diego (1975) and a PhD in Agronomy and Soil Science from the University of Hawaii (1979). His expertise is centered within the disciplines of soil science, agroecology, and plant ecophysiology. Research activities have focused on: (1) plant nutrition, root ecophysiology, soil fertility and nutrient cycling to improve fertilizer efficiency and to reduce negative effects on environmental quality; (2) crop yield potential, soil carbon sequestration, and greenhouse gas emissions in maize-based cropping systems of the USA Corn Belt; (3) the long-term sustainability of intensive crop production systems and global food security. Recently he has focused attention on the role of agriculture in contributing to renewable energy supplies through production of ethanol and biodiesel fuels from cereal, oilseed, and sugar crops and the environmental impact of expanded biofuel production from agricultural crops. He served on the California Task Force on Sustainable Agriculture (1985-86), the Board of Directors for the Nebraska Crop Improvement Association (1996-2004), the Nebraska Crop Advisors Executive Board (1996-2002), the Council on Agriculture Science and Technology (CAST) Task Force on Animal Agriculture and Global Food Security (1996-99), Chair of the Nebraska Environmental Livestock Environmental Quality Task force (1998-2001), and on the Science and Policy Committee for the 3rd International Nitrogen Conference (2003-04). In addition, he has been active as an external program reviewer for a number of scientific institutions, including: CIMMYT (1997 and 2000), IITA (2001), ICRISAT (2008), the graduate program at the Wageningen Agricultural University in the Netherlands (1998), and the Department of Soil Science at the University of Wisconsin. Professor Cassman has been elected Fellow of the American Association for the Advancement of Science, the Agronomy Association of America, the Soil Science Society of America, and the Crop Science Society of America, and has received a number of national and international awards for research excellence. His research has been widely published in seminal journals.

## EPA-SAB October 27 Meeting Abstract

Kenneth G. Cassman<sup>1</sup>, University of Nebraska

Rapid economic growth in the world's most populous countries, political instability in regions with greatest petroleum supplies, greater consumption than discovery of new petroleum reserves, and an abrupt rise in energy prices have driven global expansion of biofuel production from sugar, starch, and oil seed crops. As a result, a 50-year trend of declining real prices for the world's major crop commodities has been reversed, and we are in a demand-driven commodity market created by the convergence of energy and agriculture. Current rates of gain in crop yields are not adequate to meet this increased demand without a large expansion of crop area at the expense of rainforests, wetlands, and grassland savannah. Therefore, a large acceleration in the rate of crop yield gains on existing farm land is required, both here in the U.S. and globally, to ensure the environmental and economic sustainability of biofuel systems. But achieving yield gains while also reducing the negative environmental impacts of high-yield agriculture on soil and water quality and greenhouse gas (GHG) emissions has been an elusive goal. It requires a process of "ecological intensification" that involves interdisciplinary, systems-oriented research for which there has been little funding support from USDA, DOE, and NSF. Instead, most of our public-sector agricultural research portfolio has focused on measuring and understanding the environmental impact of agriculture without regard to crop productivity and on genetic crop improvement through biotechnology, while the private sector has emphasized productivity with little regard for environmental impact. To ensure the long-term viability of biofuel systems, these trends must change, and change quickly. A substantial increase in research investment is needed that is focused tightly on the *dual goals* of accelerating the rate of gain in crop yields and doing so in a manner that decreases the environmental footprint of agriculture. Although development of cellulosic (non-food crop) biofuels will reduce the competition between food and biofuels, large-scale commercialization of cellulosic biofuels (+4 billion L/yr annual production) is at least 7-10 years off. In the meantime, food-crop biofuels production capacity will continue to build out under present policies, and the environmental challenges embodied in this expansion must be addressed proactively.

### Citations:

Cassman, K.G. 1999. Ecological intensification of cereal production systems: Yield potential, soil quality, and precision agriculture. *Proc. National Acad. Sci. (USA)* 96: 5952-5959.

Cassman K.G. and Liska A. J. 2007. Food and fuel for all: Realistic or foolish? *Biofuels Bioprod. Biorefin.* 1:18-23. <http://www3.interscience.wiley.com/cgi-bin/fulltext/114283521/PDFSTART>

Cassman KG, Dobermann A, Walters DT, and Yang H. 2003. Meeting cereal demand while protecting natural resources and improving environmental quality. *Ann Rev Environ Resour* 28: 315-358.

Council for Agricultural Science and Technology (CAST). 2006. Convergence of Agriculture and Energy: Implications for Research and Policy. CAST Commentary QTA 2006-3. CAST, Ames, Iowa.

Liska AJ, Yang HS, Bremer V, Walters WT, Kenney D, Tracy P, Erickson G, Koelsch R, Klopfenstein T, Cassman KG. 2007. Biofuel Energy Systems Simulator: LifeCycle Energy and Emissions Analysis Model for Corn-Ethanol Biofuel (ver. 1.0, 2007). University of Nebraska, [www.bess.unl.edu](http://www.bess.unl.edu).

Liska A. and Cassman KG. 2008. Towards standardization of life-cycle assessment metrics for biofuels: Greenhouse gas emissions mitigation and net energy yield. *J. Biobased Materials and Bioenergy* 2:187-203.

Naylor RL, Liska AJ, Burke MB, Falcon WP, Gaskell J, Rozelle SD, and Cassman KG. 2007. The Ripple Effect: Biofuels, Food Security, and the Environment. *Environment*. 49: 30-4.

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<sup>1</sup> Heuermann Professor of Agronomy, and Director—Nebraska Center for Energy Sciences Research

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## **Ensuring Environmental Sustainability of Biofuel Systems**

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**Director, Nebraska Center for Energy Sciences**  
**University of Nebraska—Lincoln**  
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27 Oct 2008

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1

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## **Mega Trends Affecting the Food and Energy Supply—Demand Balances**

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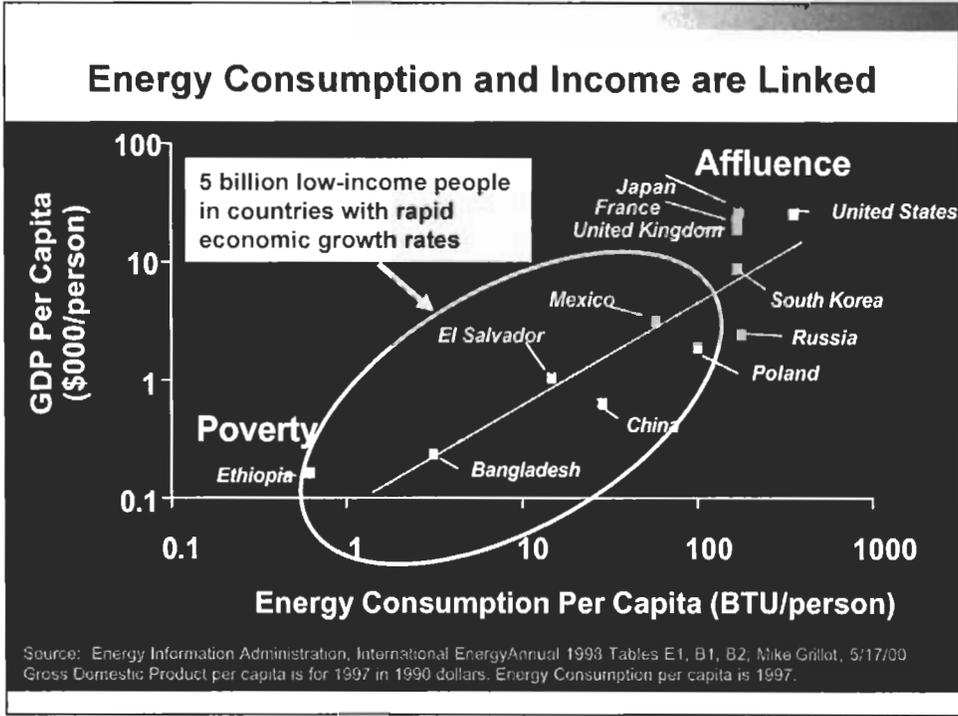
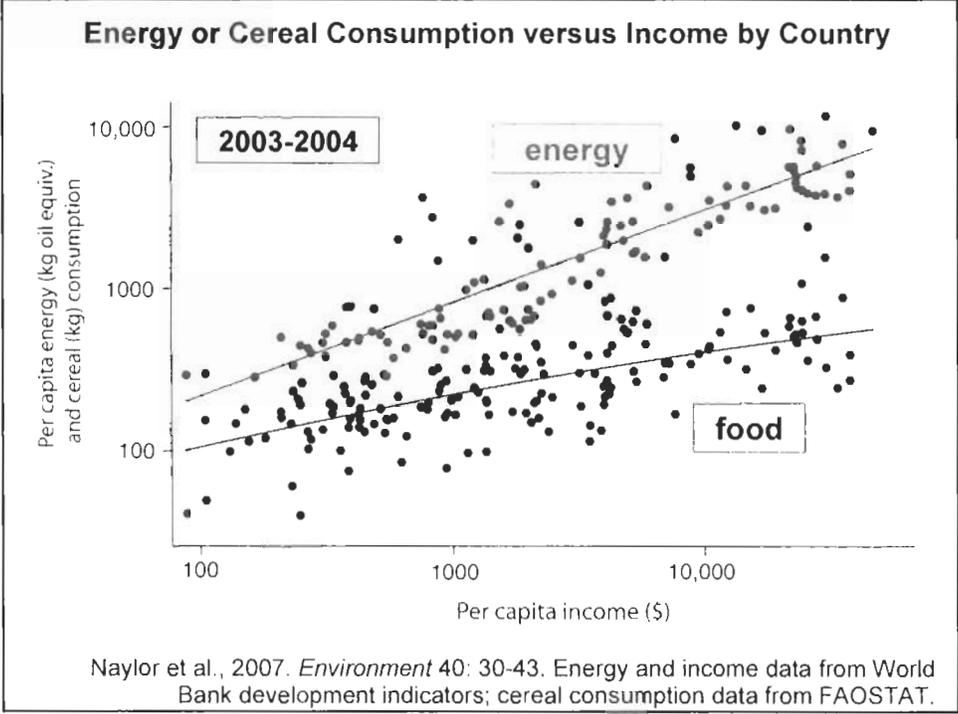
- **Rapid rate of economic growth in most populous developing countries**
  - Per capita increases in consumption of energy and livestock products
- **Uncertainty of petroleum supply**
  - Political instability in oil-producing countries
  - Decreasing replacement of petroleum reserves
  - Rising prices for petroleum and motor fuels
- **Climate change and increasing public concern about protection of environmental quality and natural resources**

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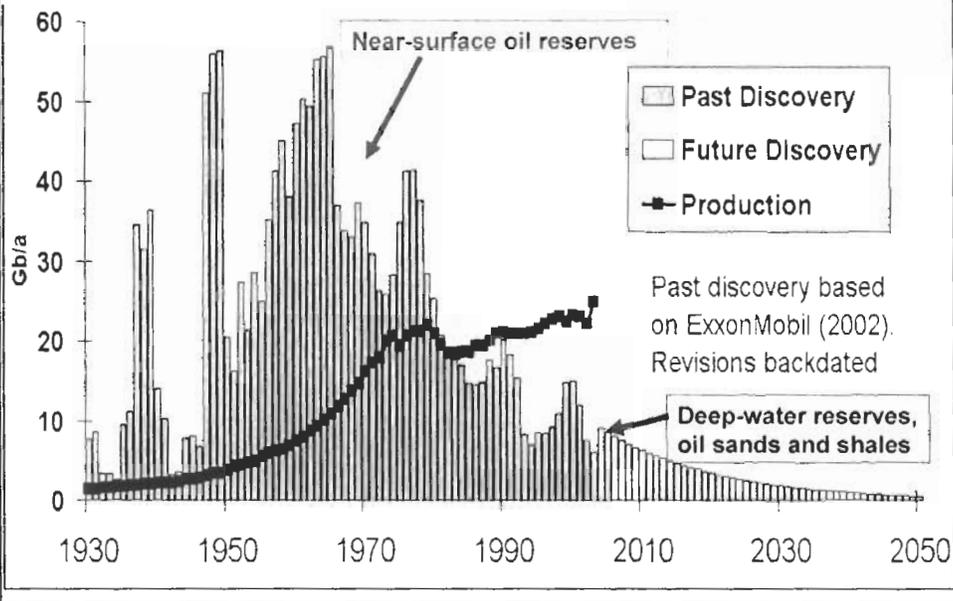
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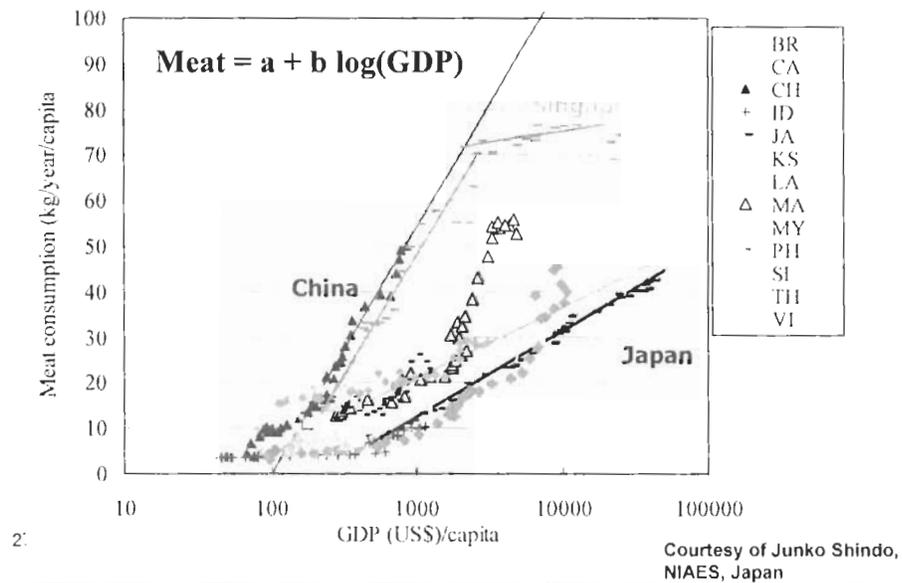
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## Oil Production vs Oil Discovery



## Increase of meat consumption with increased wealth (GDP) in Asia



## Addressing environmental challenges associated with biofuels

- Don't shoot at the caboose of a fast moving train
- Think globally, act locally
  - Population must plateau at about 9 billion by 2050
  - Requires a massive increase in wealth, energy use, and food consumption (on average) despite reduced per capita consumption in developed countries
- Must have sustainable options to meet this demand for food and energy within 10-15 yrs
  - Transitional systems vs long-term solutions

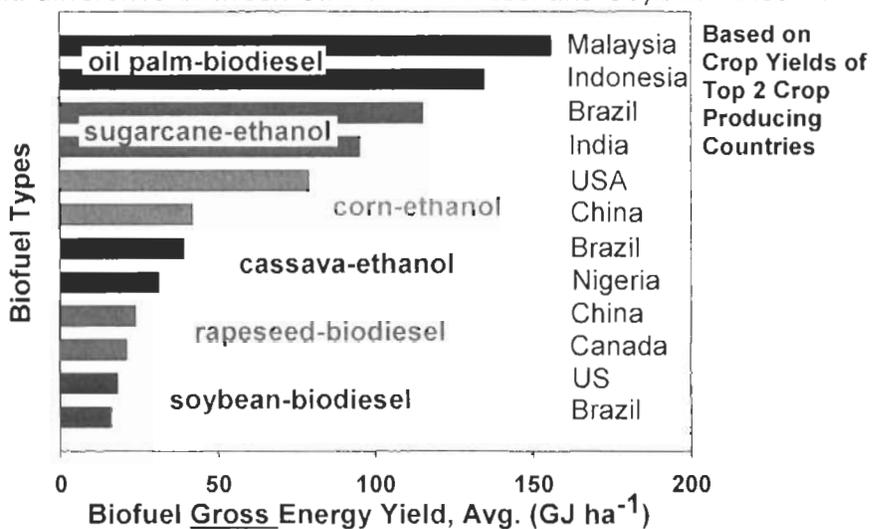
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7

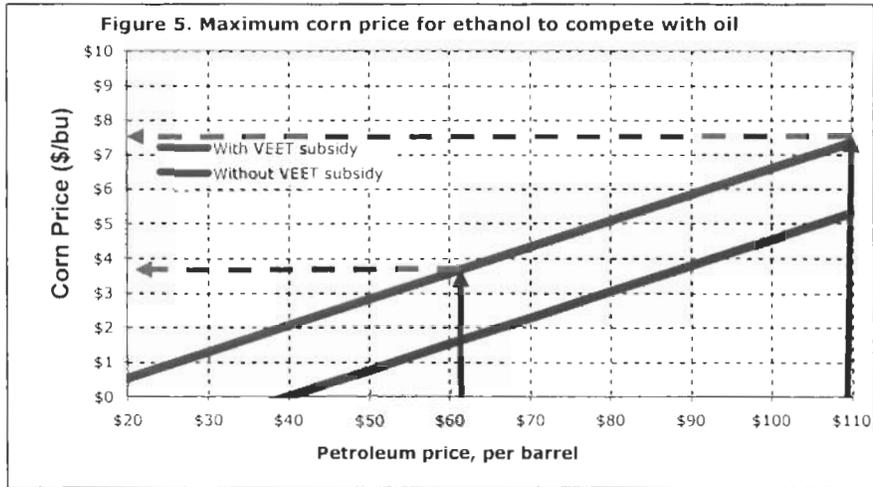
## Increasing Biofuel Energy Yield ( $\text{GJ ha}^{-1}$ ) Limits Competition with Food & Uses Land Economically

10-fold difference between Oil Palm-Biodiesel and Soybean-Biodiesel!



Source: Liska and Cassman *Journal of Biobased Materials and Bioenergy*, 2, 187-203, 2008

### Breakeven price of corn for ethanol production at different petroleum prices



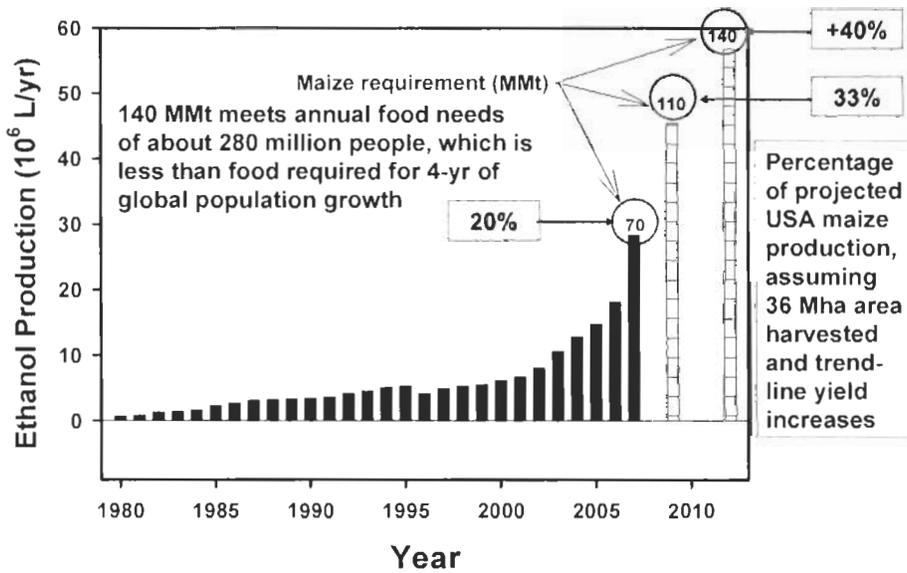
Source: R. Perrin, Univ. Nebraska

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9

### Expansion of USA Maize-Ethanol Production

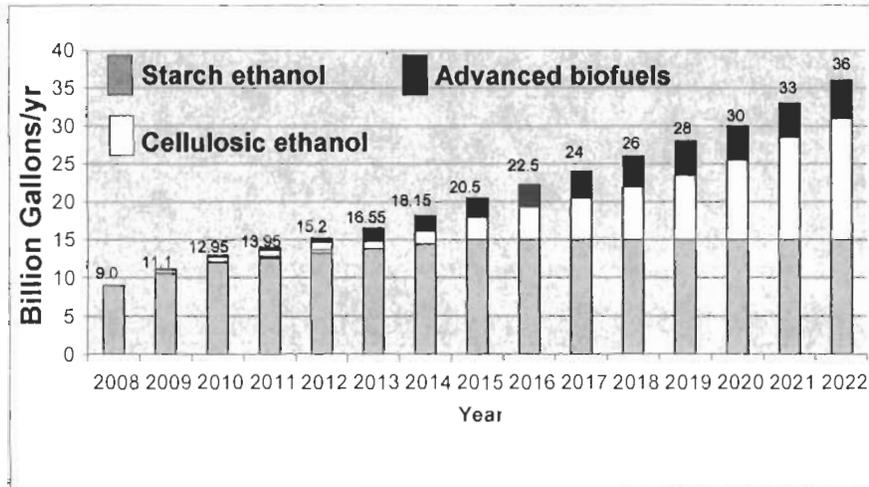


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10

## Renewable Fuel Standard Biofuel Production under the 2007 Energy Independence and Security Act (EISA)

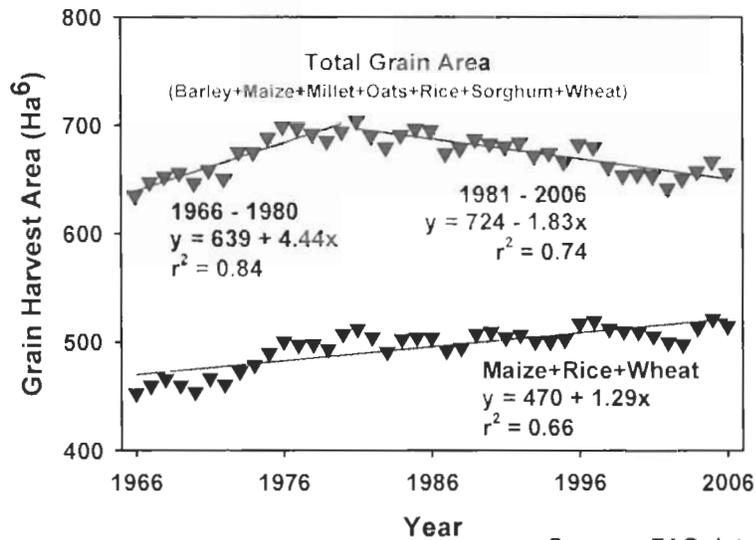


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11

## Global Cereal Area Trends, 1966-2006



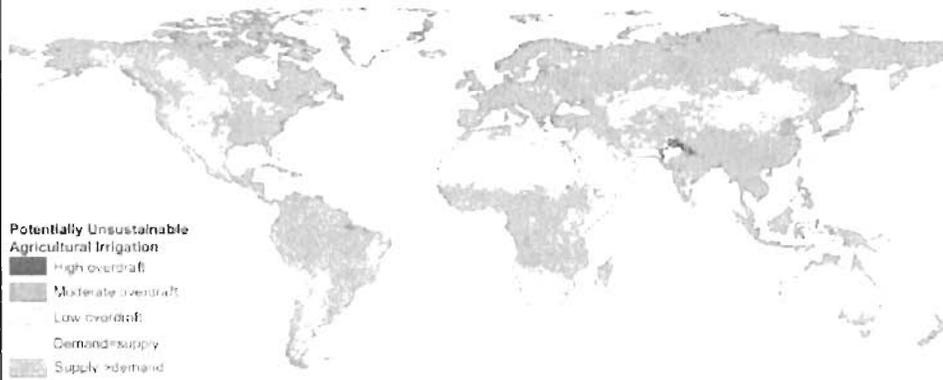
Source: FAO data archives

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12

**Decreasing water supply in all major irrigated areas**



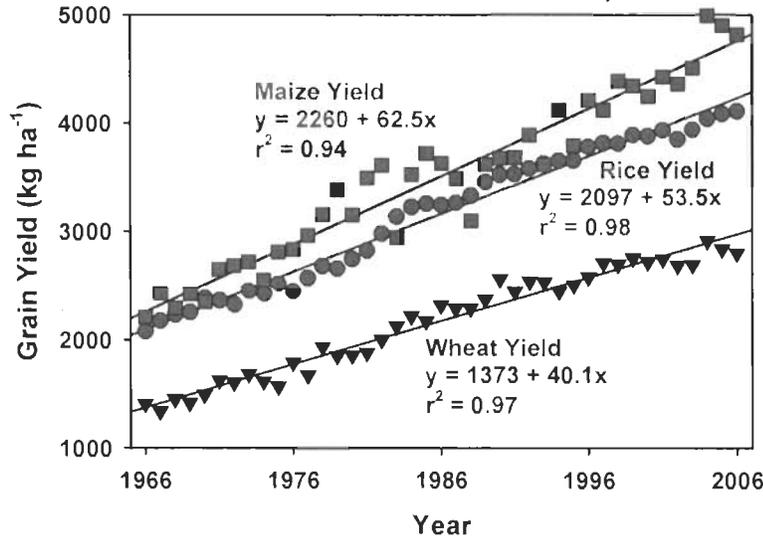
**Yet, irrigated agriculture produces 40% of global food supply on just 18% of the cropped area.**

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13

**Global Cereal Yield Trends, 1966-2006**



**THESE RATES OF INCREASE ARE NOT FAST ENOUGH TO MEET EXPETED DEMAND!** Source: FAO data archives.

14

**Rate of gain for all cereals is linear, not exponential, which means that the relative rate of gain is decreasing: relative rates of gain in 1966.**

Global rate of increase in yield of maize, rice, and wheat, 1966-2006.

Crop	Mean yield (kg ha <sup>-1</sup> )		Rate of gain* (kg ha <sup>-1</sup> yr <sup>-1</sup> )	Proportional rate of gain (%)	
	1966			1966	
Maize	2260		62.5	2.77	
Rice	2097		53.5	2.55	
Wheat	1373		40.1	2.92	

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15

**Rate of gain for all cereals is linear, not exponential, which means that the relative rate of gain is decreasing: relative rates of gain in 2006.**

Global rate of increase in yield of maize, rice, and wheat, 1966-2006.

Crop	Mean yield (kg ha <sup>-1</sup> )		Rate of gain* (kg ha <sup>-1</sup> yr <sup>-1</sup> )	Proportional rate of gain (%)	
		2006			2006
Maize		4759	62.5		1.31
Rice		4235	53.5		1.26
Wheat		2976	40.1		1.35

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16



**Potential Ripple Effect: accelerated deforestation due to abrupt increase in demand for food, feed, and biofuel crops**

**The Legal Amazon:**

*Deforestation Monitoring*



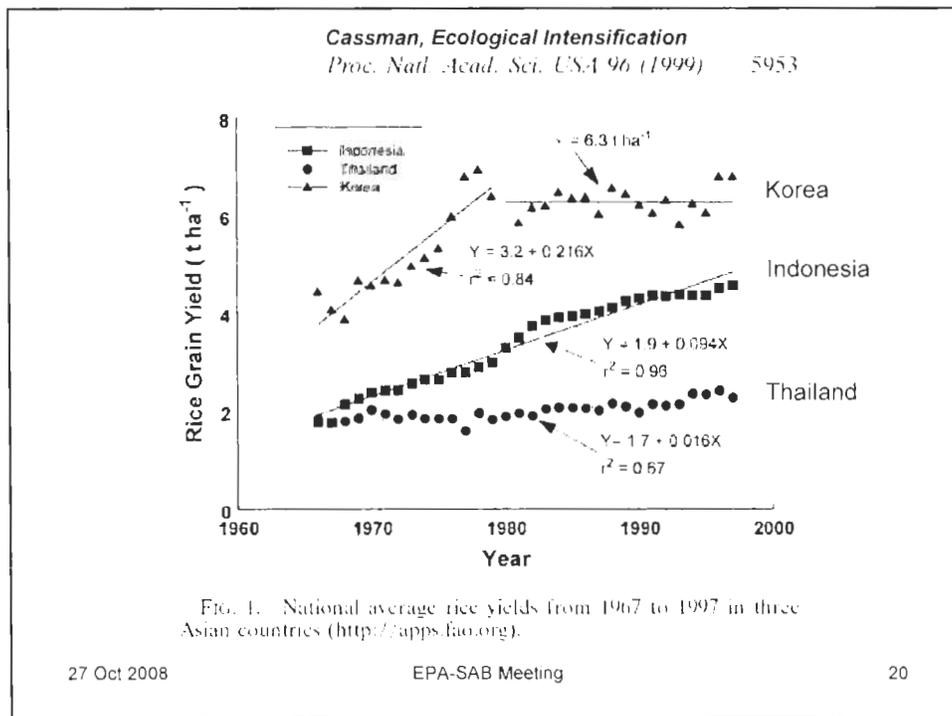
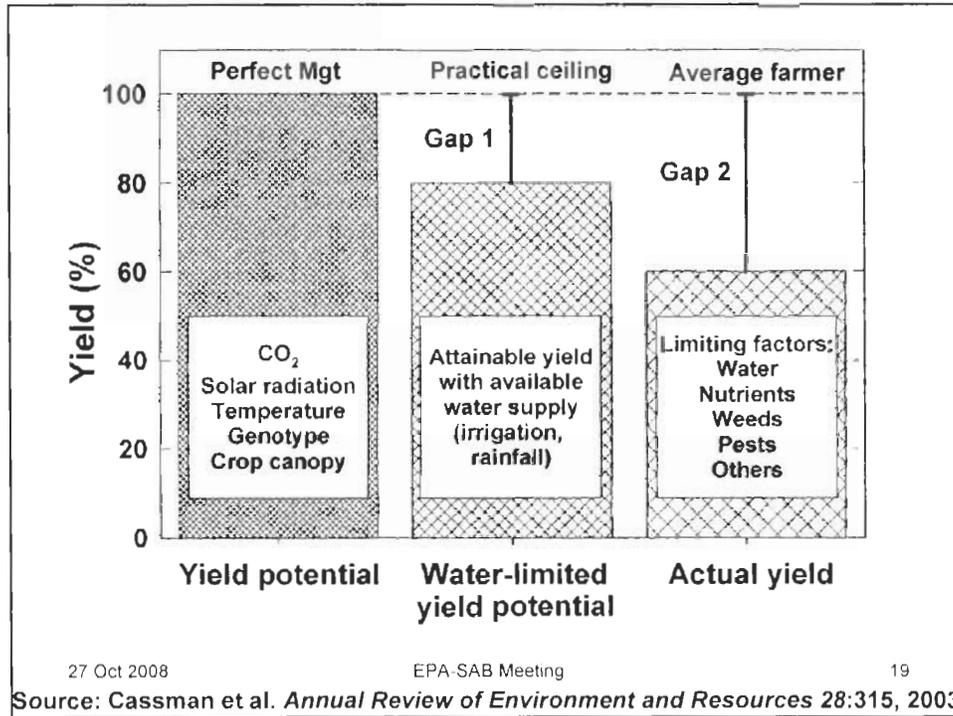
Source: INPE/PRODES

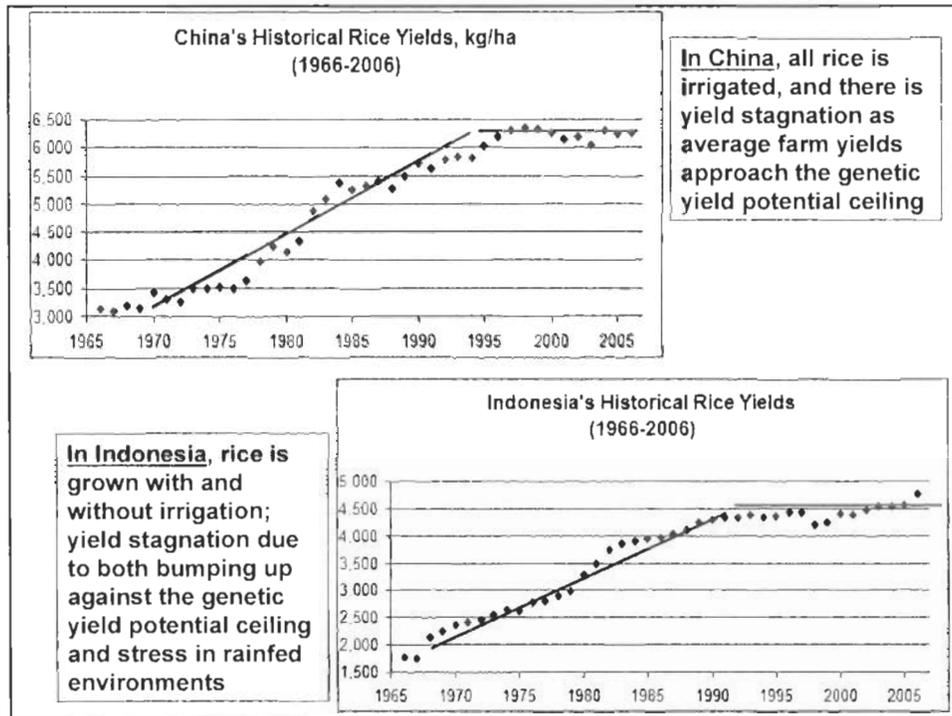
Nearly 30 million hectares of tropical forest have been cleared since 1988

- Vast majority converted into rangeland for commercial cattle production
- Deforestation is continuing at a rate of over 2.0 million hectares per year
- New rangeland provides opportunity for future field-crop cultivation

**Potential Ripple Effect: unsustainable crop production on marginal land by poor farm families without other options**

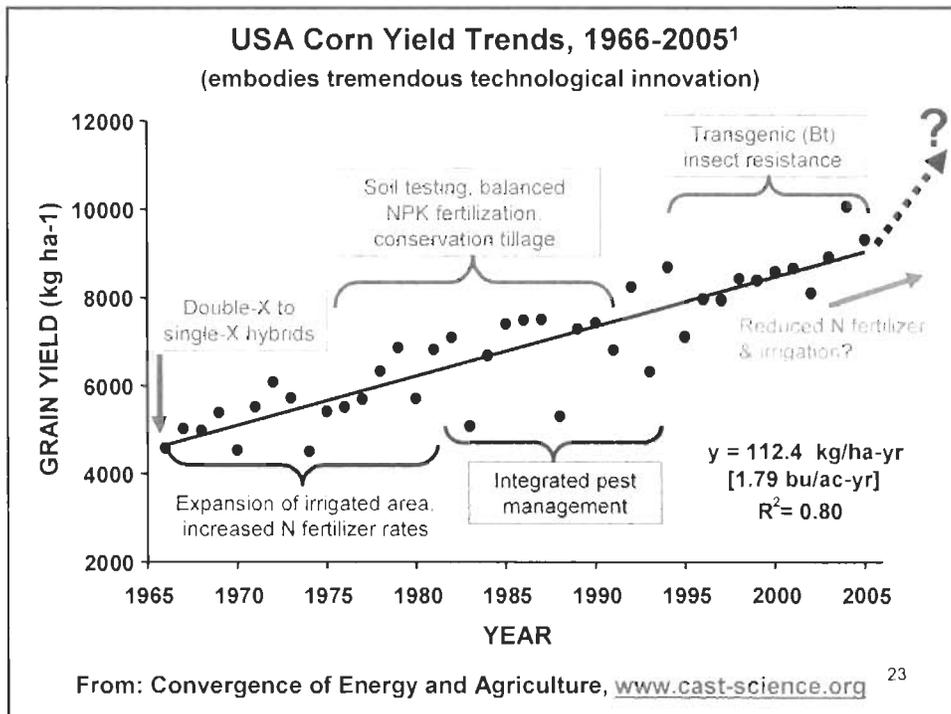






## Bottom Line on Yield Trends

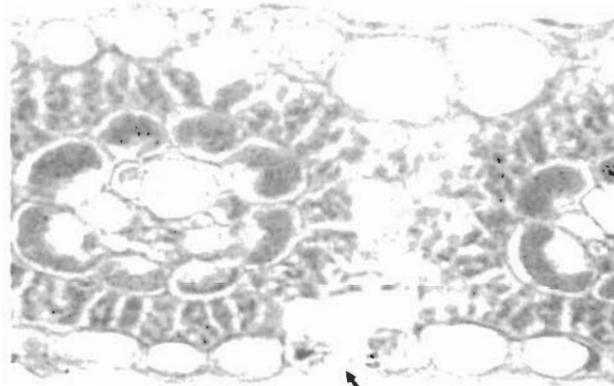
- Little increase in yield potential of maize or rice for the last 30-40 years (see publications)
- Current rates of gain in crop yields and land area available for crop production are not adequate to meet expected demand for food, feed, fiber, and fuel
- Little scope for a quantum leap in crop yields from biotechnology *despite* the hype from some major seed companies
- Little scope for increasing irrigated crop area due to competition for water with other sectors
- Expansion of crop area limited by lack of good quality arable soils and concerns about loss of wildlife habitat and biodiversity
  - USA conservation reserve land
  - Rainforests and wetlands in Latin America, SE Asia, SSA



## Will there be enough corn?

- **New York Times article, June 5 2008 :**
  - “Monsanto Offers a Plan to Increase Food Supply”, by Andrew Pollack
  - “*Monsanto, the leader in agricultural biotechnology, pledged Wednesday to develop seeds that would double the yields of corn, soybeans and cotton by 2030 and would require 30 percent less water...*”
  - “The announcement by CEO Hugh Grant came “as world leaders are meeting in Rome to discuss rising food prices and growing food shortages”
  - James E. Specht, a soybean breeder at the University of Nebraska, said he doubted it could be done. “*The hype-to-reality ratio of that one is essentially infinity,*” Mr. Specht said. “*Seeing an exponential change in the yield curve is unlikely.*”

## Basis of Crop Water Loss: Leaf architecture



Stomatal opening

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25

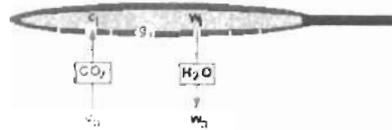


Wind



Leaf Interior  
 $c_i = 40-60 \text{ ppm}$

Atmosphere  
 $c_a = 385 \text{ ppm}$



Leaf Interior  
 $w_i = 100\% \text{ RH}$

Atmosphere  
 $w_a = 50\% \text{ RH}$

Photosynthesis:  $A = \frac{g}{1.6} (c_a - c_i)$

Transpiration:  $E = g(w_i - w_a)$

$$WUE = \frac{A}{E} = \frac{c_a - c_i}{1.6(w_i - w_a)}$$

For a sunlit soybean leaf (C3 type of photosynthesis):

During the time it takes for 1 CO<sub>2</sub> molecule to pass thru an open stomatal pore, 400 H<sub>2</sub>O molecules simultaneously escape from that same pore !!!!

( ~ 6.1g CO<sub>2</sub> per 1000g H<sub>2</sub>O ) (Nobel, 1999)

Plants must thus exchange 164 kg H<sub>2</sub>O to acquire 1 kg CO<sub>2</sub>

27 Oct

Slide provided by J. Specht, Univ. of Nebraska

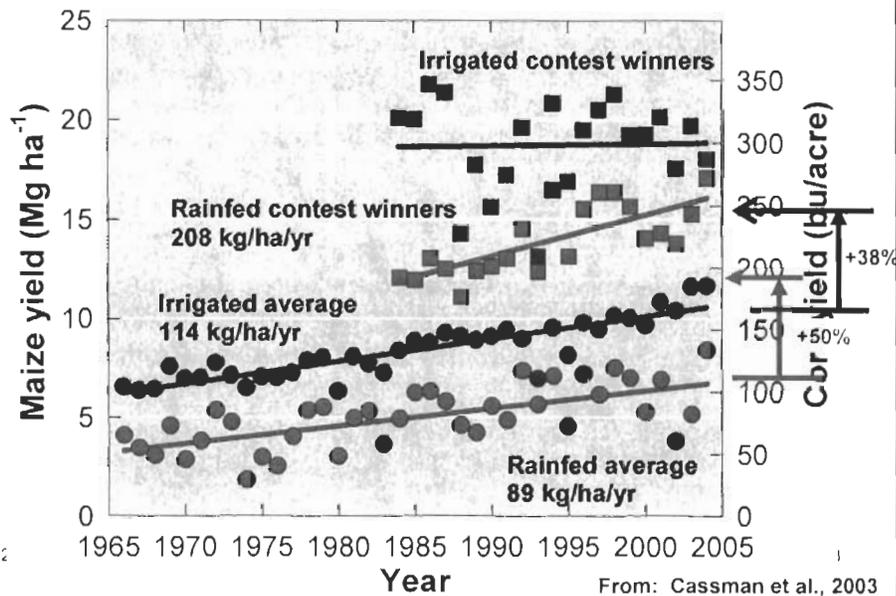
## R. Ford Denison Hypothesis: Evolution versus genetic improvement by humans<sup>1</sup>

- Evolution has already tried and rejected options for improving plant traits that give individual plants a competitive advantage against neighboring plants
  - Photosynthesis, nitrogen efficiency, drought
  - Up or down regulation of single gene expression already tested by evolution
- Evolution has not optimized traits that improve productivity of a dense community of plants of the same species, or quality traits for specific end uses
  - Greater harvest index, resistance pests/diseases in luxuriant environments (large LAI, high leaf [N])
  - Novel proteins, nutritional qualities, fine oils, pharmaceuticals

<sup>1</sup>Darwinian agriculture: When can humans find solutions beyond the reach of natural selection? 2003. Quarterly Review of Biology. 78:145-167.

### Nebraska contest-winning and average yield trends

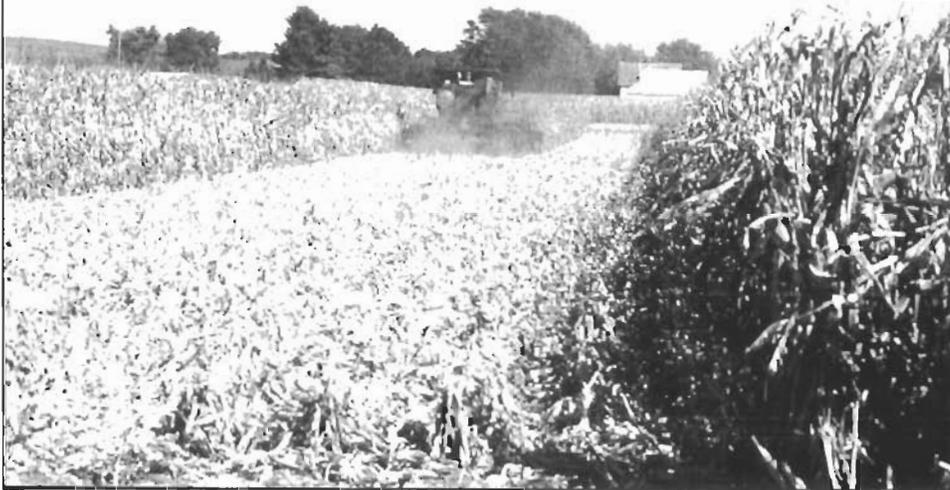
No increase in yield potential ceiling since the 1980s, but a large unexploited yield gap still exists.



**Large exploitable gap between average and record yields.**

**USA contest-winning corn field, 1997, Sterling NE.**

**310 bu/ac (ethanol yield of 800 gallons/ha): How to close the gap between highest possible yields (called yield potential) and average farm yields in an environmentally sustainable manner?**



## **Need for Ecological Intensification<sup>¶</sup>**

- **Development of high-yield crop production systems that protect soil and environmental quality and conserve natural resources**
  - Ⓔ **Characteristics of EI systems:**
    - **Yields that reach 80-85% of genetic yield potential**
    - **70-80% N fertilizer uptake efficiency (vs 30-40% now)**
    - **Improve soil quality (nutrient stocks, SOM)**
    - **Integrated pest management (IPM)**
    - **Contribute to net reduction in greenhouse gases**
    - **Have a large net positive energy balance**
    - **In irrigated systems: 90-95% water use efficiency**
- <sup>¶</sup>Cassman, 1999. *in Proc. Natl. Acad. Sci (USA):5952-5959*

## Ecological Intensification Requires

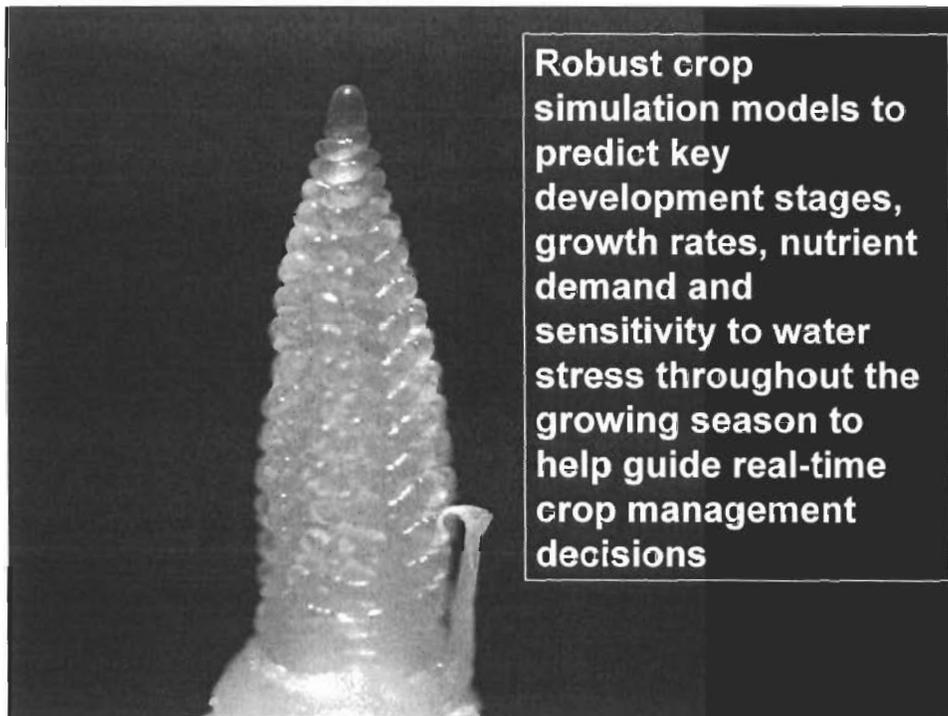
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- **Interdisciplinary, systems research**
    - agronomy, soil science, plant physiology/pathology/entomology, geology/hydrology, meteorology, conventional breeding and molecular genetics, computer science, engineering, animal science, economics and policy.,,,,,,
  - **Requires substantial funding—equivalent to support levels for genomics per FTE**
  - **Production- and landscape-scale research**
  - **An appropriate balance among simulation, validation, and measurement**
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27 Oct 2008

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31

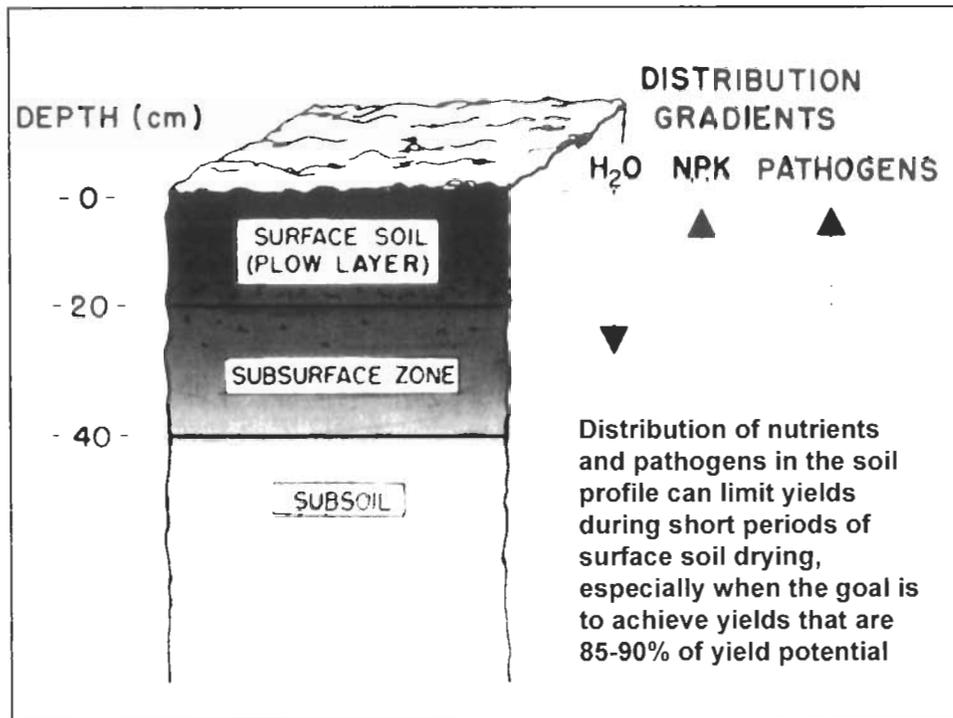


**Margin for error is razor-thin when attempting to produce crops near the yield potential ceiling----especially for N fertilizer management and for achieving a cost-effective balance of all essential nutrients in spatially variable fields**



**Nutrient-disease interactions: Severity of verticillium wilt on cotton is more severe in potassium-deficient plants; plants well-supplied with potassium have greater tolerance of verticillium wilt disease progression.**





## Energy Independence and Security Act of 2007

- Life-cycle assessment (LCA) of greenhouse gas (GHG) emissions:  
 “the aggregate quantity of GHG emissions (*including direct emissions and significant indirect emissions such as from land use changes*), related to the full fuel lifecycle, including all stages of fuel and feedstock production and distribution”
- Sets GHG emission reduction thresholds vs gasoline:
  - **Starch-ethanol (corn):** -20%
  - **Cellulosic ethanol:** -60%
  - **Advanced biofuels:** -50%
- Appropriate life-cycle methods and models will be established by the EPA by 2009

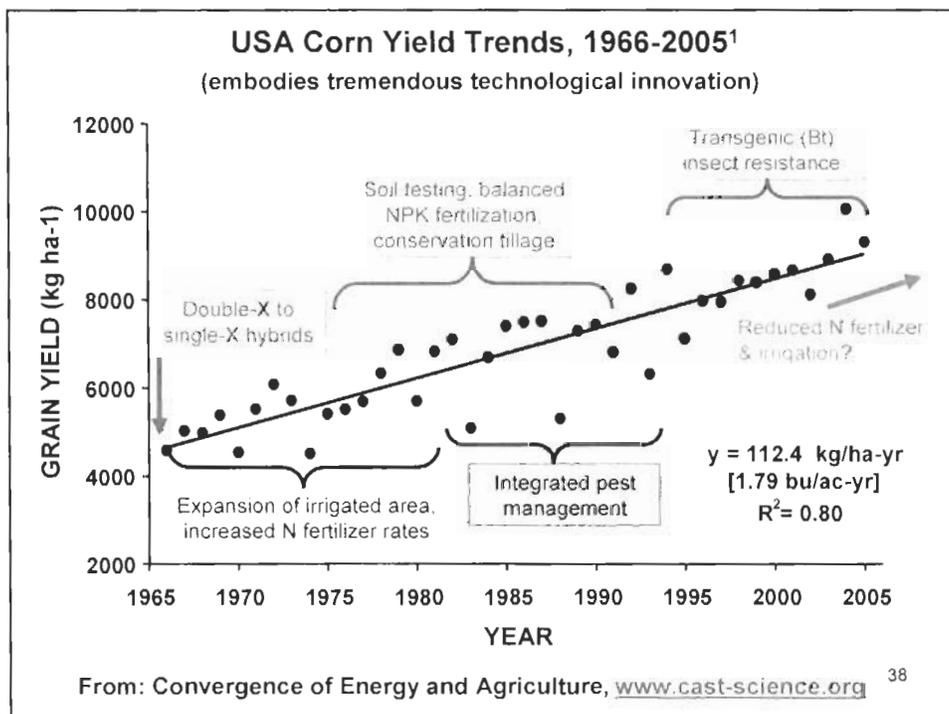
## Need to get corn-ethanol right

- **Rapid expansion of production capacity**
  - 60% of current capacity from plants that have come on line since January 2005; 75% by end of 2009
- **Direct-effect fossil fuel use and emissions can be obtained from updated data for crop production and biorefinery performance**
  - Important to use values consistent with industry performance as it currently functions: yields, inputs, energy use, DDG use
  - Exception: nitrogen losses (can use IPCC defaults)

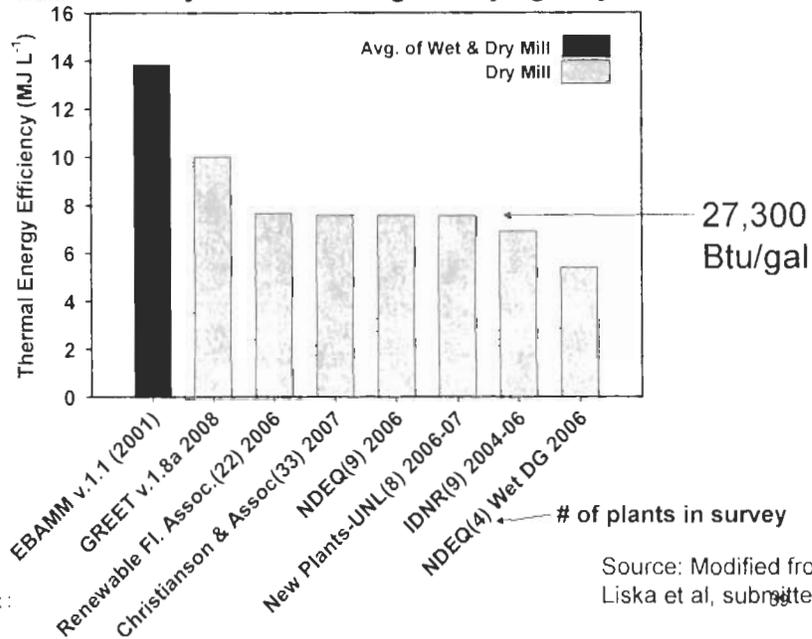
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37



**Previous biorefinery thermal efficiency estimates vs. recent surveys and state regulatory agency records**



**Corn ethanol co-product distillers grains are a nutritious livestock feed:**

- 30% CP(65% UIP), 0.8% P, 11% fat, 40% NDF
- High fiber energy source with high digestibility
- Energy content and feeding value ~125% (wet or dry) of corn; can replace 40% of beef cattle diets
- Sulfur content - .35 to 1.0%, variable



# Biofuel Energy Systems Simulator (BESS)

[available at: [www.bess.unl.edu](http://www.bess.unl.edu)]

- Most up to date estimates for direct-effect GHG emissions for corn ethanol based on best current science and input from all key disciplines (engineers, agronomists, soil scientists, animal nutritionists, industry professionals)
- User-friendly, transparent, and well documented
- Default scenarios based on state or regional-scale data, but can also be used for certification of an individual ethanol plant, its associated corn supply and co-product use
- Can be used for estimating carbon-offset credits for emissions trading with an individual ethanol plant as the aggregator
- BESS can be used for compliance and certification

27 Oct 2008

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41

The screenshot displays the BESS software interface. At the top, it says "Biofuel Energy Systems Simulator BESS N UNL". Below this are navigation tabs: "Input: Operation settings", "Output: Individual scenarios", "Output: Scenario comparison", and "Summary report". The "Input: Operation settings" tab is active. It contains several sections:

- Open a scenario:** A dropdown menu showing "US Midwest average UNL". A text box next to it contains "US Midwest, new dry-mill powered by natural gas, University of Nebraska survey".
- Scenario description (editable):** A text box with the same content as the previous one.
- Co-product selection:** Radio buttons for "Corn production", "Ethanol biorefinery", "Cattle feedlot", and "Biogasifier".
- Productivity:** Input fields for "Corn grain (dry matter), Mg/ha" (9.57) and "Soil C sequestration, Mg C/ha" (0).
- Material inputs:** Input fields for "Nitrogen, kg N/ha" (144), "Manure, kg N/ha" (5.5), "Phosphorus, kg P2O5/ha" (49.8), "Potassium, kg K2O/ha" (53.9), "Lime, kg/ha" (232), "Herbicides, kg/ha" (5.25), "Insecticides, kg/ha" (0.210), "Seed, kg/ha" (20.9), and "Irrigation water, cm" (4.98).
- Fuel consumption:** A section with a "By fuel type" radio button. It includes input fields for "Gasoline, L/ha" (15.6), "Diesel, L/ha" (67.3), "LPG, L/ha" (52.3), "Natural gas, m3/ha" (21.5), and "Electricity, kWh/ha" (105).
- By field operation:** A section with a "By field operation" radio button. It includes dropdown menus for "Diesel use by tillage type" (Chisel) and "Irrigation" (Well water). A text box below the irrigation dropdown says "Including planting, spraying, cultivation, & harvest".
- Depreciable capital energy, MWh/ha:** An input field with the value 328.
- Compute:** A large button on the right side of the interface.

At the bottom of the window, a small note reads: "All inputs and outputs refer to annual values."

**Inventory of GHG emissions from corn-ethanol life-cycle:**

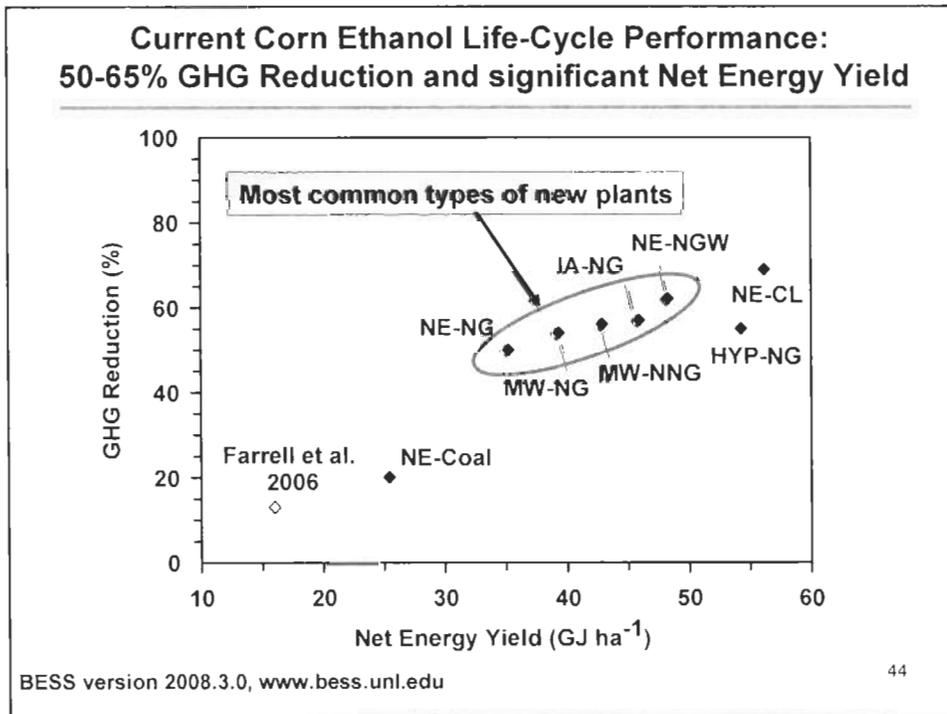
IA avg. natural gas biorefinery

N<sub>2</sub>O\* = 50% crop GHG emissions, 25% of life-cycle emissions

\*includes synthetic N, manure, crop residue, volatilization, leaching & runoff (IPCC 2006)

Component	GHG emission category	gCO <sub>2</sub> eq MJ <sup>-1</sup>	Mg CO <sub>2</sub> eq*	% of LC
<b>Crop Production</b>				
	Nitrogen fertilizer, N	4.20	33,614	7.37
	Phosphorus fertilizer, P	0.953	7,618	1.67
	Potassium fertilizer, K	0.542	4,337	0.951
	Lime	2.82	22,577	4.95
	Herbicides	1.51	12,079	2.65
	Insecticides	0.018	141	0.031
	Seed	0.193	1,540	0.338
	Gasoline	0.355	2,837	0.622
	Diesel	1.73	13,848	3.04
	LPG	1.24	9,916	2.17
	Natural gas	0	0	0
	Electricity	0.348	2,785	0.611
	Depreciable capital	0.268	2,144	0.470
	N emissions** -N <sub>2</sub> O	14.1	112,550	24.7
	<b>TOTAL</b>	<b>28.3</b>	<b>225,986</b>	<b>49.6</b>
<b>Biorefinery</b>				
	Natural Gas Input	19.7	157,356	34.5
	NG Input drying DG	0	0	0
	Electricity input	6.53	52,201	11.4
	Depreciable capital	0.458	3,663	0.803
	Grain transportation	2.11	16,851	3.69
	<b>TOTAL</b>	<b>28.8</b>	<b>230,071</b>	<b>50.4</b>
<b>Co-Product Credit</b>				
	Diesel	0.216	1,731	0.380
	Urea production	-5.10	-40,795	-8.95
	Corn production	-11.4	-91,311	-20.0
	Enteric fermentation-CH <sub>4</sub>	-2.64	-21,102	-4.63
	<b>TOTAL</b>	<b>-18.9</b>	<b>-151,476</b>	<b>-33.2</b>
	<b>EBAMM co-product credit</b>	<b>(-24.9)</b>	<b>(-198,975)</b>	<b>(-43.6)</b>
	Transportation of Ethanol from Biorefinery	1.40	11,196	2.46
	<b>LIFE-CYCLE NET EMISSIONS</b>	<b>39.5</b>	<b>315,777</b>	<b>70.0</b>
	GHG-intensity of ethanol, g CO <sub>2</sub> eq MJ <sup>-1</sup>	39.5	315,777	
	GHG-intensity of gasoline***, g CO <sub>2</sub> eq MJ <sup>-1</sup>	92.0	735,715	
	<b>GHG reduction relative to gasoline, %</b>	<b>52.5</b>	<b>419,938</b>	<b>57.1%</b>

BESS version 2008.3.0 Source: Liska et al, submitted



**Our Recommendation to California Air Resources Board\*:  
Create 3 classes of Ethanol Facilities for GHG Regulation**

- 1) Title V permitted facilities; major source, e.g. 100 tons VOC/yr (includes all wet mills and coal powered facilities in Nebraska and Iowa, 9 out of 31 facilities in 2006)
- 2) Dry mills using natural gas (largest group)
- 3) Dry mills using natural gas, with advanced efficiencies (e.g. high cattle densities, closed-loop facilities, DG as energy source)

Class	I	II	III
Description	Title V (coal with dry DG)	Natural Gas dry mills, dry DG	Natural gas dry Mills, wet DG
Thermal Energy, MJ L-1	12.81	7.61	5.44
BESS Life-cycle GHG emissions reduction	7%	51%	62%

27 Oct 2008

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45

\*March 26, 2008 memo to CARB

**Most sensitive input parameters on GHG emissions reductions & net energy yield of corn-ethanol**

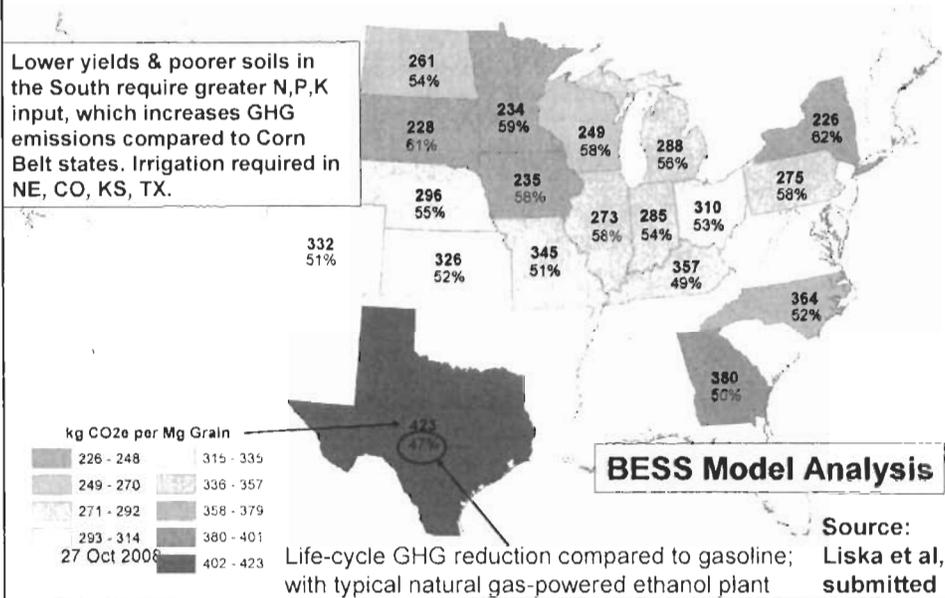
1. Crop yield and nitrogen fertilizer efficiency
2. Biorefinery thermal energy inputs: MJ per liter (e.g. wet vs. dry distillers grains)
3. Conversion yield: liters ethanol per kg grain
4. Biorefinery electricity use

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46

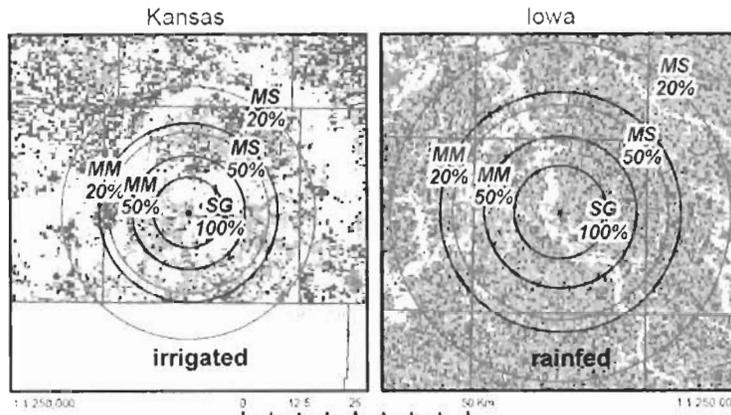
**Regional Variability in GHG-Intensity of Corn Production and Life-Cycle GHG Reductions from Corn-Ethanol Assuming a New Nat. Gas Biorefinery**



**Challenges to large-scale development of the cellulosic biofuels industry**

- Harvest, handling, storage of huge amounts of biomass
- More cost-effective pretreatment and enzyme technologies
  - Can they utilize multiple feedstock sources?
- Improved options for use of co-products
  - Feedstock for industrial chemicals?
- Large-scale deployment (>1 billion gallon/yr) is 7-10 years off
  - Meantime, biofuel production capacity builds out until prices of maize, sugarcane, and oil palm reach breakeven point as a biofuel feedstock

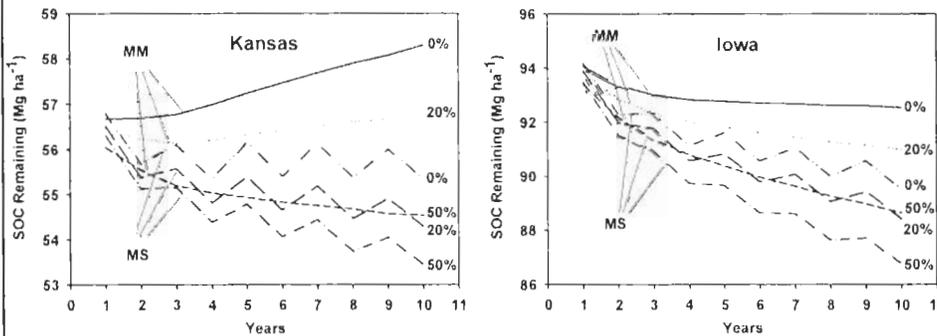
**Cellulosic Ethanol Life-Cycle Assessment:  
Biomass Cultivation Area for Switchgrass or Maize Crop  
Residue, with Removal Levels & Crop Rotations**



Maize acres (yellow), soybean (green), wheat and sorghum (brown), other crops (gray), non-crop acres (white), and water (blue); SG 100%, switchgrass complete harvest; MS 50% and MS 20%, maize-soybean rotation, with either 50% or 20% maize residue removal, respectively; MM 50% and MM 20%, continuous maize with either 50% or 20% residue removal.

Source: BESS-Cellulosic ethanol, BETA version

**Loss of Soil Organic Carbon (SOC) under  
Continuous Maize (MM) and Maize-Soybean (MS)  
Rotation with Differing Residue Removal Levels  
for Cellulosic Ethanol Production**



Soil C trends estimated using D-K Model

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50

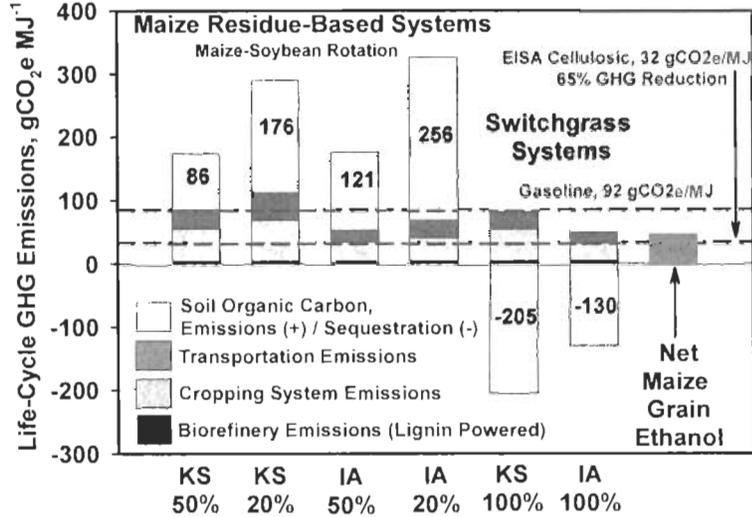
Source: unpublished data, manuscript in progress

## Cellulosic Ethanol Life-Cycle GHG Emissions

in Kansas (KS) and Iowa (IA):

NET gCO<sub>2</sub>e MJ<sub>e</sub><sup>-1</sup>:

175 291 177 328 -122 -79 45



27

Cellulosic Ethanol Systems

Source: unpublished data, manuscript in progress

indirect land use change not considered

## Conclusions

- We must plan to meet food and energy demand of 9 billion people (much wealthier on average than today) by ~2040
  - Will require ~75% more food production and 2-3x more energy use even with major efforts to improve energy efficiency and conservation
- It is possible to develop biofuel systems that contribute to reduced demand for imported oil and mitigate GHG emissions without sacrificing food security
  - Corn-ethanol has potential to be a component, but only if the food vs fuel trade-off can be avoided
- Current USA & global research portfolio will not get us there, neither for corn or other crops, without an explicit focus on accelerating crop yield gains on existing farmland while reducing the environmental footprint of agriculture
- **Ecological intensification** of agricultural is the only means to achieve food security, expanded biofuel-bioproduct production, and protection of ecosystem services
- For cellulosic ethanol, yield density determines economic viability, soil C sequestration is key for environmental sustainability

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52

## Final Conclusions

- **Developing effective environmental policies, regulation, and incentive framework depends on projections of future environmental impact under different scenarios**
- **Unfortunately, the balance between research investment in developing simulation models without adequate underpinning investment in measurement and monitoring of driving forces and environmental indicators can lead to huge differences in estimates of current and future environmental impact**
  - Soil carbon sequestration or loss
  - Impact of climate change on crop yields
  - Nitrous oxide emissions from agriculture
  - Nr deposition rates and emissions from agriculture
  - N fertilizer use efficiency of major crops and future biofuel crops

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53

## Citations

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- Yang Y., Dobermann A., Cassman K.G., and Walters D.T. 2006. Features, Applications, and Limitations of the Hybrid-Maize Simulation Model. *Agron. J.* 98:737-748; Hybrid-Maize Simulation Model: [www.hyridmaize.unl.edu](http://www.hyridmaize.unl.edu)

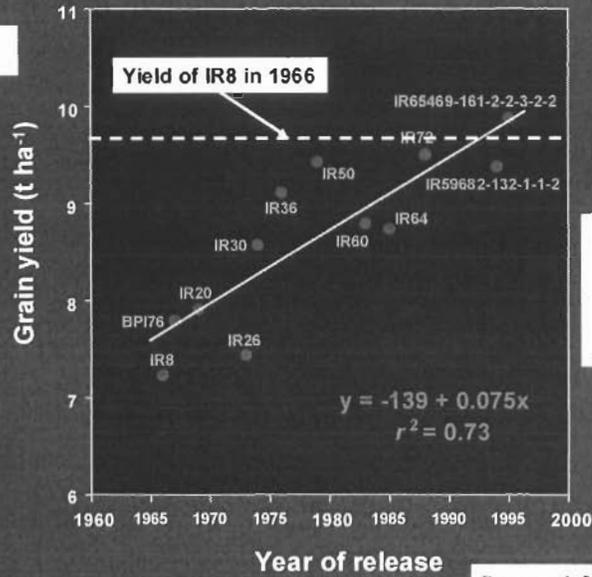
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54

## Yield trend of IRRI cultivars and lines developed since 1966

**RICE**

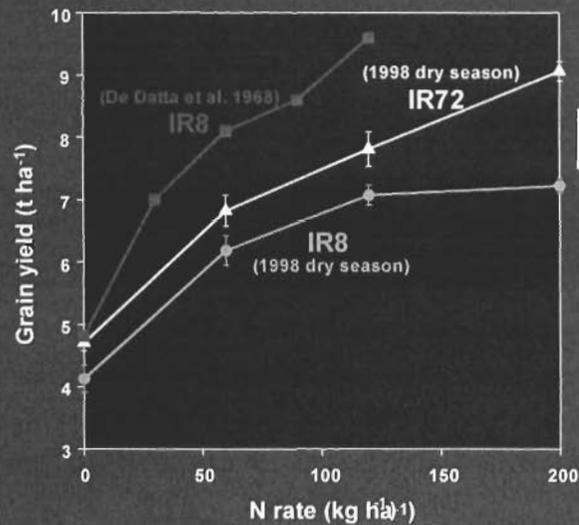


17 October 2007

152nd BIFAD meeting

Peng et al. 2000; Crop Sci 40:307

## Grain yield of IR8 grown in the late 60s and 1998

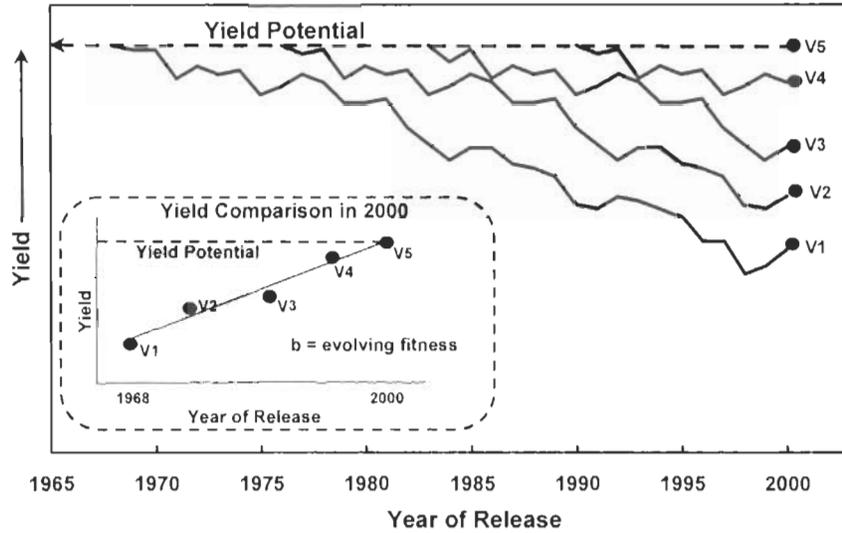


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46

**Conceptual framework for stagnant yield potential and red-queen breeding to maintain disease/insect resistance and adaptation to evolving agro-ecosystems (soils, [CO<sub>2</sub>], climate change)**



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57

From: Cassman et al., 2003, ARER

## **Dr. G. David Tilman**

### **University of Minnesota**

Dr. G. David Tilman is Regents Professor of Ecology and holds the McKnight University Presidential Chair in Ecology at the University of Minnesota. He is an experimental and mathematical ecologist studying the impacts of the loss of biological diversity and of other types of human-driven global change on the functioning and stability of ecosystems and on the services that ecosystems provide society. David Tilman is deeply interested in the interface of science, society, ethics and environmental policy. He has given expert testimony to committees of the US Senate and House and to the White House's Office of Management and Budget, has had his scientific findings on biodiversity added to the Congressional Record by a member of congress, and given invited briefings to the Minnesota House and Senate. He has served on scientific advisory committees for the White House (the Biodiversity and Ecosystems Panel of the President's Committee of Advisors on Science and Technology), for Public Radio International's The World, and for the National Academy of Sciences (Board on Environmental Studies and Toxicology). In 1996 he founded a new publication, *Issues in Ecology*, to foster communication among ecologists, the public and governmental decision makers. He served as its Editor-in-Chief for eight years. He has also served on the editorial boards of scientific publications including *Science*, *Proceedings of the National Academy of Science*, and *Ecology*. Honors include selection as a Guggenheim Fellow, and election as a Fellow of the American Association for the Advancement of Science, as a Fellow of the American Academy of Arts and Sciences and as a member of the National Academy of Science. Prizes and awards include Sweden's Per Brink Award, Pew Scholar in Conservation Biology, and the Ecological Society of America's Cooper Award and MacArthur Award. In 2001 he was designated the most highly cited environmental scientist for the decade by the Institute for Scientific Information, an honor he also received in 2003 and 2005 for the decades from 1992-2002 and 1995-2005. After earning his Ph. D. at the University of Michigan in 1976, Dr. Tilman has spent his academic career at the University of Minnesota, but also has served as a Member of Princeton's Institute for Advanced Study, a Senior Visiting Fellow at Princeton University, and a Fellow of the National Center for Ecological Analysis and Synthesis.

## *Lifecycle Environmental and Health Costs and Benefits of Fossil and Renewable Fuels*

by David Tilman, University of Minnesota\*

Negative environmental and health consequences of fossil fuels and concerns about petroleum supplies have spurred the search for renewable transportation biofuels. To be a viable alternative, a biofuel should provide, in total across its full lifecycle, net energy gains and environmental benefits, be economically competitive, and be producible in large quantities without reducing food supplies. We use these criteria to evaluate, through life-cycle accounting, ethanol from corn grain, biodiesel from soybeans and cellulosic biofuels derived from alternative crops transformed into biofuels via either biochemical or thermochemical processes.

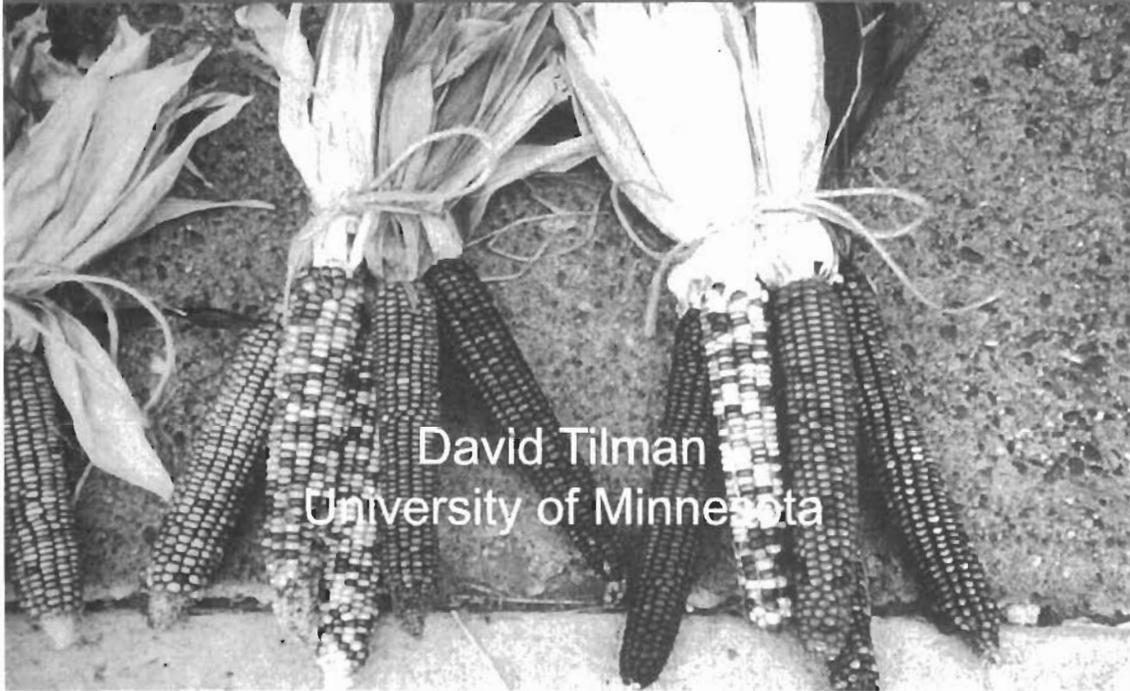
Corn ethanol yields 25% more energy than the energy invested in its production, whereas soybean biodiesel yields 93% more. Compared with ethanol, biodiesel releases just 1.0%, 8.3%, and 13% of the agricultural nitrogen, phosphorus, and pesticide pollutants, respectively, per net energy gain. Relative to the fossil fuels they displace, greenhouse gas emissions are reduced 12% by the production and combustion of ethanol and 41% by biodiesel. Biodiesel also releases less air pollutants per net energy gain than ethanol. These advantages of biodiesel over ethanol come from lower agricultural inputs and more efficient conversion of feedstocks to fuel. Neither corn ethanol nor soybean biodiesel can replace much petroleum without greatly impacting food supplies. Even dedicating the full 2005 U.S. corn and soybean crops to biofuels would meet only 12% of gasoline demand and 6% of diesel demand. Because of fossil energy needed to produce these crops and convert them to biofuels, the net energy gain from converting all US corn and soybeans to biofuels for each would only be 3% of current gasoline and diesel energy use.

Whether or not a given biofuel offers carbon savings and other environmental benefits relative to a fossil fuel depends on how the biomass crop is produced. Converting rainforests, peatlands, savannas, or grasslands to cropland to produce food-based biofuels in Brazil, Southeast Asia, and the United States creates a 'biofuel carbon debt' by releasing 17 to 420 times more CO<sub>2</sub> than the annual greenhouse gas (GHG) reductions these biofuels provide by displacing fossil fuels. In contrast, biofuels made from waste biomass or from biomass grown on abandoned agricultural lands planted with perennials incur little or no carbon debt and offer immediate and sustained GHG advantages. If grown with low inputs of agrichemicals, they also offer potentially great increases in the quality of surface and ground waters.

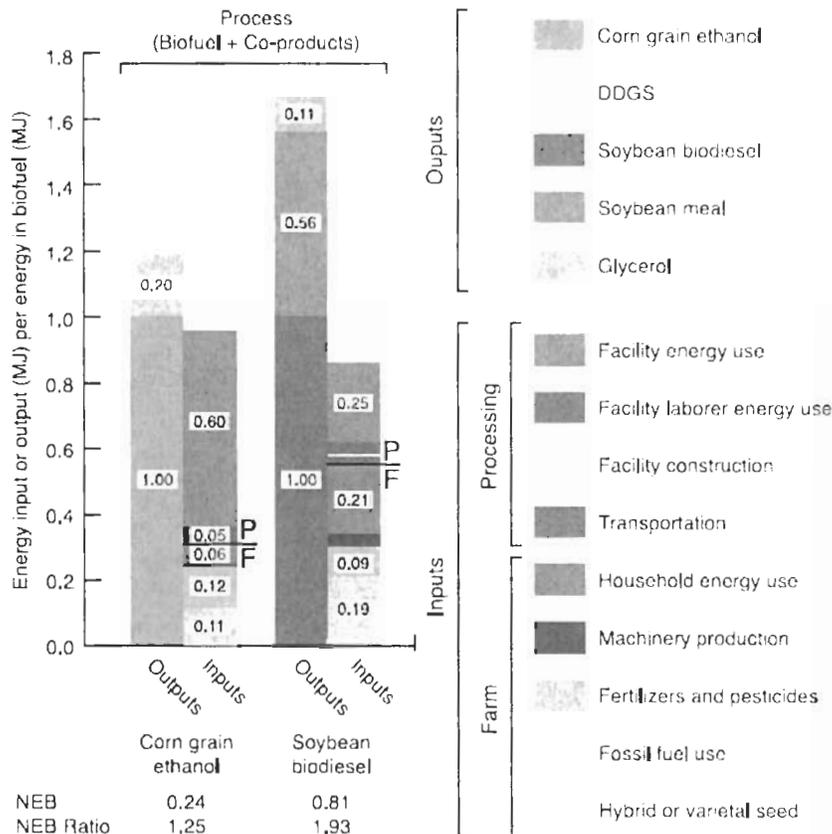
Fine particulate matter (PM<sub>2.5</sub>) emissions from fossil fuels and biofuels, which can potentially impose large health costs on society, are another environmental concern that must be used in evaluating alternative energy sources. By using the EPA's RSM and BenMAP analytical tools on a county-by-county basis for the US, we quantified and then monetized the lifecycle climate and health effects of greenhouse gas (GHG) and fine particulate matter (PM<sub>2.5</sub>) emissions from gasoline, corn ethanol, and cellulosic ethanol, we found that, for each billion ethanol-equivalent gallons of fuel produced and combusted in the US, climate and health costs are about \$500 million for gasoline, about \$600–1000 million for corn ethanol depending on biorefinery heat source (natural gas, coal, or corn stover), but only \$100–200 million for cellulosic ethanol depending on feedstock (corn stover, switchgrass, prairie biomass, or *Miscanthus*). Moreover, a spatially-explicit lifecycle analysis that tracked PM<sub>2.5</sub> emissions and exposure relative to US population shows regional shifts in health costs dependent upon fuel production systems. Because climate and PM<sub>2.5</sub> health costs are roughly equal, the total monetized benefit of shifting from gasoline to properly-produced cellulosic biofuels is twice as large as when only GHG benefits are considered.

\*Based on collaborative projects with J. Hill, S. Polasky, E. Nelson, H. Huo, L. Ludwig, D. Bonta, D. Tiffany, J. Neumann, H. Zheng, J. Fargione, and P. Hawthorne

# Environmental Impacts of Food versus Cellulose-Based Biofuels



David Tilman  
University of Minnesota

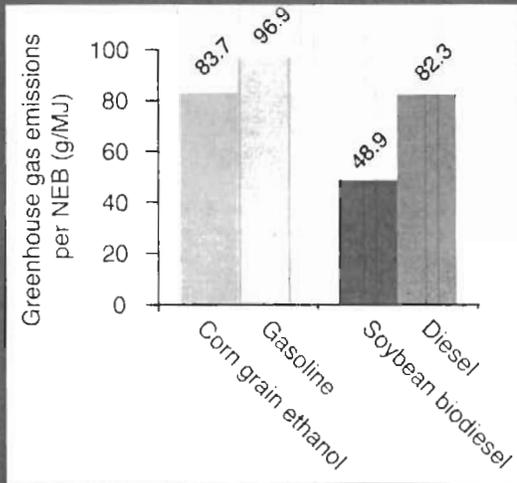


Lifecycle  
Energy  
Inputs  
And  
Outputs  
For Corn  
Ethanol  
&  
Soybean  
Biodiesel  
(Hill et al. 2006)

# Lifecycle Emissions

(relative to fossil counterpart; H=Higher; L = Lower)

- Greenhouse gasses



- Criteria pollutants

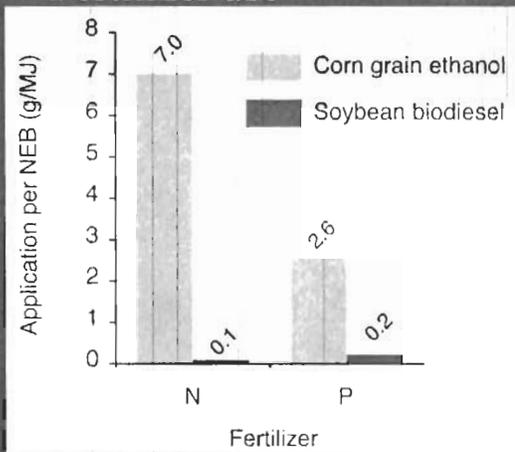
	VOC	CO	PM 10	SO <sub>x</sub>	NO <sub>x</sub>
Corn grain ethanol	H	H	H	H	H
Soybean biodiesel	L	L	L	L	H

**Corn Ethanol:  
14% less GHG than Gasoline**

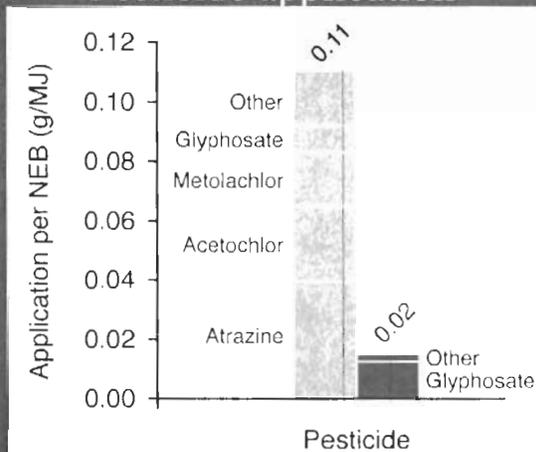
(Hill et al. 2006)

## Environmental Effects of Corn Ethanol and Soybean Biodiesel

- Fertilizer use



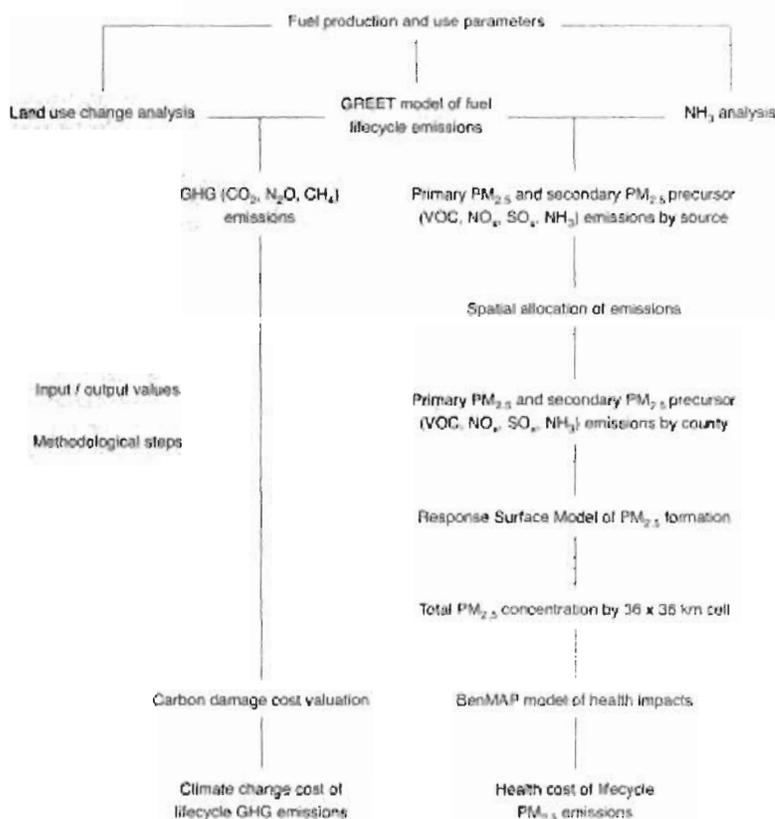
- Pesticide application



Much of N, P and pesticide enter surface and ground waters  
Increased corn from irrigation uses 5000 gallons of water for each gallon of ethanol made

# Potential of US Food-Based Biofuels

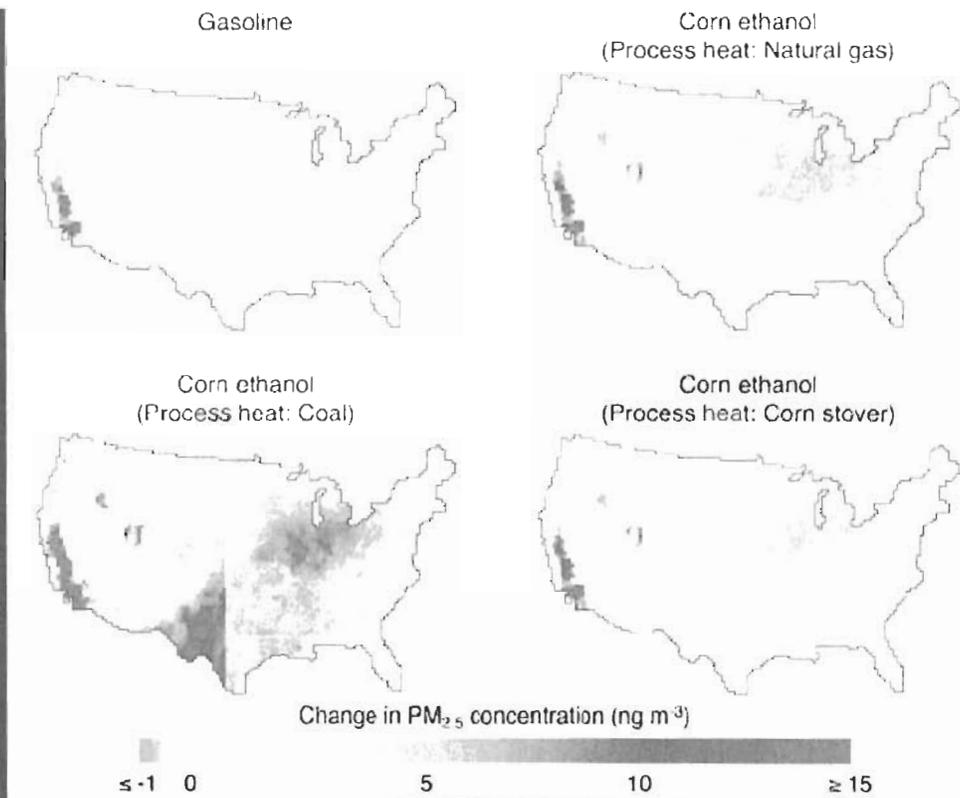
	<b>Entire 2005 US crop to biofuel</b>
Corn ethanol	12% of gasoline <b>2.5% Energy Gain</b>
Soybean biodiesel	6% of diesel <b>3% Energy Gain</b>



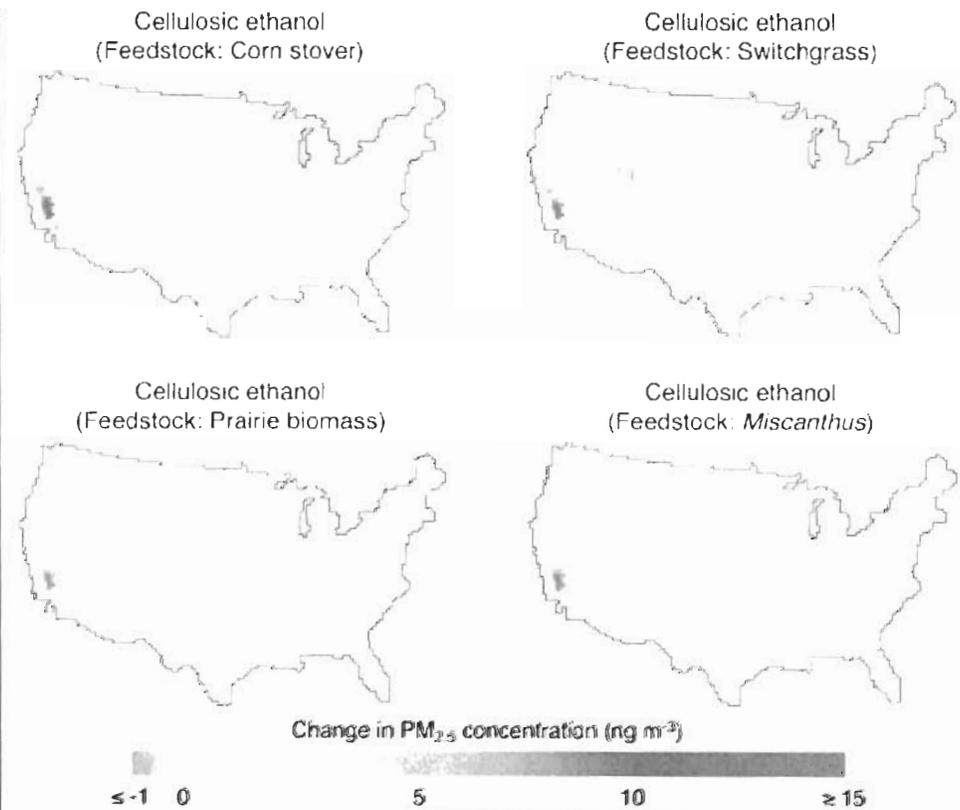
Lifecycle Health and CO<sub>2</sub> Costs Of Alternative Biofuels:

Ethanol from Corn or from Perennial Grasses or from Corn Stover

(Hill et al., in review)

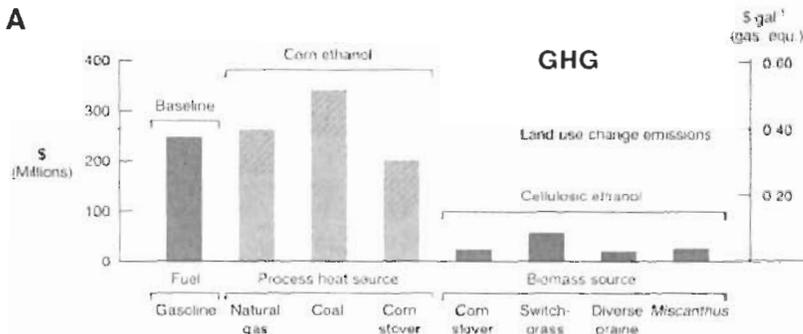


(Hill et al., in review)

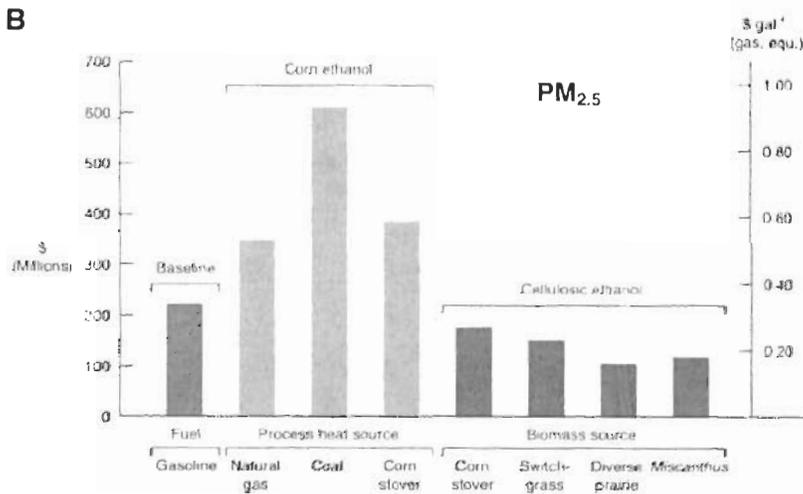


(Hill et al., in review)

A



B

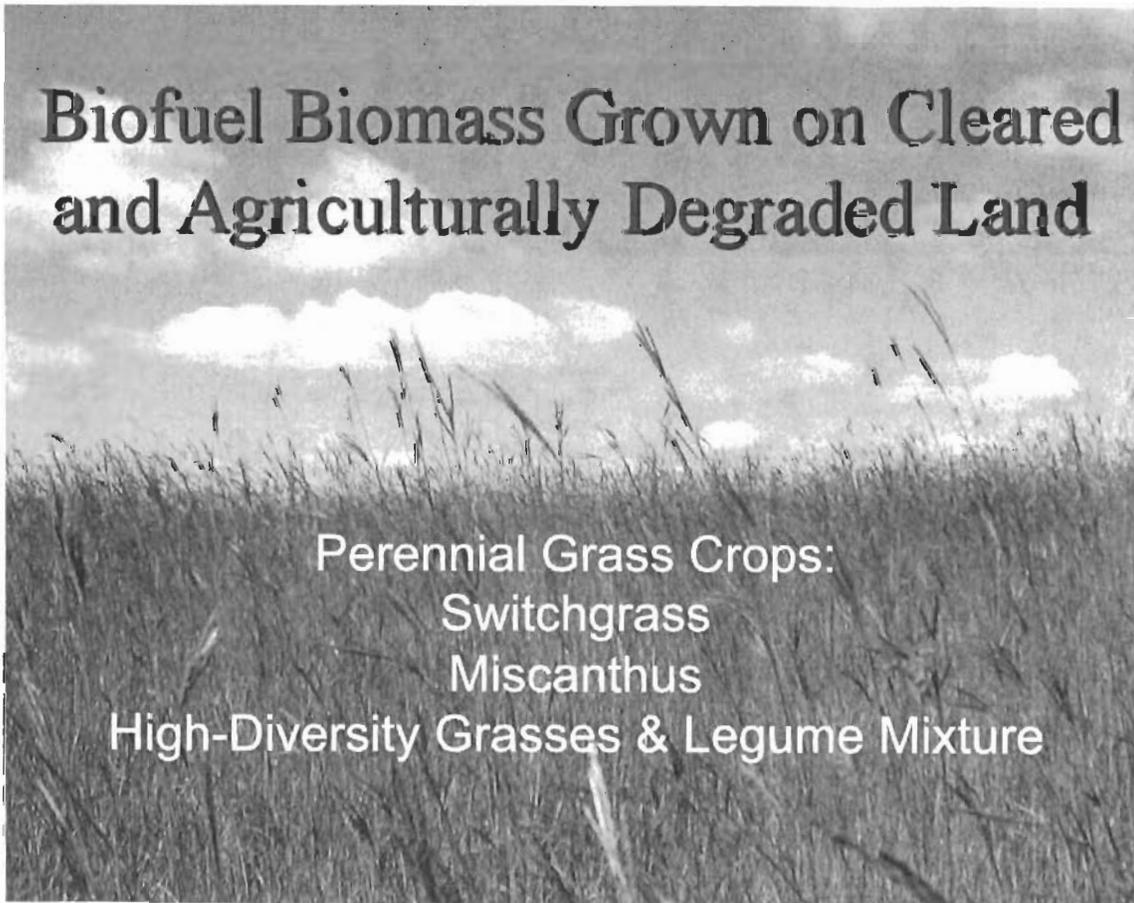


**GHG Impacts Of Corn Ethanol Are Similar To Gasoline**

**PM-2.5 Health Impacts of Corn Ethanol Are Higher Than for Gasoline**

**Cellulosic Fuels Offer Major Benefits**

## Biofuel Biomass Grown on Cleared and Agriculturally Degraded Land

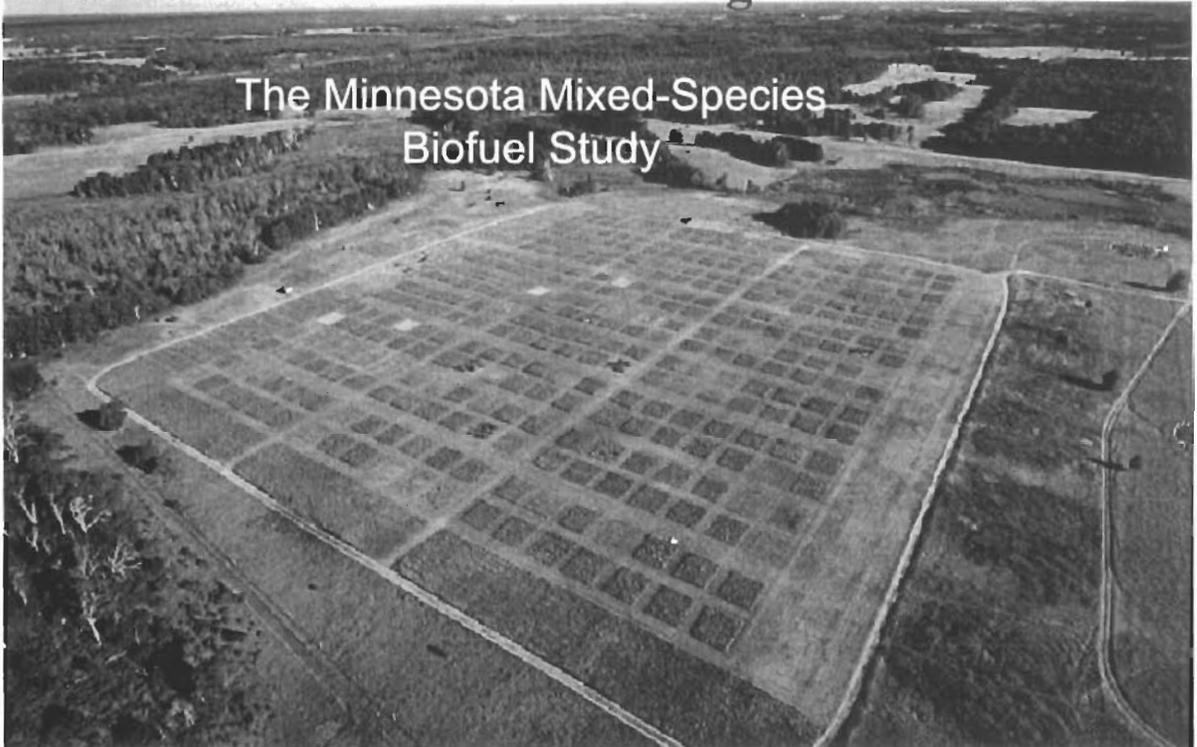


Perennial Grass Crops:  
Switchgrass  
Miscanthus

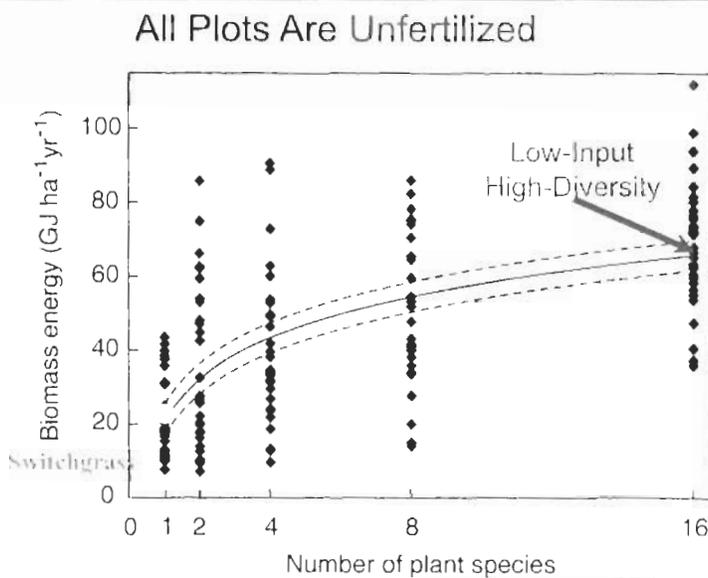
High-Diversity Grasses & Legume Mixture

# Biofuels from High-Diversity Mixtures of Native Grasses Grown on Degraded Lands

The Minnesota Mixed-Species Biofuel Study



## High Diversity Grasslands Produced 238% More Biofuel Than Monocultures



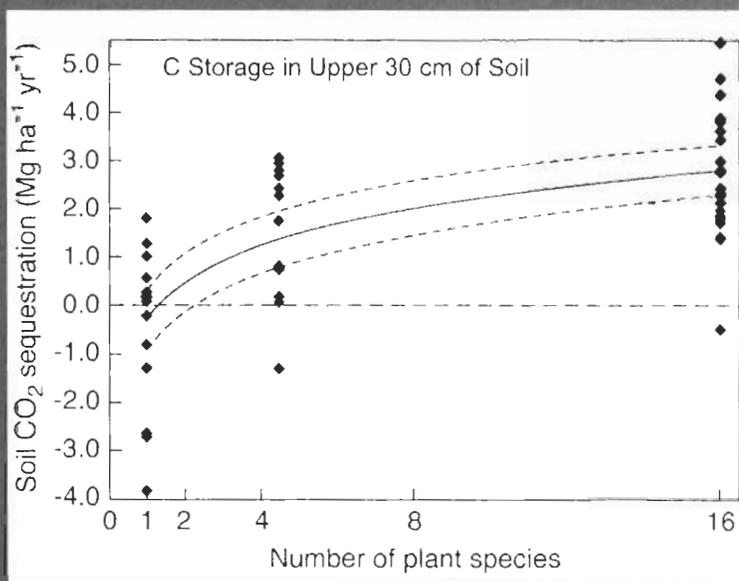
Effects of diversity came from legumes that fixed N and perennial warm-season grasses that efficiently used converted this N to make biomass

(Tilman et al. 2006 *Science*)

## Soil Is A Major Carbon Sink

- Small changes in soil carbon storage can have a large impact on atmospheric carbon dioxide levels.
- The carbon stored in the world's soils is about 1,400 billion tons.
- This is more than twice the carbon in trees and other plants (560 billion tons).
- This is about twice that in the atmosphere (750 billion tons as carbon dioxide).

## Diverse Prairie Stores More C in Soil



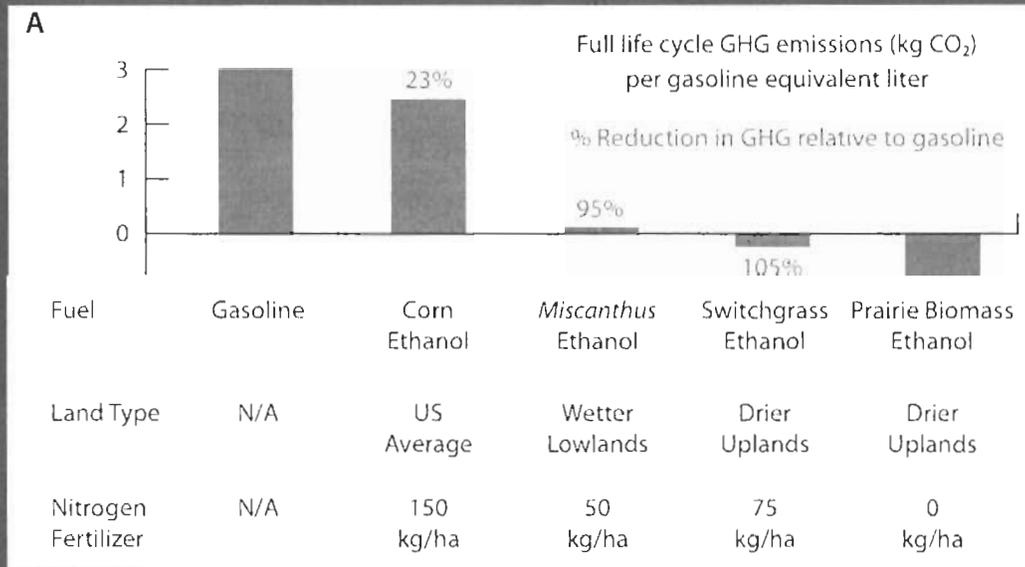
High-Diversity  
Prairie Biofuels  
Are Carbon  
Negative

4.4 t/ha CO<sub>2</sub> Storage in  
Soil (0-100 cm depth)  
and Perennial Roots;  
0.3 t/ha Fossil CO<sub>2</sub>  
Released to  
Produce Biofuel

Net Sequestration of 4.1 t/ha of CO<sub>2</sub> (1.8 tons/acre)  
After Biofuel Production and Use

(Tilman et al. 2006 *Science*)

## Greenhouse Gas Reductions for Next Generation Biofuels Based on GREET Analyses Using Latest Data (US Average Data for Corn Yields & Inputs)



Each Biomass Crop Will Have Its Own Optimal Conditions for Growth

## US Biofuel Potential?

Residual ('Waste') Biomass And  
Dedicated Plantings Of Switchgrass, Diverse  
Mixed Prairie And Other Perennials Can Give  
Sustainable Liquid Fuel Yields Of  
~30 Billion Gallons Per Year Of Ethanol  
That Exceed the GHG  
Benefits Mandated in 2007 EISA  
(giving GHG reductions  
~75% to 100% less than gasoline)

## Biofuels and Greenhouse Gas Benefits

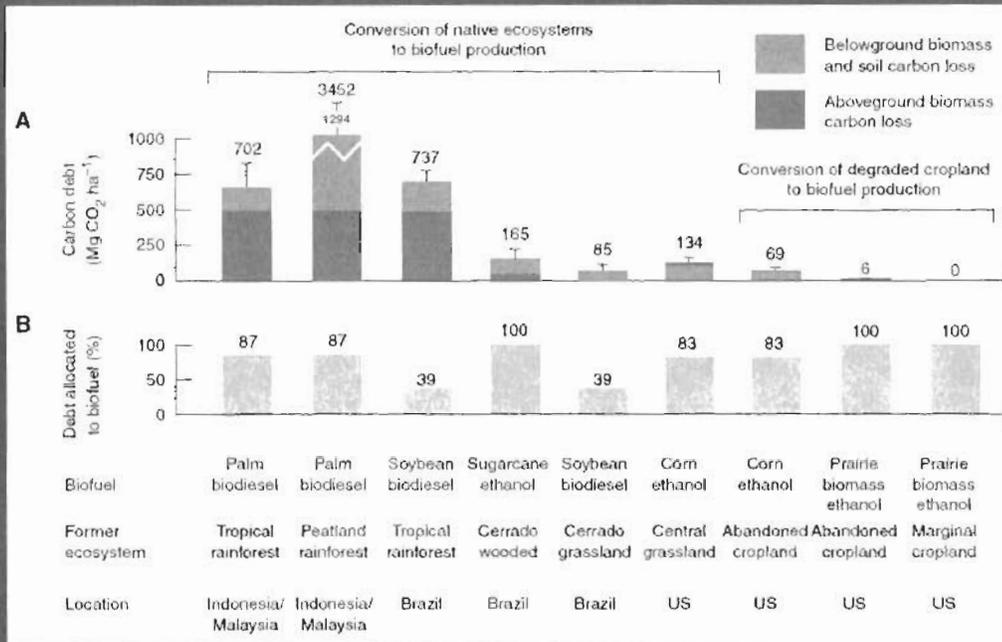
If properly produced, biofuels can provide major greenhouse gas benefits relative to gasoline and other fossil fuels.

But , the direct or indirect clearing of land to grow biofuel crops can release immense amounts of carbon dioxide



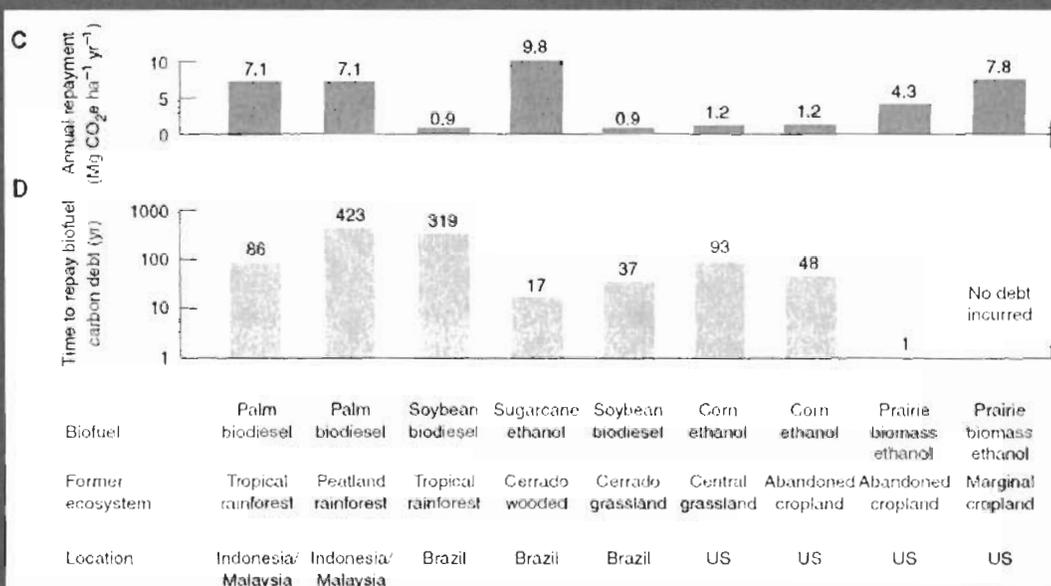
**Direct Land Clearing for Biofuel  
Biomass Production:  
Most New Land from Clearing  
Tropical Forest & Savanna**

## Greenhouse Gas Release (as CO<sub>2</sub> equivalents) from Land Clearing for Biofuel



(Fargione et al. 2008)

## Greenhouse Gas Repayment Rates and Times for Various Biofuels



# Food Crops for Biofuels?

- 50% of US corn crop is used to feed livestock
- Remainder is exported, processed for human consumption, or converted to ethanol
- Soybean oil
- constitutes 80%
- of US edible oil consumption



## Indirect Land Use Change

Diverting Croplands to Biofuel Crops

### Use of U.S. Croplands for Biofuels Increases Greenhouse Gases Through Emissions from Land-Use Change

Timothy Searchinger,<sup>1\*</sup> Ralph Heimlich,<sup>2</sup> R. A. Houghton,<sup>3</sup> Fengxia Dong,<sup>4</sup> Amani Elobeid,<sup>4</sup> Jacinto Fabiosa,<sup>4</sup> Simla Tokgoz,<sup>4</sup> Dermot Hayes,<sup>4</sup> Tun-Hsiang Yu<sup>4</sup>

Most prior studies have found that substituting biofuels for gasoline will reduce greenhouse gases because biofuels sequester carbon through the growth of the feedstock. These analyses have failed to count the carbon emissions that occur as farmers worldwide respond to higher prices and convert forest and grassland to new cropland to replace the grain (or cropland) diverted to biofuels. By using a worldwide agricultural model to estimate emissions from land-use change, we found that corn-based ethanol, instead of producing a 20% savings, nearly doubles greenhouse emissions over 30 years and increases greenhouse gases for 167 years. Biofuels from switchgrass, if grown on U.S. corn lands, increase emissions by 50%. This result raises concerns about large biofuel mandates and highlights the value of using waste products.

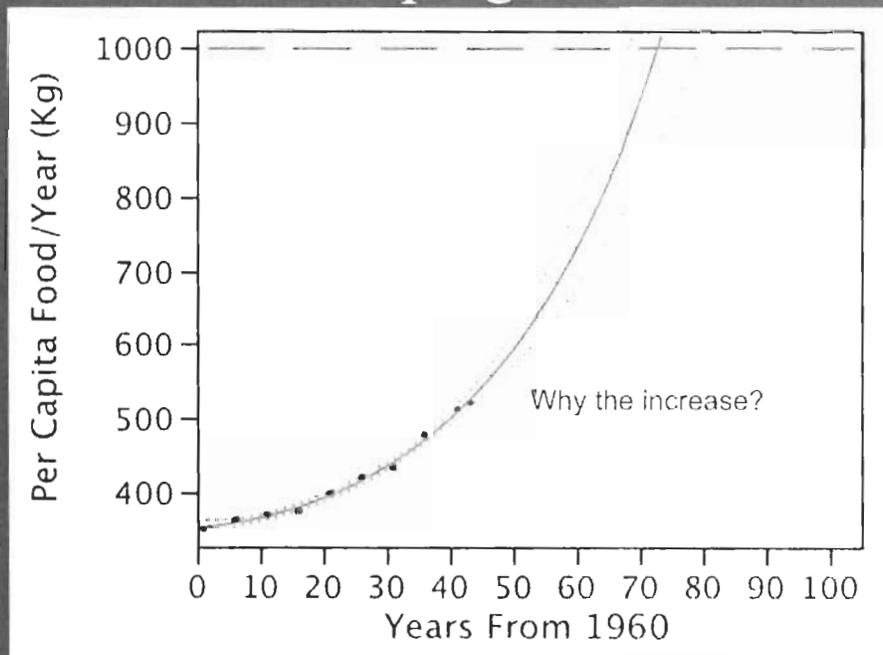
# Food is an International Commodity

All nations of the world are linked via agricultural trade

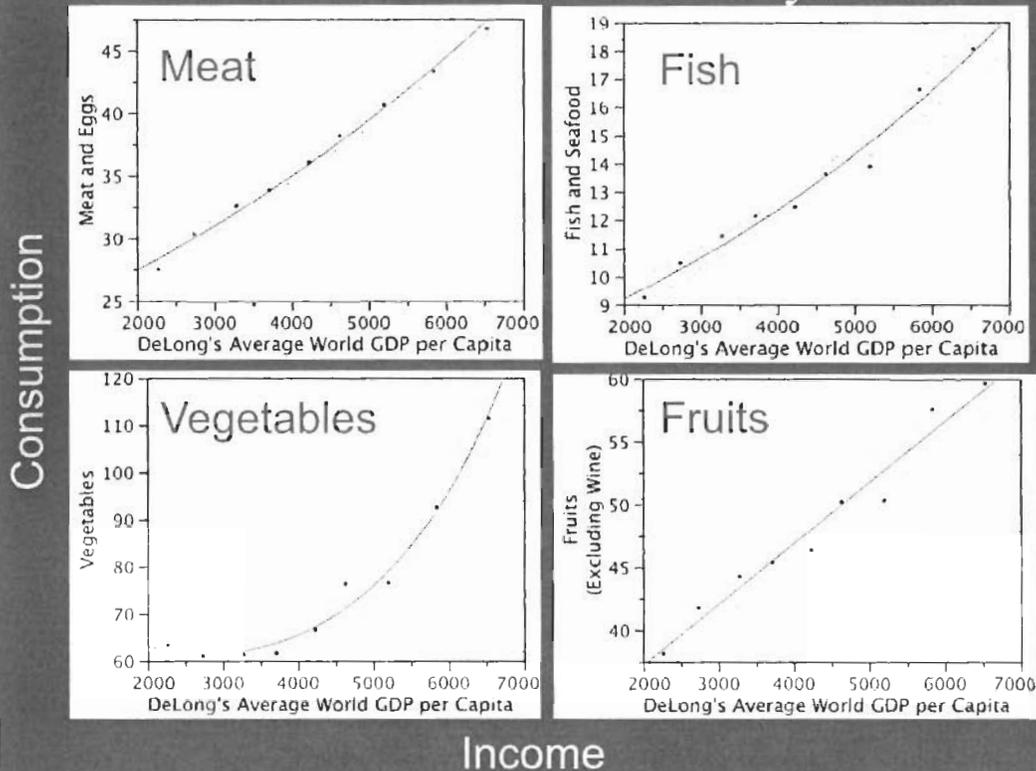
Food & fertile agricultural land diverted to biofuels in one nation impact its own food supply and that of other nations

FAPRI and other general equilibrium agricultural models

## Per Capita Food Consumption in Developing Countries



## Income and Global Dietary Shifts



## Future Global Food Demand

Based on projected global increases in population and per capita incomes, and on observed dietary shifts with income, total global food demand would increase 120% to 170% in 50 years

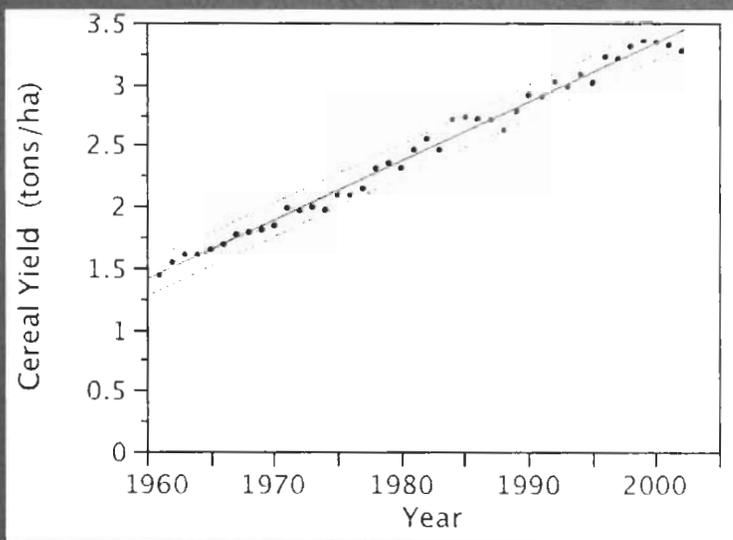
## *Increase Yield or Land?*

$$\text{Production} = \text{Yield} \cdot \text{Land Area}$$

[tons/hectare • hectares]

Environmental Impacts of Global  
Food Production at 120% to 170%  
More Than Current Levels

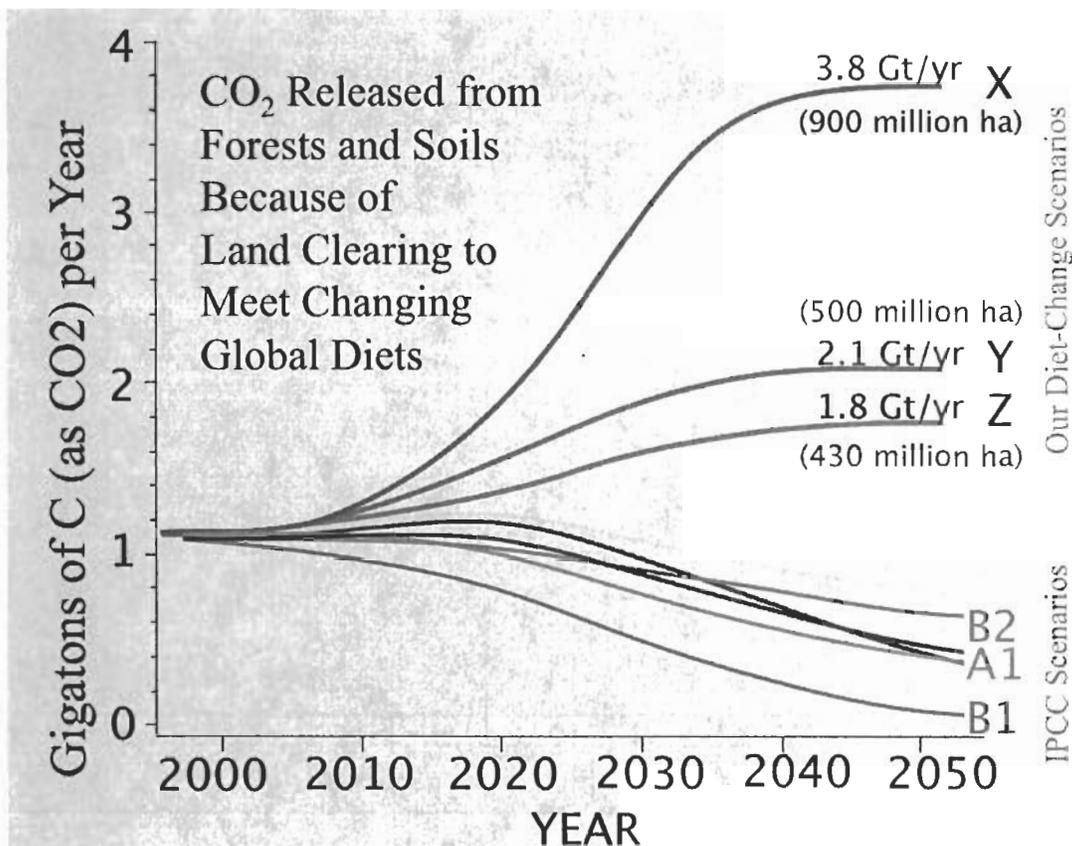
## Global Cereal Yield Trends



If This Rate Of  
Yield Gain Could  
Be Maintained For  
50 More Years,  
Global Cereal  
Yields Would  
Increase By 70%

For a Weighted Mix of All Major Crops Combined, Global  
Yields Are on Trajectory to Increase 66% in 50 Years

With These Projected Yield  
Increases, Food Production  
Increase of 120% to 170%  
Also Would Require from 35% to  
65% More Crop Land  
(~500 to 950 million hectares)  
And, about 540 More Million  
Hectares of Pasture Land for Meat/  
Dairy Production



## Meat and Dairy Greenhouse Gas Loading by 2050

If current per capita meat and dairy consumption trends in the developed, developing and least developed nations continue, methane and nitrous oxide from livestock would have a GHG equivalence of about 3 gigatons/year of C emissions.

## **Biofuels Have the Potential to be Beneficial or Harmful**

To Assure that Domestic or Imported Biofuels are Beneficial, there must be a Full Lifecycle Analysis and a Certifiable and Auditable Documentation Trail of this Lifecycle

## Dr. Christopher Field

### Carnegie Institution

Dr. Christopher Field is the director of the Carnegie Institution's Department of Global Ecology and professor by courtesy in the Department of Biological Sciences at Stanford University. Trained as an ecologist, Chris has conducted environmental research from tropical rainforests to deserts to alpine tundra in the Americas, Asia, Africa, and Australia. He is a specialist in global-change research. He has developed an evolutionary approach to understanding the spatial organization of plant canopies and the adaptive significance of leaf aging. These studies led to work on the role of nitrogen in regulating plant growth and photosynthesis. They also suggested ways that plant physiological responses could be summarized with a few parameters, providing a basis for predicting many aspects of ecosystem function at very large scales. Recently, he has emphasized formalizing approaches for summarizing plant responses into models that simulate ecosystem exchanges of carbon, water, and energy at the global scale. These models, which synthesize surface data on climate and soils, satellite data on vegetation type and canopy development, and functional generalizations from physiology and ecology, help test hypotheses and understand the future status of terrestrial ecosystems, especially responses to and influences on global change factors like increased atmospheric carbon dioxide or altered climate. Field is active in developing the international community of global change researchers, with involvement in organizations like SCOPE, IGBP, and the Global Carbon Project. An author of more than 100 scientific papers, he is a member of the US National Academy of Sciences and a leader in several national and international efforts to provide the scientific foundation for a **sustainable future**.

Biofuels potential: The climate protective domain  
Chris Field  
Department of Global Ecology  
Carnegie Institution for Science

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[www.dge.ciw.edu](http://www.dge.ciw.edu)

- Biofuels are the only currently viable option for powering the world's existing vehicle fleet, using fuels that potentially release less CO<sub>2</sub> than gasoline or diesel.
- Combined with geological storage, biofuels represent one of the few options for an energy source with negative CO<sub>2</sub> emissions, one that leads to a net decrease in atmospheric CO<sub>2</sub>
- Many countries are investing in large biofuels programs, motivated by concerns over global change, energy security, and rural development.
- Liquid biofuels already provide some developing and developed countries with a local renewable energy resource and jobs for rural populations.
- There are many ways to do biofuels wrong, so that the costs in damage to the environment or to human well-being exceed the benefits, but there are also some ways to do biofuels right.
- Current crops used to produce liquid biofuels are all food crops. With these crops, increasing the fraction allocated to biofuels can decrease the availability of food, and increasing the area can lead to loss of natural ecosystems rich in biodiversity or carbon stocks.
- Biofuels from waste, from crops grown with a focus on improving marginal or abandoned land, and from diverse natural ecosystems have the potential for net benefits in terms of climate, energy security, and rural development, with low or no costs in environmental degradation or human well-being
- Global production and use of liquid biofuels have tripled since 2000, with much more to come if current policy targets are implemented. With larger and larger levels of production, it becomes increasingly difficult to successfully manage environmental impacts.

#### Suggested reading

- Fargione, J., J. Hill, D. Tilman, S. Polasky, and P. Hawthorne. 2008. Land Clearing and the Biofuel Carbon Debt. *Science* **319**:1235.
- Field, C. B., J. E. Campbell, and D. B. Lobell. 2008. Biomass energy: the scale of the potential resource. *Trends in Ecology & Evolution*.
- Gallagher, E. 2008. The Gallagher Review of the indirect effects of biofuels production. The Renewable Fuels Agency, Hastings, East Sussex.
- Hill, J., E. Nelson, D. Tilman, S. Polasky, and D. Tiffany. 2006. Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. *Proceedings of the National Academy of Sciences* **103**:11206.
- Searchinger, T., R. Heimlich, R. A. Houghton, F. Dong, A. Elobeid, J. Fabiosa, S. Tokgoz, D. Hayes, and T. H. Yu. 2008. Use of US Croplands for Biofuels Increases Greenhouse Gases Through Emissions from Land-Use Change. *Science* **319**:1238.


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October, 2008

**Biomass Energy: the Climate Protective Domain**

Chris Field

[cfield@ciw.edu](mailto:cfield@ciw.edu)  
<http://dge.ciw.edu>


 Stanford University  
 Global Climate & Energy Project

- Food/Biomass energy interactions
  - Roz Naylor, Holly Gibbs
- Biomass in areas converted to bioenergy
  - Greg Asner, Scott Loarie
- Albedo feedbacks from bioenergy agriculture
  - David Lobell, Matt Georgescu
- Available land, potential yield, GHG balance
  - Chris Field, Elliott Campbell

**Biomass energy -- landscape**

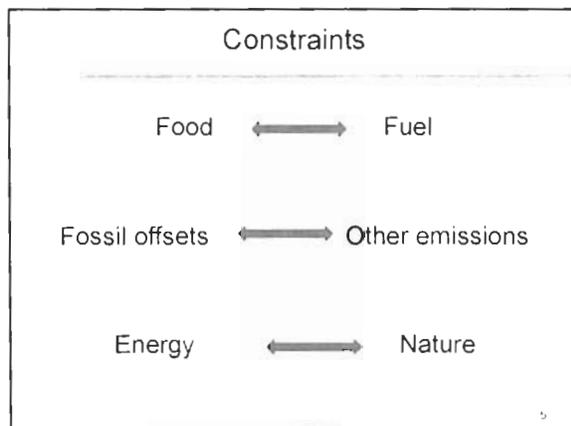
- Only currently viable option for powering existing vehicle fleet, using potentially lower CO<sub>2</sub> fuels
- Many countries investing, motivated by concerns over global change, energy security, and rural development.
- Global production and use of liquid biofuels tripled since 2000, with much more to come.
- Already provide some countries with a local renewable energy resource and rural jobs
- Current crops used to produce liquid biofuels are almost all food crops. Increasing the fraction allocated to biofuels can decrease the availability of food, and increasing the area can lead to loss of natural ecosystems rich in biodiversity or carbon stocks

3

**Biomass energy –moving forward**

- Biofuels from waste, from crops grown with a focus on improving marginal or abandoned land, and from diverse natural ecosystems have the potential for net benefits in terms of climate, energy security, and rural development, with low or no costs in environmental degradation or human well-being
- There are many ways to do biofuels wrong, so that the costs in damage to the environmental or to human well-being exceed the benefits, but there are also some ways to do biofuels right.
- Liquid biofuels for transportation almost always yield less useful energy and more create more environmental challenges than biomass used for direct combustion
- With larger and larger levels of production, it becomes increasingly difficult to successfully manage environmental impacts
- Combined with geological storage, biofuels represent one of the few options for an energy source with negative CO<sub>2</sub> emissions, one that leads to a net decrease in atmospheric CO<sub>2</sub>

4



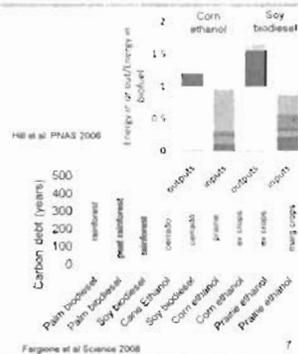
**Climate-protective biofuels**

- Grow more plants
  - Without more environmental downsides
- Get more energy per unit of plant biomass
- Figure out where it does and doesn't make sense to produce biofuels

6

## Climate protection issues

- Usable energy out fossil energy in
- Carbon debt – C losses from conversion
- Food security - Indirect conversion

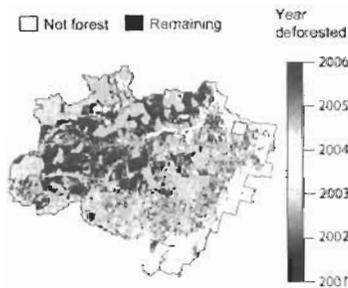


## Net energy balance ratio

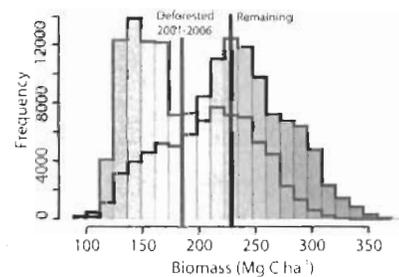
(biomass energy out/fossil energy in)

- Corn ethanol ~1.2
- Sugarcane ethanol ~ 8
- Soy biodiesel ~ 2
- Palm biodiesel ~ 9
- Cellulosic ~5(?)

## Year-by-year deforestation



## Greater biomass in remaining forests

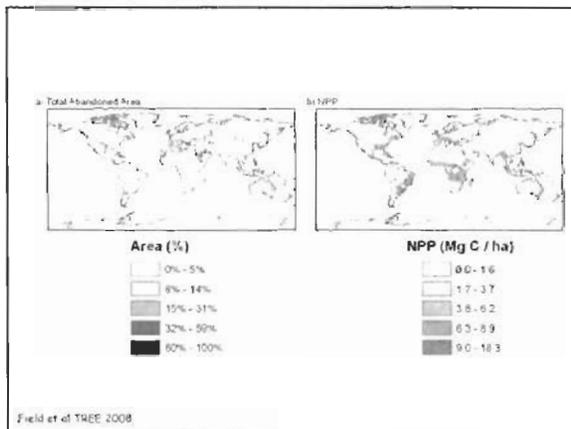
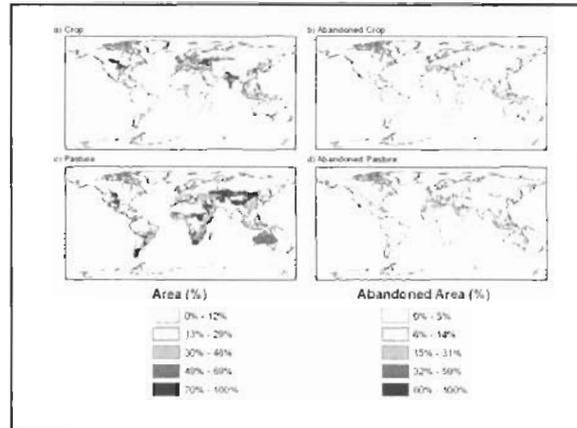
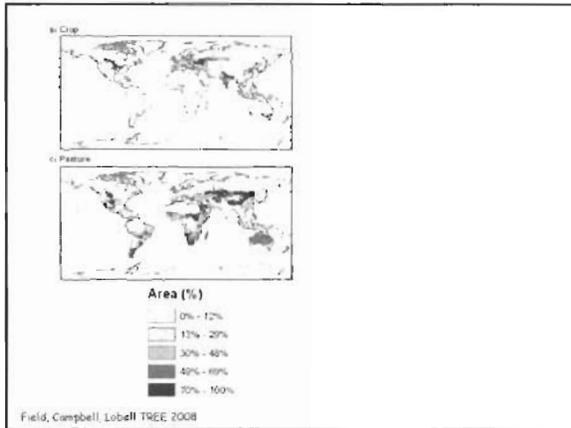


## Energy in ag and pastures?

Land Type	Area (Mha)	Mean NPP (ton C/ha/y)	Total NPP (Pg C/y)	Total Energy* (EJ/y)
Global Crop	1,445	4.6	6.7	119
Global Pasture	3,321	3.4	11.3	200
US Crop	173	5.7	1.0	18
US Pasture	226	3.5	0.8	14

Global Primary Energy = 480 EJ/y  
 \* In 1/2 biomass (to allow for roots), assume 45% C

- Ag in relation to natural NPP  
 – Ag/NPP -- Globally about 65%
- Global average crop yields unlikely to exceed natural NPP for at least the next several decades



### Potential from abandoned land

Land Type	Area (Mha)	Mean NPP (ton C / ha / yr)	Total NPP (Pg C / yr)
Global			
Crop	1,445	4.6	6.7
Pasture	3,321	3.4	11.3
Abandoned	474-579	4.7	2.2-2.7

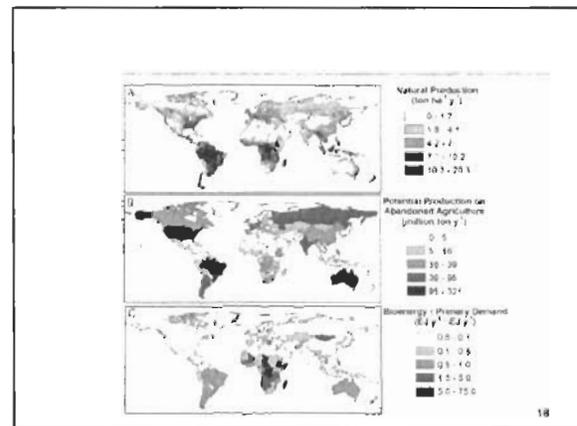
Campbell et al ESAT 2008

### From available abandoned land

Land Type	Area (Mha)	Mean NPP (ton C / ha / yr)	Total NPP (Pg C / yr)
Global			
Crop	1,445	4.6	6.7
Pasture	3,321	3.4	11.3
Abandoned	474-579	4.7	2.2-2.7
In Forest	72	6.5	0.5
In Urban	18	5.0	0.1
In Other	385-472	4.3	1.6-2.1

$1.6 - 2.1 \text{ Pg C} \times 2 \text{ g Plant/g C} \times 0.5 \text{ g top/g plant} \times 20 \text{ EJ/Pg} = 32 - 41 \text{ EJ}$   
 = 7-8% of current global energy system

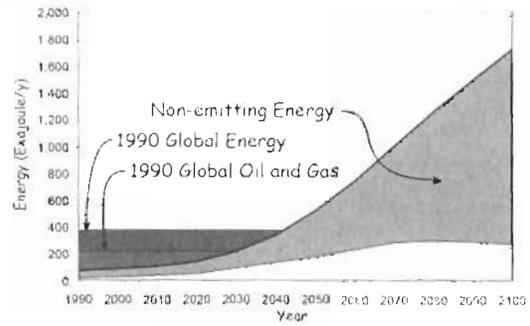
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### Bioenergy

- Climate impact depends on pre-existing ecosystem
- Indirect as well as direct paths to carbon loss
- Natural NPP reasonable proxy for potential yield under ag management
- Available land resource limited
  - Quantity and quality
- Big potential in absolute terms
- But a small slice of present or future demand

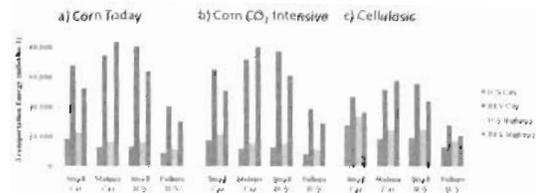
### Future energy needs: Many times current



### Biomass energy

- Corn \$146/ton
- Coal Power River \$16/ton  
Central Appalachia \$148/ton
- Crude oil \$466/ton

### Ferment or burn?



## **Attachment 3 – Epigenomic Speakers’ Biosketches, Abstracts, and Handouts**

## **Dr. Mark Hanson**

### **University of Southampton**

Dr. Mark Hanson is a British Heart Foundation Professor of Cardiovascular Science at the University of Southampton and President of the International Society for Developmental Origins of Health and Disease. He has worked in the field of fetal and developmental physiology, and its implications for medicine, for nearly 30 years, establishing a research group at Reading University in 1979, moving to a joint appointment in Obstetrics & Gynaecology and Physiology at UCL in 1990, and founding the Centre for Developmental Origins of Health and Disease at Southampton University in 2000. Early achievements focused on defining neural, hormonal and local mechanisms involved in cardio-respiratory, behavioural and metabolic control in the fetus and neonate, initiating new thinking on fetal adaptations and responsiveness to the prenatal environment. The Centre was the first to make recordings demonstrating unequivocal arterial chemoreceptor function in late gestation, opening avenues for studying fetal reflex responses to hypoxia. This work was extended to the effects of acute and chronic hypoxia in altricial species (e.g. cat) to large precocial species (llama, sheep) to gain insights from differing maturational strategies. The Centre's seminal studies established the concept of postnatal resetting of chemoreceptor sensitivity, explored its mechanisms and relevance to respiratory failure, and developed a test of chemoreflex sensitivity which was applied to human babies, including those at high risk of sudden infant death. Its research simultaneously played a leading role in establishing brainstem processes involved in the characteristic reduction in breathing activity seen in the hypoxic fetus and newborn, and examined interactions between thermoregulation and breathing, e.g. bacterial endotoxin-induced pyrexia. Throughout his career he has collaborated with clinical scientists in developing methods for studying the human fetus, including heart rate variability, Doppler ultrasonic measurement of vascular impedance, cardiac volume imaging and near infrared measurement of tissue oxidative state. This work has contributed to developments in human fetal monitoring. Extending the concept of fetal adaptive responses, his research group was the first to show perturbations in fetal cardiovascular and endocrine function induced by mild nutritional challenges without reductions in fetal growth. It was in the forefront in focusing on the importance of early gestation challenges, and in performing long-term follow up to adulthood of animals in which additional postnatal nutritional challenges were imposed. This demonstrated that prenatal nutrition can condition the animal's later cardiovascular, metabolic and hypothalamo-pituitary adrenal axis responses, relevant to later pathophysiology. This research has now shown that dietary, hormonal and pharmacological interventions can reverse aspects of the phenotype induced in early life, and this may have therapeutic implications. He has conducted detailed investigation of underlying epigenetic mechanisms, showing changes in DNA methylation, histone methylation and acetylation and small non-coding RNAs following a prenatal nutritional challenge and affecting expression of non-imprinted genes in a range of tissues. Recent studies have examined the ways in which epigenetic processes can induce the equivalent of polyphenisms in mammals, and also the effects of endocrine disruptor chemicals. With Peter Gluckman he developed the influential concept of predictive adaptive responses, extending evolutionary and developmental biology concepts to human populations and we have extended this work to champion the field of evolutionary medicine. His recent studies utilise Southampton's human epidemiological cohorts, showing the importance of pre-pregnancy maternal body composition and diet to later fetal cardiovascular function. They will facilitate the translation of mechanistic insights to new early life markers of risk of later chronic disease and to methods of monitoring interventions. In collaboration with organisations such as The World Bank and WHO he is attempting to define the human cost of a poor start to life.

## **Developmental origins of health and disease – role of epigenetic mechanisms**

M.A. Hanson<sup>1</sup>, P.D. Gluckman<sup>2</sup>, G.C. Burdge<sup>1</sup>, K.A. Lillycrop<sup>1</sup>, K.M. Godfrey<sup>1</sup>

<sup>1</sup> Division of Developmental Origins of Health & Disease, University of Southampton,

<sup>2</sup> Liggins Institute, University of Auckland

Epidemiological and animal studies show that small changes in the environment during development, e.g. in nutrient provision or balance, induce phenotypic changes which affect an individual's responses to their later environment. These may in turn alter the risk of chronic disease resulting from inadequate responses, e.g. to a rich environment leading to metabolic syndrome or cardiovascular disease. Recent research shows that animals exposed to such a mismatch between pre- and postnatal environment develop obesity, reduced activity, leptin and insulin resistance, elevated blood pressure and vascular endothelial dysfunction. We have found an important role for molecular epigenetic processes in producing such effects, processes which are targeted to promoter regions of specific genes in specific tissues but which also include changes in histone structure and post-transcriptional processes involving miRNAs. Such fine control of gene expression endorses the view that the mechanisms have been retained through evolution as a result of the adaptive advantage which they confer, rather than representing extreme effects of developmental disruption akin to teratogenesis. Moreover there may be adaptive advantage in a developmental cue inducing a phenotypic change in generations beyond the immediately affected pregnancy, and there is now a range of human and animal data which support this concept. Such effects – which might be termed non-genomic inheritance – may be mediated by a range of effects including alterations in maternal adaptations to pregnancy in successive generations or behavioural influences. Recent data however also show that epigenetic effects such as DNA methylation can be passed to successive generations. This suggests that they might persist through meiosis. Environmental toxins, including endocrine disruptors, can play a role in inducing greater risk of chronic disease even at low exposure levels, especially if they act via the normal epigenetic processes involved in developmental plasticity. Current research in this area is important for mechanistic understanding and for developing novel prognostic markers of later disease risk. It also emphasizes the long-term multi-generational effects which appropriate interventions may confer to reduce the risk of chronic disease in subsequent generations.

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1. Gluckman PD, Hanson MA, Cooper C, Thornburg KL (2008). Effect of in utero and early-life conditions on adult health and disease. *New England Journal of Medicine* 359:61-73
1. Godfrey KM, Lillycrop KA, Burdge GC, Gluckman PD, Hanson MA (2007). Epigenetic mechanisms and the Mismatch concept of the Developmental Origins of Health and Disease. *Pediatric Research* 61 ( Pt2):5R-10R
3. Burdge GC, Hanson MA, Slater-Jefferies JL, Lillycrop KA (2007). Epigenetic regulation of transcription: a mechanism for inducing variations in phenotype (fetal programming) by differences in nutrition during early life? *British Journal of Nutrition* 97:1036-1046

*MAH and GCB are supported by the British Heart Foundation and PDG by the National Research Centre for Growth and Development*

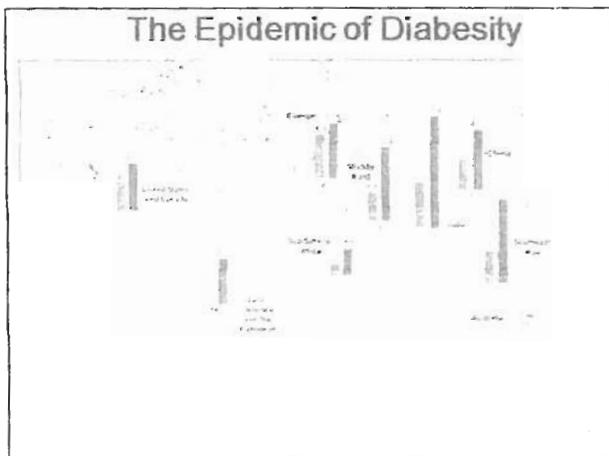
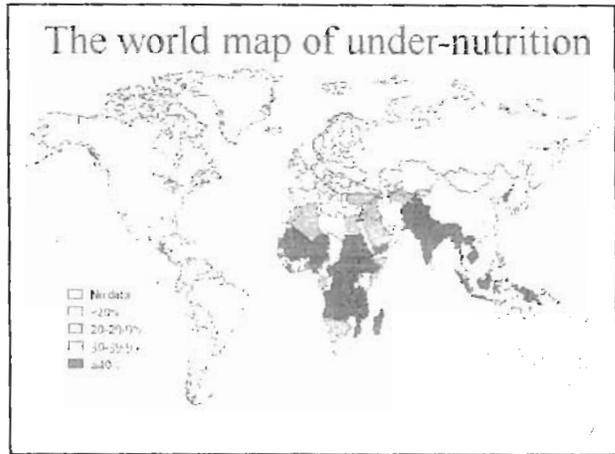
UNIVERSITY OF Southampton  
School of Medicine

*Developmental Origins of Health & Disease - the role of epigenetic mechanisms*

Mark Hanson

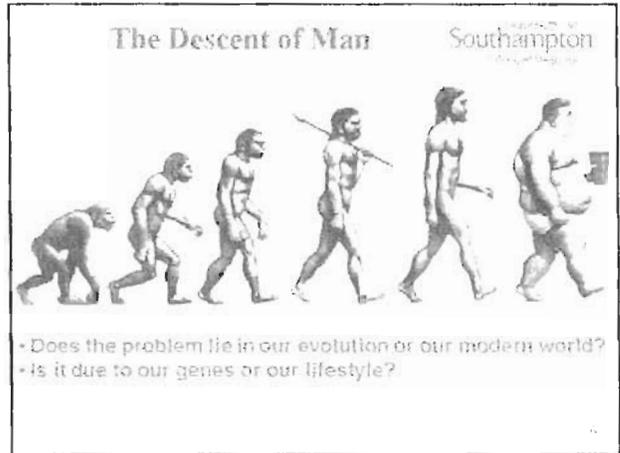
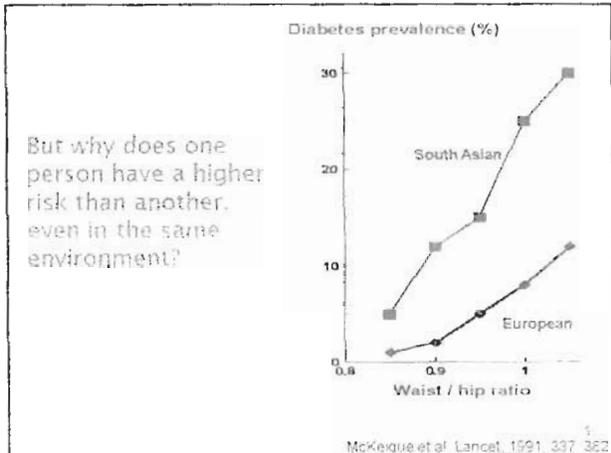



dohadoc.org

Southampton

Challenge to humans  
at least as big as  
global warming

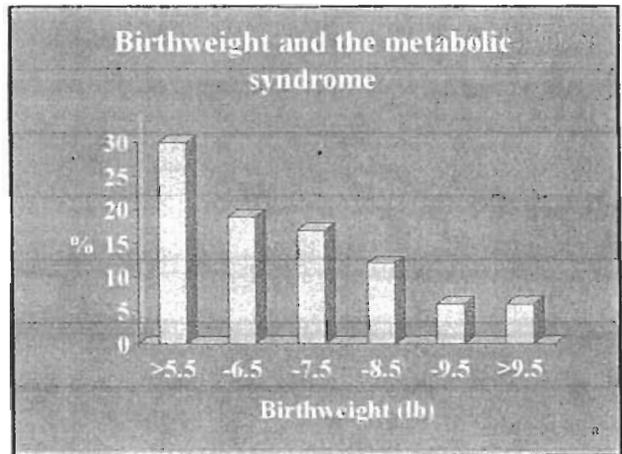


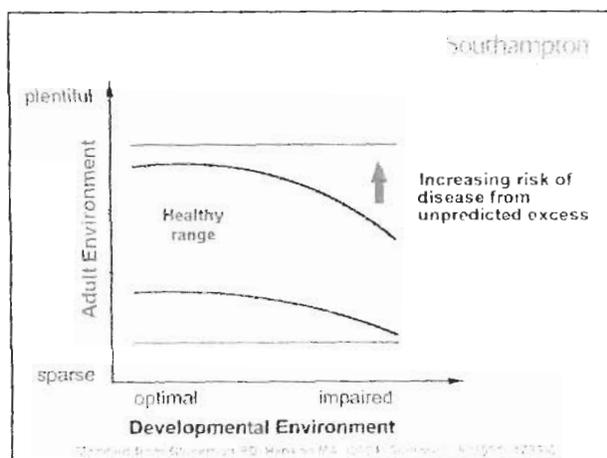
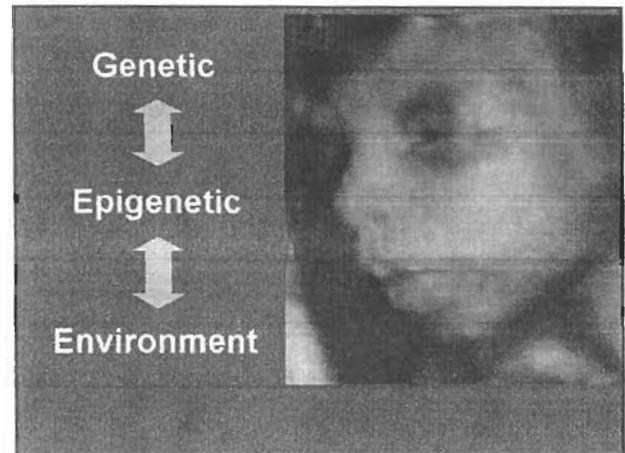
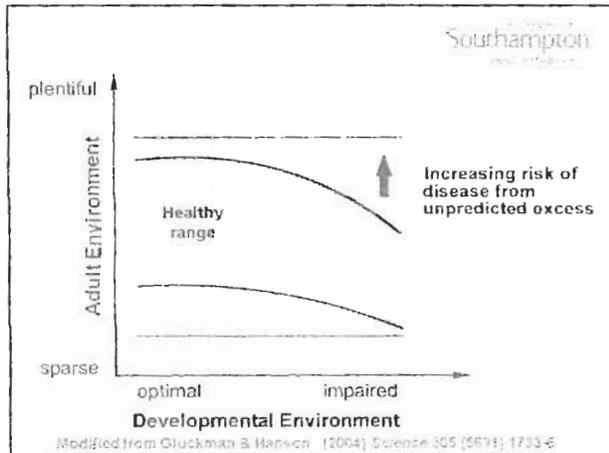
**BMJ**

**Metabolic syndrome**

Globally 16% of adults over age 20 have the metabolic syndrome and it's increasing

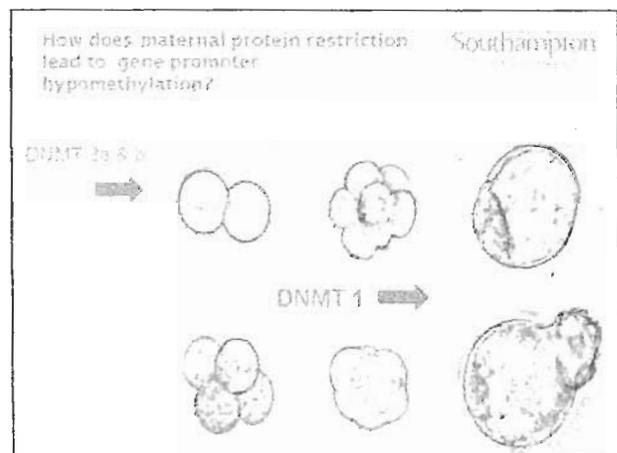
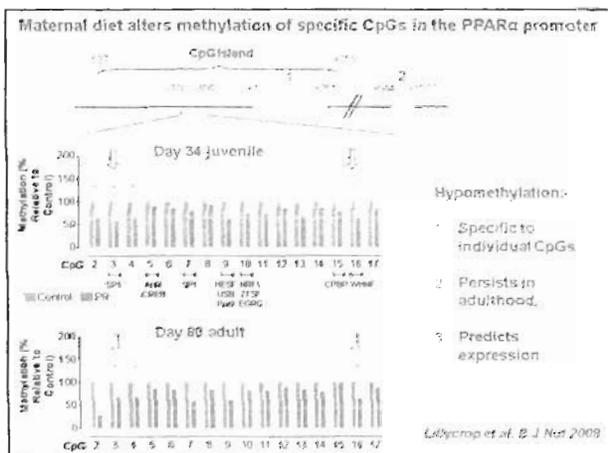
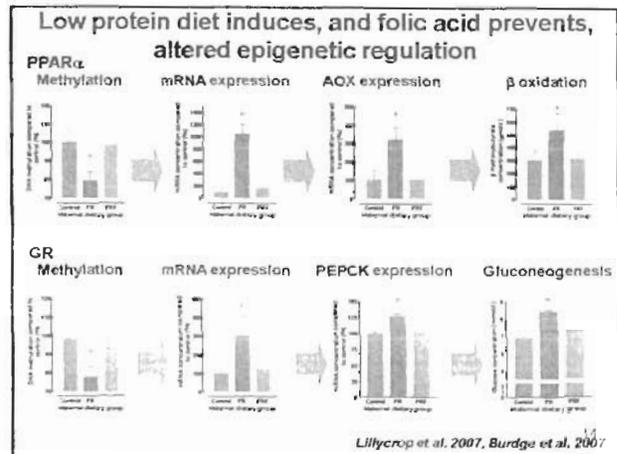
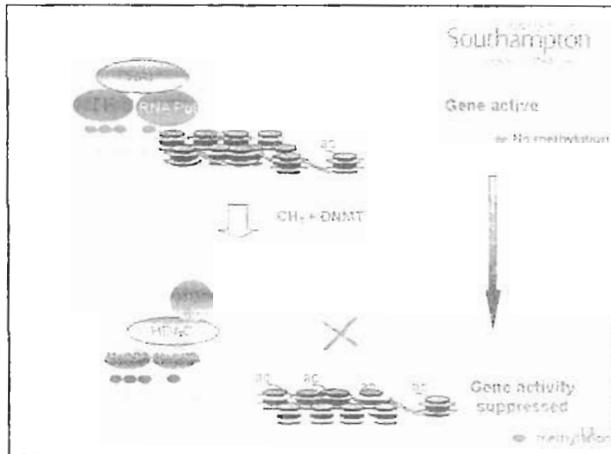
- Central Obesity
- High blood pressure
- Disordered blood lipids
- High blood sugar - Type 2 diabetes
- High risk of coronary heart disease

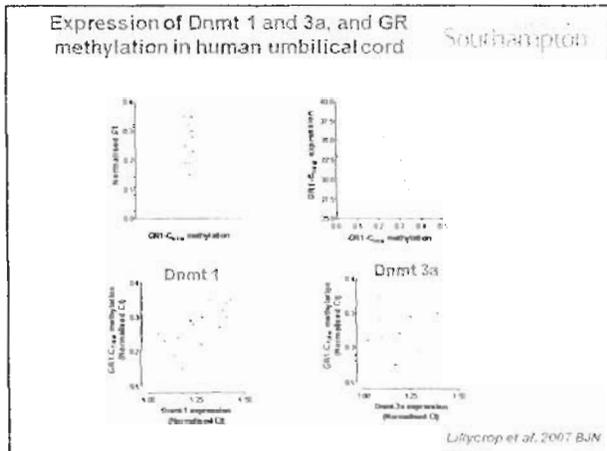
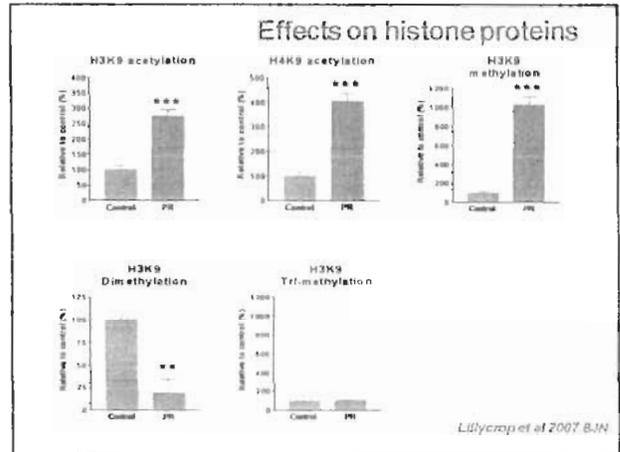
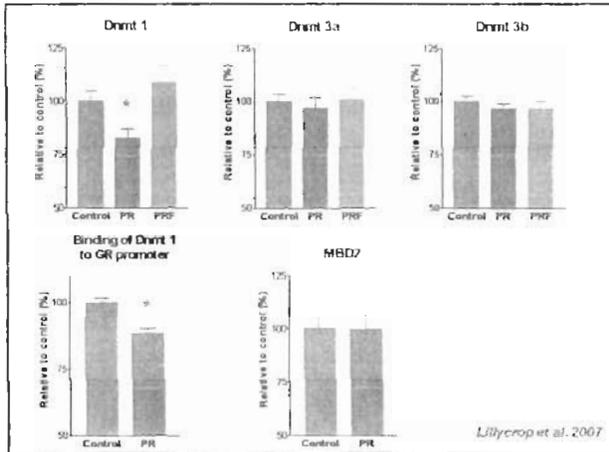


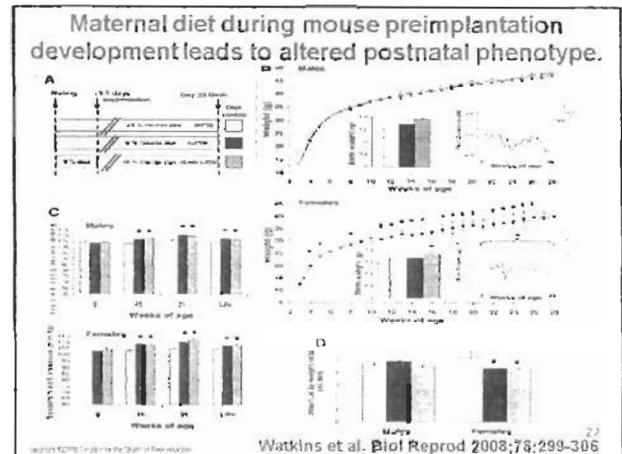
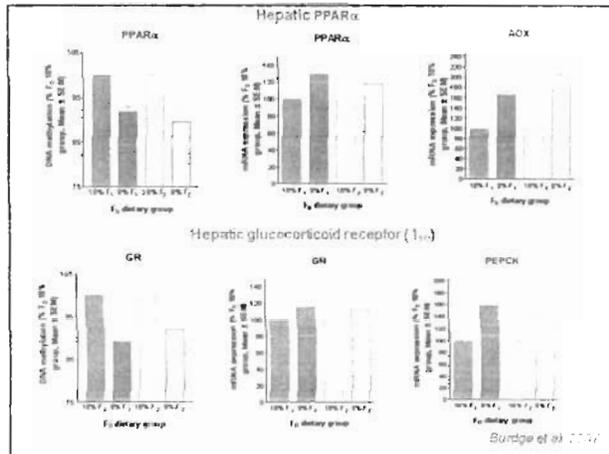


### Epigenetic effects

- Allow several phenotypes to be produced from one genotype, depending on the environment
- Affect gene expression without changing genetic code
- Don't just involve imprinted genes
- DNA methylation
- Histone acetylation, phosphorylation, methylation, ubiquitination....
- Small non-coding RNAs



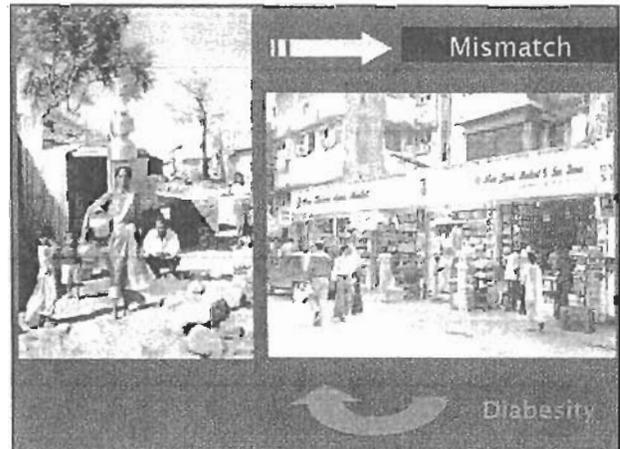


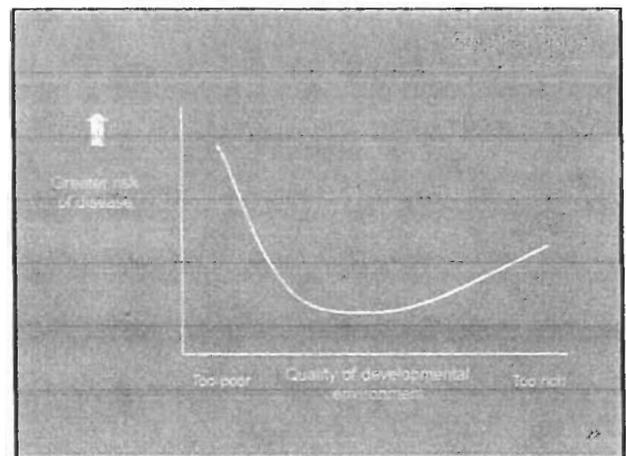
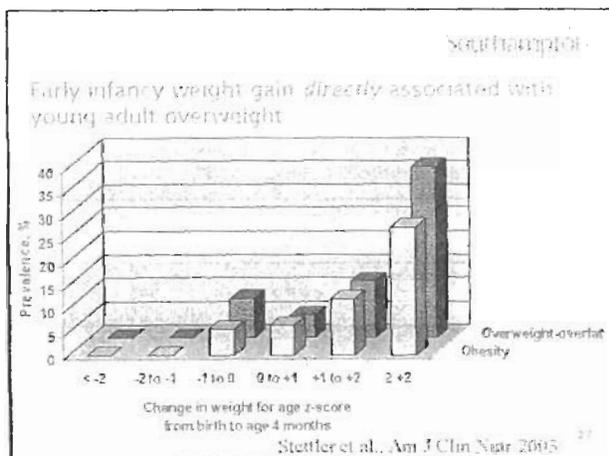
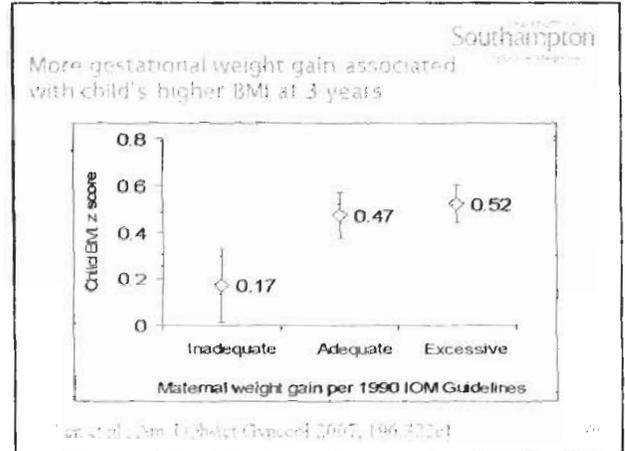
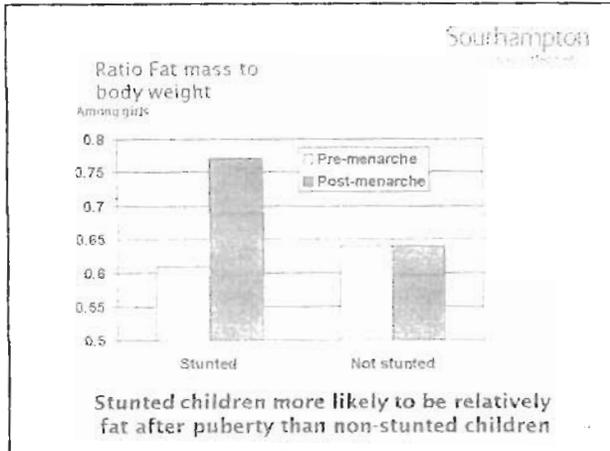


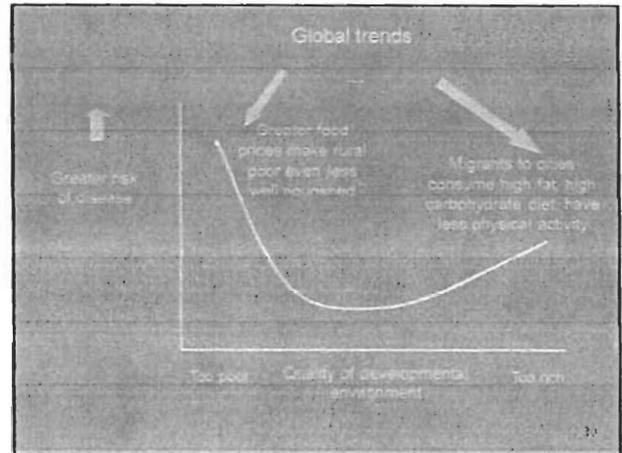
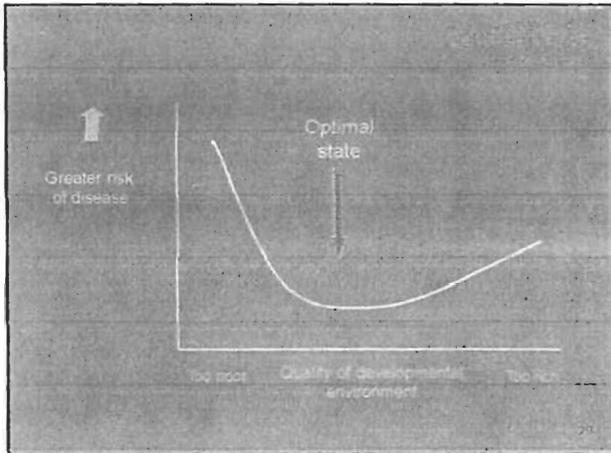
**Ceelen M et al J Endoc Metab. 2008 May 93(5):1682-8. Cardiometabolic differences in children born after in vitro fertilization: follow-up study.**

Systolic and diastolic blood pressure levels were higher in 8-18 yr. old IVF children than in controls (109+/-11 mm Hg vs 105+/-10, P<0.001; 61+/-7 mm Hg vs 59+/-7, P=0.001, respectively). Children born after IVF were also more likely to be in the highest systolic and diastolic blood pressure quartiles (OR= 2.1, 95% CI: 1.4, 3.3; OR= 1.9, 95% CI: 1.2, 3.0, respectively). Furthermore, higher fasting glucose levels were observed in pubertal IVF children (5.6+/-0.4 mmol/l vs 4.8+/-0.4 in controls, P=0.009). Blood pressure and fasting glucose differences could neither be explained by current body size, birth weight and other early life factors nor by parental characteristics including subfertility cause.

34



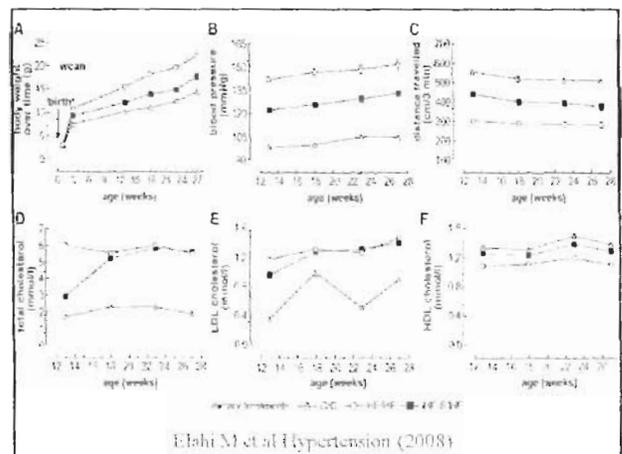




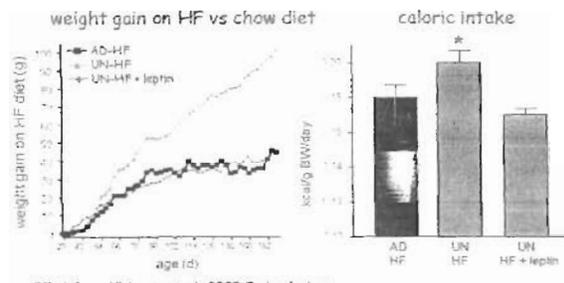
Southampton

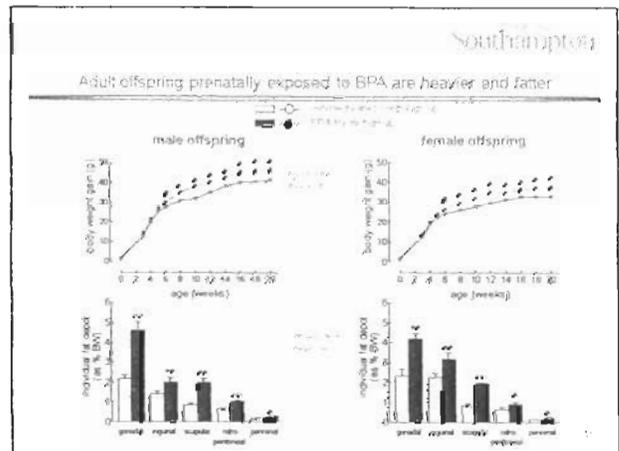
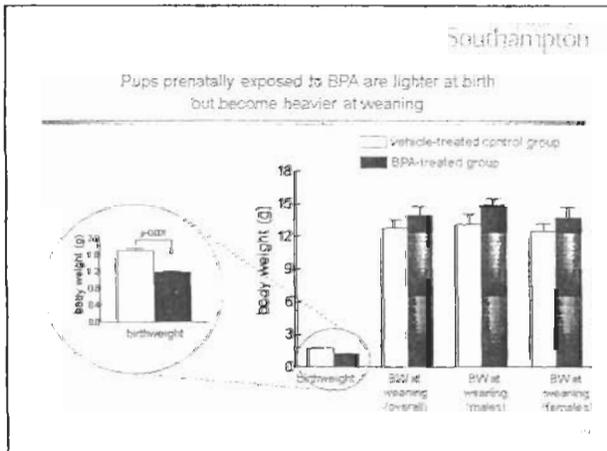
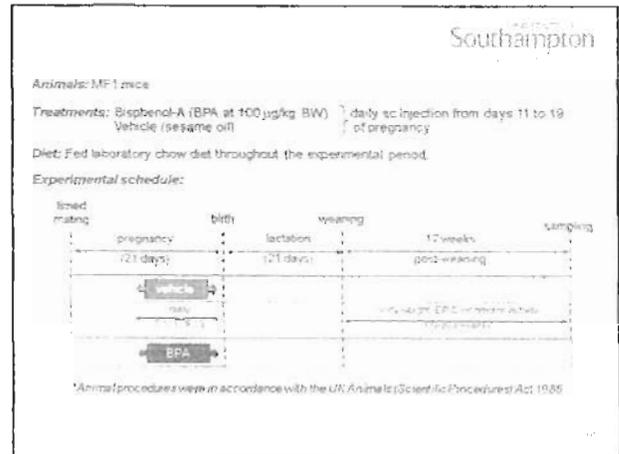
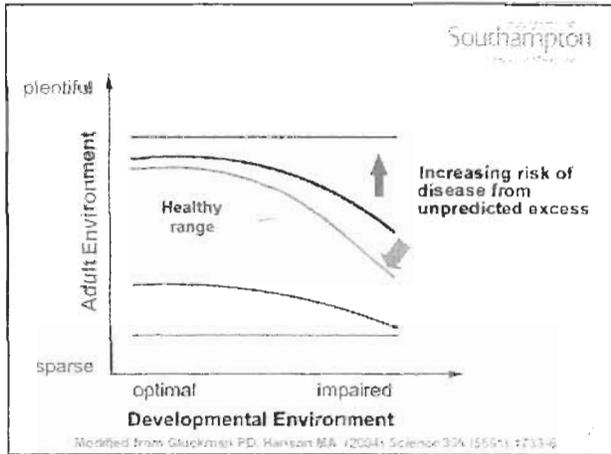
### Reversibility?

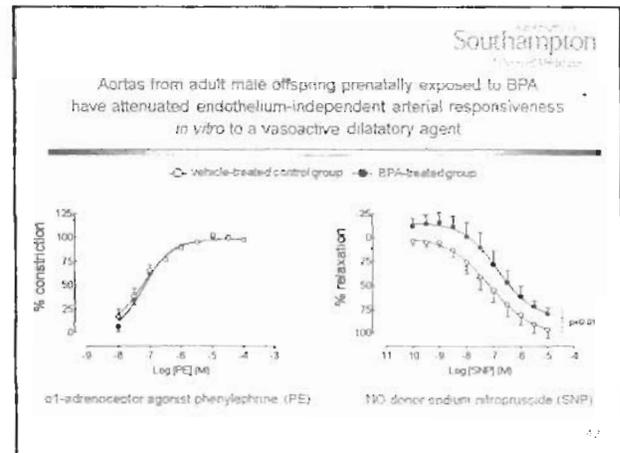
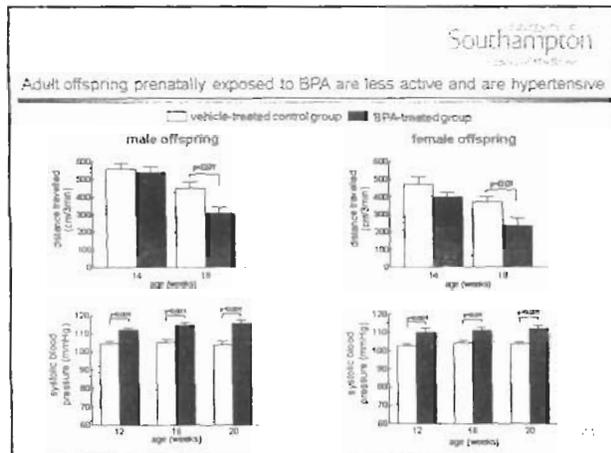
- Micronutrients - folic acid/ choline
- Statins in late pregnancy (Elahi et al Hypertension 08)
- Neonatal leptin (Gluckman et al PNAS 07)



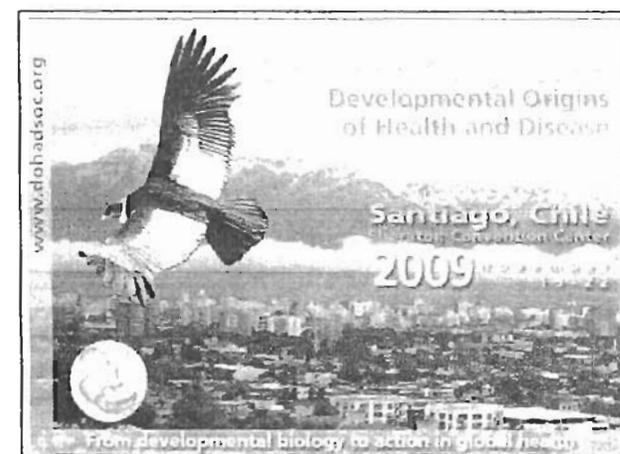
Early postnatal leptin treatment alleviate the obesogenic effects of post weaning high fat (HF) diet in rat offspring

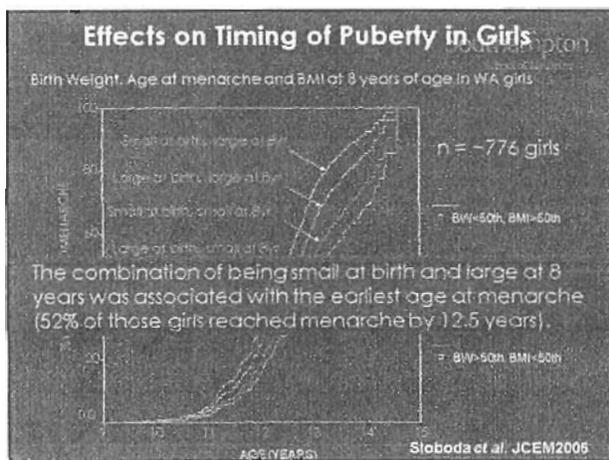
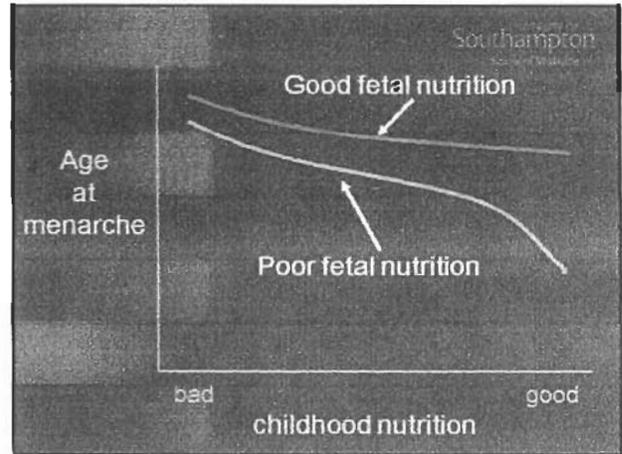
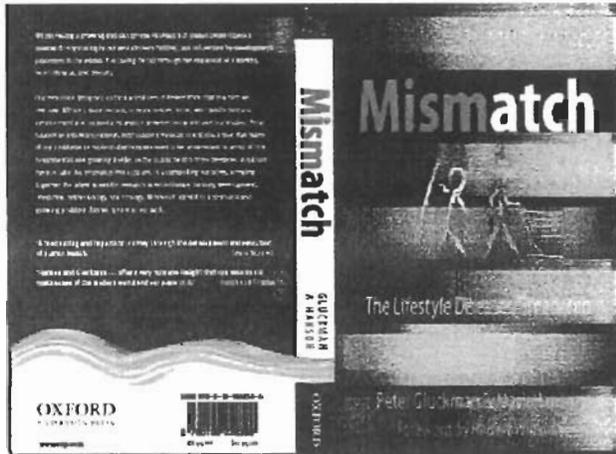






- University of Southampton  
Southampton
- ### Conclusions
- Diseases of developmental origin are a new medical category
  - They are reaching epidemic proportions in both developed and developing societies
  - Part of the risk of disease is influenced by gene - environment interactions during development
  - The underlying epigenetic mechanisms are now becoming understood
  - They comprise novel opportunities for prognosis and for intervention
  - Research in this area will pay major health and social - and so economic - dividends





## Dr. Randy Jirtle

### Duke University

Dr. Randy L. Jirtle is a professor of radiation oncology and an associate professor of pathology at Duke University, Durham, NC, where he has been a faculty member since 1977. He graduated with a B.S. degree in nuclear engineering in 1970 and a Ph.D. degree in radiation biology in 1976, both from the University of Wisconsin-Madison. Jirtle's research interests are in epigenetics, genomic imprinting, and the fetal origins of disease susceptibility. He identified the imprinted IGF2R as a tumor-suppressor, and showed its inactivation increases tumor resistance to radiotherapy. Jirtle discovered a novel imprinted domain at human 14q32, and identified the Callipyge or beautiful buttocks locus in the homologous region of sheep. He subsequently traced the mammalian origin of genomic imprinting from monotremes to placental mammals. These studies provided the crucial data that allowed him to complete the first genome-wide mapping of human imprinted genes using a bioinformatic approach. The effort yielded candidate imprinted genes in chromosomal regions linked to complex human diseases and neurological disorders. Jirtle also demonstrated that maternal dietary supplementation of Avy mice during pregnancy, with either methyl donors or genistein, decreases adult disease incidence in the offspring by increasing DNA methylation at the Agouti locus. Moreover, these nutritional supplements were shown to block CpG hypomethylation caused by the endocrine disruptor, bisphenol A. Jirtle holds two U.S. patents on imprinted genes and another one is pending approval. He has published more than 160 peer-reviewed articles, including ten publications featured on journal covers. His research has been featured in popular press accounts ranging from American Scientist and Discover to Allure. He was also a featured scientist this past year on the NOVA and ScienceNow television programs on epigenetics, and National Public Radio programs, The People's Pharmacy and The DNA Files. His enthusiasm for promoting the public understanding of epigenomics led him to create the website [www.geneimprint.org](http://www.geneimprint.org), which has been designated by the scientific publisher Thomson ISI as an 'Exemplary Website in Genetics.' Jirtle has organized five international meetings and been an invited speaker at dozens of others. He has delivered five endowed lectures, and was invited to present his research at the 2004 Nobel Symposium on Epigenetics. He was honored in 2006 with the Distinguished Achievement Award from the College of Engineering at the University of Wisconsin-Madison. In 2007, Jirtle received an Esther B. O'Keeffe Charitable Foundation Award and capped off the year with a nomination for Time Magazine's "Person of the Year." He was the inaugural recipient of the Epigenetic Medicine Award in 2008.

## ABSTRACT

### Epigenetics: The New Genetics of Disease Susceptibility

Randy L. Jirtle, Ph.D.

Department of Radiation Oncology

Duke University Medical Center, Durham, NC USA 27710

Human epidemiological and animal experimental data indicate that the risk of developing adult-onset diseases, such as asthma, diabetes, obesity, and cancer, is influenced by persistent adaptations to prenatal and early postnatal exposure to environmental conditions such as nutritional privation [1]. Moreover, the link between what we are exposed to *in utero* and disease formation in adulthood appears to involve epigenetic modifications like DNA methylation at metastable epiallele and imprinted gene loci.

Genomic imprinting is an epigenetic form of gene regulation that results in monoallelic, parent-of-origin dependent gene expression [2]. Since imprinted genes are functionally haploid, only a single genetic or epigenetic event is needed to dysregulate their function. This vulnerability means that imprinted genes are prime candidates for causative roles in human diseases that have a parental inheritance bias and an environmental component in their etiology. We recently developed computer-learning algorithms that predicted the presence of 600 imprinted genes in mice [3] and 156 imprinted genes in humans [4]. Not only are humans predicted to have fewer imprinted genes than mice, but there is also a mere 30% overlap between their imprinted gene repertoires. By mapping the human candidate imprinted genes onto the landscape of disease risk defined by linkage analysis, we are now poised to determine the importance of imprinting in the etiology of complex human diseases and neurological disorders.

Genes with metastable epialleles have highly variable expression because of stochastic allelic changes in the epigenome rather than mutations in the genome. The viable yellow agouti ( $A^{vy}$ ) mouse harbors a metastable *Agouti* gene because of an upstream insertion of a transposable element. We have used the  $A^{vy}$  mouse to investigate the importance of nutrition in determining the susceptibility of offspring to adult diseases [5,6]. We have shown that maternal dietary supplementation during pregnancy, with either methyl donors (i.e. folic acid, vitamin B<sub>12</sub>, choline and betaine) [5] or genistein [6], decreases adult disease incidence in the offspring by increasing DNA methylation at the  $A^{vy}$  locus. Moreover, these nutritional supplements can counteract the CpG hypomethylation caused by the endocrine disruptor, bisphenol A [7]. (Supported by NIH grants ES13053, ES08823, ES015165 and T32-ES07031, and DOE grant DE-FG02-05ER64101)

## References

1. Jirtle, R.L., and Skinner, M.K. Environmental epigenomics and disease susceptibility. *Nat. Rev. Genet.* 8: 253-562, 2007
2. Jirtle, R.L., and Weidman, J.R. Imprinted and more equal. *Am. Sci.* 95: 143-149, 2007.
3. Luedi, P.P., Hartemink, A.J., and Jirtle, R.L. Genome-wide prediction of imprinted murine genes. *Genome Res.* 15: 875-884, 2005.
4. Luedi, P.P., Dietrich, F.S., Weidman, J.R., Bosko, J.M., Jirtle RL, and Hartemink, A.J. Computational and experimental identification of novel human imprinted genes. *Genome Res.* 17: 1723–1730, 2007..
5. Waterland, R.A., and Jirtle, R.L. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Cell. Mol. Biol.* 23: 5293-5300, 2003.
6. Dolinoy, D.C., Weidman, J.R., Waterland, R.A., and Jirtle, R.L. Maternal genistein alters coat color and protects  $A^{vy}$  mouse offspring from obesity by modifying the fetal epigenome. *Environ. Health Perspect.* 14: 567-572, 2006.
7. Dolinoy, D.C., Huang, D., and Jirtle, R.L. Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. *Proc. Natl. Acad. Sci. USA* 104: 13056-13061, 2007.

# Epigenetics: The New Genetics of Disease Susceptibility

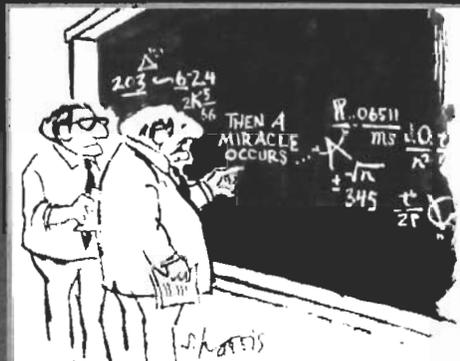
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Department of Radiation Oncology  
Duke University Medical Center  
Durham, NC 27710



Origins  
Artist: Collin Murphy  
Portland, OR

# Fetal Origin of Adult Disease Susceptibility

Miracle: Epigenetic Modifications

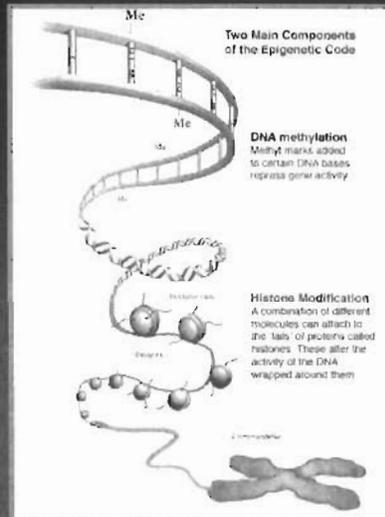


"I think you should be more explicit here in step two."

From *What's So Funny about Science?* by Sidney Harris (1977)

<http://www.geneimprint.com>

# What is Epigenetics?

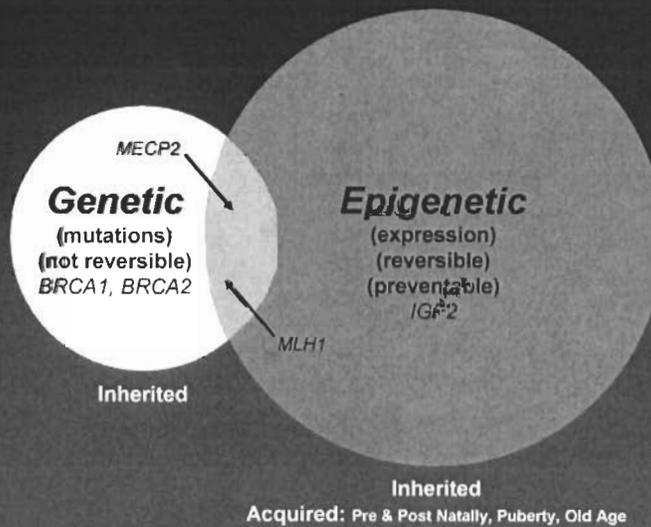


*Epi-ge-net-ics* - "above genetics"

Epigenetics research is the study of heritable changes in gene function that occur without a change in the sequence of the DNA. (i.e. DNA methylation & chromatin structure)

<http://www.geneimprint.com>

# Cancer Susceptibility



<http://www.geneimprint.com>

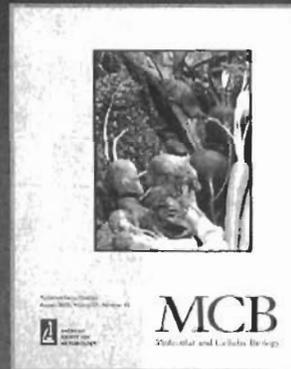
# Epigenetically Labile Genes



Artist: Nancy Jittle

**Imprinted Genes**

**Metastable Epialleles**



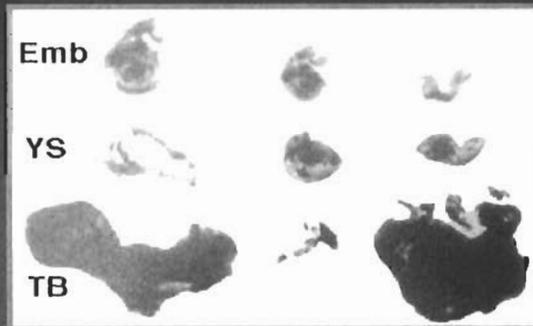
<http://www.geneimprint.com>

**“All animals are equal, But some animals are more equal than others.”** *George Orwell*



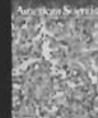
<http://www.geneimprint.com>

# Maternal and Paternal Genomes not Functionally Equivalent



McGrath and Solter Cell 37: 179-183, 1984  
Surani et al. Nature 308: 548-550, 1984

<http://www.geneimprint.com>



# Imprinted Genes

## Autosomal Genes with a Sex

"Imprinting results in parent-of-origin  
dependent monoallelic expression."

<http://www.geneimprint.com>

# Imprinting Evolution

Species, Tissue, and Time Dependent  
Gene Expression



Artist: James Jirtle

*IGF2R* & *IGF2*  
Imprinting Evolved  
(150 M Years Ago)

*IGF2R*  
Imprinting Lost  
(75 M Years Ago)

\* *Nnat, Meg3, Dlk1*

Primates  
Scandentia  
Dermoptera  
Rodents  
Ungulates  
Marsupials  
Monotremes  
Birds

Euarchonta

Killian *et al.* Mol. Cell 5: 707-716, 2000  
Killian *et al.* Hum. Mol. Genet. 10: 1721-1728, 2001  
Evans *et al.* Mol. Biol. Evol. 22: 1740-1748, 2005

<http://www.geneimprint.com>

## Consequence of Divergent Evolution of Imprinting

- Biological responses due to imprinting dysregulation will be difficult to extrapolate between species.
- Mice are not humans!

<http://www.geneimprint.com>



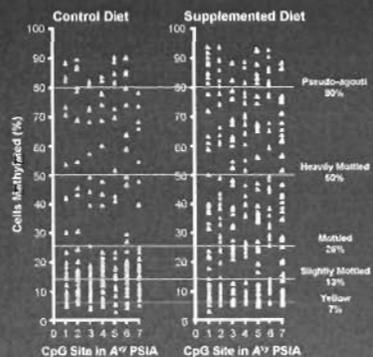
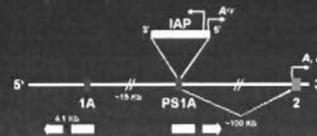
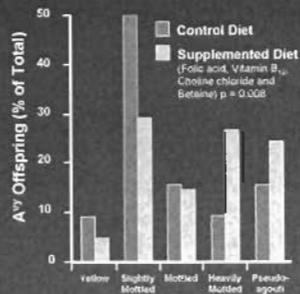
The Agouti Sisters



# Metastable Epialleles

<http://www.geneimprint.com>

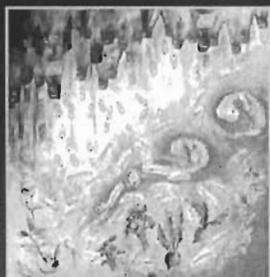
## Methyl Donor Supplementation Viable yellow Agouti ( $A^{vy}$ ) Locus



Waterland et al. Mol. Cell Biol. 23: 5293-5300, 2003

<http://www.geneimprint.com>

# Food is Medicine!



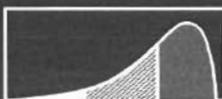
Artist: Collin Murphy

'Let food be thy medicine, and medicine be thy food.' *Hippocrates*

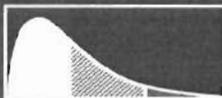
## Agouti Coat Color Distribution



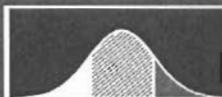
Control Diet



Methyl Donor or Genistein Supplementation



BPA Exposure



BPA Exposure plus Methyl Donor or Genistein Supplementation

Doinov et al. *PNAS* 104: 13058-13061, 2007

<http://www.geneimprint.com>

# You are What You Eat!



<http://www.geneimprint.com>

Neo-Rosetta Stone



Artist: James Jirtle

## Future Objectives

Identify epigenetically regulated targets in the human genome.

- Imprinted genes
- Metastable epialleles

Luedi et al. Genome Res. 17: 1723-1730, 2007

<http://www.geneimprint.com>

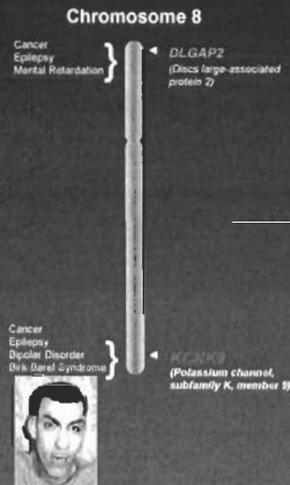
## Genome-wide Prediction of Imprinted Genes



Jirtle Luedi Hartemink

Mouse  
600 genes

Human  
156 genes



Barel et al. ASHG 83: 192-199, 2008

"The proper study of Mankind is Man." Alexander Pope

Luedi et al. Genome Res. 15: 875-884, 2005  
Luedi et al. Genome Res. 17: December, 2007

<http://www.geneimprint.com>

# Take Home Message

Human risk assessment must be based not only on the ability of an agent to alter the genome, but also the epigenome.

<http://www.geneimprint.com>

## **Dr. Michael Skinner**

### **Washington State University**

Dr. Michael K. Skinner is Professor and Director of Center for Reproductive Biology at Washington State University. He holds a Ph.D. from Washington State University and a B.A. from Reed College. His primary research addresses on molecular and cellular aspects of reproduction (testis/ovary biology) and transgenerational epigenetic mutagenesis. He has investigated how different cell types in a tissue interact and communicate to regulate cellular growth and differentiation, with emphasis in the area of reproductive biology. He has initiated an investigation of the effects of environmental toxicants on gonadal development has been initiated and found that the impact of endocrine disruptors on embryonic testis and ovary development demonstrated an epigenetic transgenerational phenotype on adult male fertility. Exposure of the embryonic testis at the time of sex determination caused an epigenetic reprogramming of the male germ-line that causes a variety of disease states in the adult and this phenotype is transferred through the male germ-line to all subsequent generations. His laboratory is investigating the underlying mechanism and phenotype of this epigenetic transgenerational phenomenon.

***Epigenetic Transgenerational Actions of Endocrine Disruptors on Reproduction and Disease:  
The Ghosts in Your Genes***

Michael K. Skinner - Center for Reproductive Biology, School of Molecular Biosciences, Washington State University, Pullman, Washington.

Transgenerational effects of environmental toxicants (e.g. endocrine disruptors) significantly amplify the impact and health hazards of these compounds. One of the most sensitive periods to endocrine disruptor exposure is during embryonic gonadal sex determination when the germ line is undergoing epigenetic programming and DNA re-methylation. The model endocrine disruptors tested were vinclozolin, which acts as an anti-androgenic compound, and methoxychlor, that has metabolites that are estrogenic. Previous studies have shown that these endocrine disruptors can effect embryonic testis development to subsequently cause an increase in spermatogenic cell apoptosis in the adult. Interestingly, this spermatogenic defect is transgenerational (F1, F2, F3 and F4 generations) and hypothesized to be due to a permanent altered DNA methylation of the germ-line. This appear to involve the induction of new imprinted-like DNA methylation sites that regulate transcription distally. The expression of over 200 genes were found to be altered in the embryonic testis and surprisingly this altered transcriptome was similar for all generations (F1-F3). In addition to detection of the male testis disorder, as the animals age transgenerational effects on other disease states were observed including tumor development, prostate disease, kidney disease and immune abnormalities. Recent observations suggest transgenerational effects on behaviors such as sexual selection and anxiety. Therefore, the transgenerational epigenetic mechanism appears to involve the actions of an environmental compound at the time of sex determination to alter the epigenetic (i.e DNA methylation) programming of the germ line that then alters the transcriptomes of developing organs to induce disease development transgenerationally. The suggestion that environmental factors can reprogram the germ line to induce epigenetic transgenerational disease is a new paradigm in disease etiology not previously considered.

Anway M, Cupp AS, Uzumcu M and MK Skinner (2005) Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science* 308:1466-1469.

Anway MD, Leathers C and MK Skinner (2006) Endocrine Disruptor Vinclozolin Induced Epigenetic Transgenerational Adult Onset Disease. *Endocrinology* 147:5515-5523.

Crews D, Gore AC, Hsu TS, Dangleben NL, Spinetta M, Schallert T, Anway MD, Skinner MK (2007) Transgenerational epigenetic imprints on mate preference. *Proceedings of the National Academy of Sciences of the United States of America*. 3;104(14):5942-6.

Anway MD, Rekow SS, and MK Skinner (2008) Transgenerational epigenetic programming of the testis transcriptome by endocrine disruptor exposure at sex determination. *Genomics* 91:30-40.



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October 23, 2008

Mark A. Greenwood  
202-508-4605  
202-383-7785 fax  
mark.greenwood@ropesgray.com

Dr. Deborah Swackhamer  
Chair, Science Advisory Board  
U.S. Environmental Protection Agency  
1200 Pennsylvania Avenue, N.W.  
Washington, DC 20460

Dear Dr. Swackhamer:

On behalf of the Coalition for Effective Environmental Information ("CEEI"), we are providing comments regarding the U.S. Environmental Protection Agency's ("EPA's") plan on strategic directions for environmental research, which is currently under discussion by the Science Advisory Board ("SAB"). In particular, we urge the SAB to emphasize and amplify its recommendations regarding risk communication research, which is addressed in its draft report.<sup>1</sup>

CEEI is a group of leading corporations and business groups interested in the policies guiding how agencies collect, manage, use and disseminate information about health and environmental matters.<sup>2</sup> CEEI has a particular interest in promoting "information stewardship" – the obligation of agencies to present information about health and environmental matters in an accurate, balanced and understandable way.

CEEI believes that effective risk communication should be one of the highest policy and institutional priorities for EPA. While the Agency often thinks of itself as a regulatory agency, most Americans actually experience EPA primarily as an information source. The public sometimes obtains health and environmental information directly from EPA. More often, however, the public receives EPA information about health and environmental issues through intermediary parties, which may include state or local governments, media outlets of all kinds, business organizations, academic institutions and environmental organizations.

In all of these contexts, however, EPA has struggled at times to find ways to communicate its perspective in an understandably way. An ongoing example of the problem has been how EPA describes the hazards of chemicals to the public. EPA uses various forms of hazard labels that are intended to summarize the Agency's perspective. Some of these labels suggest higher levels of

<sup>1</sup> SAB Draft Report dated February 6, 2008 for Board Review, p. 19.

<sup>2</sup> CEEI includes representatives from the aerospace, chemical, automobile, petroleum, electronics and consumer products industries.

public danger than EPA truly intends (e.g., "likely human carcinogen" to express a weight of evidence evaluation, "chemicals of high concern" in describing priorities for further toxicity testing.)

EPA needs to improve its institutional capability to provide the public with useful, understandable information about health and environmental risk. Too often EPA pursues an "end of pipe" strategy to risk communication, viewing this function as essentially the issuance of a press release at the end of a project. In fact, EPA would better serve the public if it treated risk communication as an essential and ongoing component of its risk assessment and risk management responsibilities, drawing an analogy to how EPA would view environmental protection as a linchpin of sustainable development.

To accomplish this objective, EPA should make a greater commitment to risk communication on several fronts:

- Make risk communication an ongoing, rather than a concluding, component of its risk assessment and risk management policies;
- Incorporate risk communication responsibilities into the budgets for specific programs and projects;
- Establish a center of excellence on risk communication within the Agency that develops the knowledge base on risk communication research and provides pragmatic advice to program offices on specific issues;
- Provide relevant training on risk communication to EPA employees and reward employees for innovation in the field;
- Engage relevant stakeholders, the public health community and key public audiences to understand the public's expectations for useful information on risk-related matters; and
- Establish a policy-relevant research strategy on risk communication issues.

Our hope is that the SAB can work with EPA to identify a set of risk communication research topics that address the Agency's priority needs. We know that the SAB has several leading national experts in this field who could undoubtedly provide valuable insights on what issues warrant attention. While we do not claim to have a comprehensive plan for such research, we offer a few topics for your consideration, which reflect our experience with risk communication challenges:

- As suggested earlier in this letter, EPA uses hazard labels in its chemical risk assessment programs that sometimes convey a greater sense of public danger than EPA actually intends. How should EPA design hazard labels to match its own intended message?
- With the increasing sophistication of analytical techniques, EPA is able to detect and quantify the levels of chemicals in the body and in environmental media with much more precision. Over the last several decades, environmental data is moving steadily from parts per million measurements to parts per trillion measurements. EPA has had

difficulty explaining the health and environmental significance of low numbers that it can measure, finding itself unable to answer the question "Should I be concerned about this data?"

- It is difficult to address public concerns about low-probability, high-impact events. Yet emerging environmental issues are presenting more examples where this type of scenario is present. In the context of important topics like climate change or the environmental implications of new technology (e.g., biotechnology, nanotechnology), the public will sometimes hear experts discuss scenarios with Draconian outcomes (e.g., loss of major cities to flooding, uncontrolled self-replicating sources of disease or material destruction) that are theoretically possible but unlikely to occur as a practical matter. How should EPA communicate with the public about such matters, helping the public understand what is possible and what is probable?
- In its risk assessment activities of the last several years, EPA has emphasized the importance of characterizing the uncertainty that often surrounds risk assessment on particular topics. While such analysis can be helpful to policymakers, it is not clear how the various forms of uncertainty analysis (e.g., a risk range with a central tendency) are perceived or used by members of the public. Are there effective ways to discuss uncertainty with the public without conveying confusion and indecisiveness?
- When making decisions about the "acceptability" of health or environmental risks, consumers typically weigh an array of factors concerning the alternatives they have, the benefits of the risk-related activity, the social "fairness" of the risk and other values-based considerations. It is rare for EPA, or any government agency, to provide useful contextual information addressing those factors when communicating about health or environmental risk. It would be valuable to learn more about how individuals weigh various factors in interpreting risk-related information so that government agencies could more effectively provide relevant information to the public.

CEEI offers these ideas, which are recurring challenges for EPA, in the hope that we can stimulate broader discussion of a risk communication research agenda for the Agency. We certainly recognize that EPA and the SAB may identify other priority issues. Our primary goal, however, is to emphasize the overall importance of developing greater risk communication awareness and competence at the Agency.

The need to focus on this objective could never be greater. The public is receiving increasing flows of information about health and environmental issues, particularly from online sources. This flow of information is occurring at a time when the science of risk assessment is becoming more sophisticated, challenging even environmental professionals to understand the new techniques for conceptualizing, measuring and characterizing the interaction between environmental conditions and health or environmental effects. All of this is occurring as public interest, and anxiety, is increasing about matters as diverse as climate change, tainted consumer products from foreign countries, the potential for pandemics and possible threats from new technology.

The two topics that the SAB will be discussing at its upcoming meeting on October 27-28, 2008 are good examples of emerging issues that present risk communication challenges. The public is certainly receiving mixed messages about the economic and social impacts of biofuels. When Congress was enacting the Energy Independence and Security Act in December 2007, the public heard a fairly consistent message that greater use of home-grown biofuels was a critical national strategy that would yield advantages for the environment, energy prices and our national security. Within a few months, however, a variety of experts were characterizing the shift to biofuels as a primary cause of world hunger and higher prices for Americans at the grocery store.

The field of epigenomics is a new addition to the list of "nomics" research that seeks to explain how pollutants may interact with the body to cause adverse effects. As EPA pursues these new areas of scientific inquiry, it would be helpful for the Agency to offer an explanation of how these fields of research do and do not relate to basic questions of public health. Otherwise, the public could easily see the new "nomics" research as uncovering new forms of human disease, a fate that would be similar to the confusing messages about topics like endocrine disruption.

As the SAB discusses these two topics and considers the larger issues around the strategic directions for environmental research, we hope you will give high priority to the need for a strong risk communication research agenda and the development of EPA's institutional capabilities in this area. This is an essential mission-critical function for the Agency. The best scientific work that EPA can achieve will ultimately be a policy failure if it does not provide responsive and understandable answers to citizen questions about public health and safety.

Please let us know if we can assist this effort in any way. Thank you for your attention to this topic.

Respectfully submitted,



Mark A. Greenwood

10/1/08 Draft

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This draft SAB panel report has been prepared for quality review  
and approval of the chartered SAB.

This report does not represent EPA policy



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON D.C. 20460

OFFICE OF THE ADMINISTRATOR  
SCIENCE ADVISORY BOARD

Date to be inserted

Honorable Stephen L. Johnson  
Administrator  
U.S. Environmental Protection Agency  
1200 Pennsylvania Avenue, N.W.  
Washington, DC 20460

Subject: Review of EPA's, "Toxicological Review of Acrylamide".

Dear Administrator Johnson:

In response to a request from EPA's Office of Research and Development (ORD), the Science Advisory Board (SAB) convened an expert panel to conduct a peer review of EPA's draft Integrated Risk Information System (IRIS) assessment entitled, "*Toxicologic Review of Acrylamide*". This draft document updates EPA's current evaluation of the potential health effects of acrylamide.

The SAB was asked to comment on the hazard characterization and dose-response assessment of acrylamide, including the Agency's selection of the most sensitive non-cancer health endpoint, the use of a pharmacologically-based toxicokinetic (PBTK) model, the derivation of a proposed oral reference dose (RfD), an inhalation reference concentration (RfC) for non-cancer endpoints, as well as the cancer descriptor, oral slope factor, and inhalation unit risk for acrylamide. The SAB Panel's report contains a number of recommendations that are aimed at making the assessment more transparent and improving the scientific bases for the conclusions presented. The Panel's key points and recommendations are highlighted below:

- The Panel agreed with the EPA's conclusion that based on the existing toxicity data base for acrylamide, neurotoxicity does appear to be the most sensitive non-cancer endpoint, and therefore, the most appropriate for developing the RfD and RfC for non-cancer health effects.
- The Panel believed that the use of the benchmark dose methodology in this assessment was deemed scientifically supportable, given the nature and robustness of the data sets available on the endpoint of concern.
- The Panel supported the Agency's conclusions that exposure to acrylamide in animals leads to heritable gene mutations and that these results indicate that it may also pose a hazard to humans. In addition, the Panel supported the Agency's conclusions that the available data on heritable gene mutations are not adequate to conduct a robust assessment of this endpoint at this time. The

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**and approval of the chartered SAB.**  
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Panel urges further research on acrylamide-induced heritable germ cell mutations, given the serious nature of such effects.

- The Panel concluded that the rationale and justification for acrylamide being a “*likely human carcinogen*” via a mutagenic mechanism was well described and the conclusion was scientifically supportable, although it should be further elaborated.
- The Panel encouraged the Agency to use the two main chronic bioassays in rats for deriving the oral cancer slope factor and include an in depth discussion of the strengths and limitations of both studies.
- The Panel commends EPA for using the PBTK model for developing the RfD, RfC and cancer slope factor for acrylamide. The Panel did however provide some recommendations to the Agency for improving the model as they revise their draft document. The Panel notes that the use of internal dose metrics combined with a fairly robust understanding of the mechanism of action may replace the use of the default interspecies factor for toxicokinetic differences. Internal dose may be derived using the PBTK model or through application of other pharmacokinetic approaches indicated in the Panel report.
- The Panel agreed with the use of PBTK modeling to conduct dose-route extrapolation and commended the EPA for using the PBTK model to fill the gap resulting from the absence of robust animal toxicology studies investigating neurotoxicity via the inhalation route that would support the development of an RfC. In estimating the cancer slope factor and unit risk, human-rodent differences in pharmacokinetics were taken into account with the PBTK model, whereas pharmacodynamic differences were not, but should be, through the application of a standard factor.
- Finally, the Panel agreed that the use of the age-dependent adjustment factors (ADAF) to adjust the unit risk for early life exposure is well justified and transparently and objectively described.

The Panel appreciates the opportunity to provide EPA with advice on this important subject. A more detailed description of the technical recommendations is contained in the body of the report. We look forward to receiving the Agency’s response.

Sincerely,

Dr. Deborah Cory-Slechta, Chair  
SAB Acrylamide Review Panel

Dr. Deborah Swackhammer, Chair  
EPA Science Advisory Board

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## TABLE OF CONTENTS

1		
2	EXECUTIVE SUMMARY .....	7
3	Selection of Endpoint	7
4	Mechanism of Action	7
5	Derivation of RfD	8
6	Heritable Germ Mutations	8
7	Physiologically-Based Toxicokinetic (PBTK) modeling	9
8	Uncertainty Factors	9
9	Carcinogenicity	10
10	Derivation of the RfC	11
11	INTRODUCTION .....	13
12	Background	13
13	RESPONSES TO THE CHARGE QUESTIONS .....	14
14	Charge Question 1.	14
15	Charge Question 2.	16
16	Charge Question 3.	17
17	Charge Question 4.	19
18	Charge Question 5.	21
19	Charge Question 6.	22
20	Charge Question 7.	24
21	Charge Question 8	25
22	Charge Question 9.	31
23	Charge Question 10.	32
24	Charge Question 11.	32
25	Charge Question 12.	33
26	Charge Question 13.	34
27	Charge Question 14.	35
28	Charge Question 15.	37
29	Charge Question 16.	37
30	Charge Question 17.	38
31	Charge Question 18.	40
32	Charge Question 19.	42
33	Charge Question 20.	43
34	Charge Question 21.	45
35	Charge Question 22.	48
36	Charge Question 23.	48
37	Charge Question 24.	49
38	Charge Question 25.	50
39	Charge Question 26.	51
40	ABREVIATIONS .....	52
41	REFERENCES .....	53
42	APPENDIX A Charge Questions .....	63
43	APPENDIX B Proposed MOAs for Acrylamide Neurotoxicity.....	71

## **EXECUTIVE SUMMARY**

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3           This report was prepared by the Science Advisory Board (SAB) Acrylamide Review  
4 Panel (the “Panel”) in response to a request by EPA’s Office of Research and Development  
5 (ORD) to review the Draft IRIS Toxicological Review of Acrylamide (hereafter referred to as  
6 the draft document). The Panel deliberated on the charge questions (see Appendix A) during a  
7 March 10-11, 2008 face-to-face meeting and discussed its draft report in a subsequent conference  
8 call on July 16, 2008. There were 26 charge questions that focused on the selection of the most  
9 sensitive non-cancer health endpoint, the use of a PBTK model, the derivation of a proposed oral  
10 reference dose (RfD), an inhalation reference concentration (RfC) for non-cancer endpoints, as  
11 well as the cancer descriptor, oral slope factor, and inhalation unit risk for acrylamide. The  
12 Panel encourages the Agency to review relevant data which has been published since their draft  
13 assessment was completed as they revise and finalize the IRIS document.

14           This Executive Summary highlights the Panel’s major findings and recommendations as  
15 a result of their deliberations. The responses that follow represent the views of the Panel.

### *Selection of Endpoint*

16  
17  
18           In the draft document, EPA identified neurotoxicity as the most sensitive non-cancer  
19 effect from exposure to acrylamide. This endpoint was based on an extensive database of animal  
20 and human studies. Other endpoints were also considered, such as reproductive toxicity and  
21 heritable germ cell effects. The Panel agreed that based on the existing toxicity data base for  
22 acrylamide, neurotoxicity does appear to be the most sensitive non-cancer endpoint, and  
23 therefore, the most appropriate for developing the RfD and RfC for non-cancer effects from  
24 exposure to acrylamide.

### *Mechanism of Action*

25  
26  
27           The Panel discussed two hypotheses regarding the mechanism of acrylamide  
28 neurotoxicity. The Panel did not attempt to resolve the debate over a definitive or single MOA  
29 for neurotoxicity; however, there was agreement that the discussion of MOA is important for  
30 inclusion in the draft document. The Panel found the separation of the discussion of MOA(s) for

1 neurotoxicity in two different sections of the document confusing and recommended their  
2 incorporation into a single section. A more complete presentation by the Panel of these MOAs  
3 has been appended (see Appendix B) to this report for EPA's consideration as they revise their  
4 draft document.

5

6 *Derivation of RfD*

7 EPA's proposed RfD (0.003 mg/kg-day) for acrylamide is based on a benchmark dose  
8 analysis of the dose-response relationship for neurotoxicity in two chronic drinking water  
9 exposure bioassays using Fischer 344 rats. Uncertainty factors and a PBPK model were used to  
10 extrapolate the animal dose-response to a human equivalent dose-response in the derivation of  
11 the RfD. The Panel afforded considerable discussion to the question of whether the Friedman et  
12 al. (1995) and Johnson et al. (1986) studies were the best choices for derivation of the  
13 quantitative RfD (and RfC). The main concerns with these studies are that they were primarily  
14 designed as cancer bioassays and therefore did not include the most sensitive measures of  
15 neurotoxicity. Nevertheless, the Panel agreed that the selected studies did have some important  
16 strengths, including reasonable statistical power due to the relatively large number of animals,  
17 chronic dosing, and the fact that the NOAELs for the endpoint in the two studies were similar,  
18 implying some precision in the effect estimate measured. Several Panel members noted that the  
19 lack of sensitive functional/behavioral assessments is a significant data gap that should be  
20 considered in the context of setting a database uncertainty factor. Use of the benchmark dose  
21 methodology in this assessment was deemed scientifically supported, given the nature and  
22 robustness of the data sets available on the endpoint of interest. The calculations and choices  
23 made were described clearly and at an appropriate level of detail.

24

25 *Heritable Germ Mutations*

26 EPA's draft document concluded that data also exist that reveal acrylamide's capacity to  
27 induce heritable germ cell effects at doses somewhat above those at which neurotoxicity has  
28 been observed, but that there are as yet no studies providing an in-depth examination of dose-  
29 response or identification of credible no-effect levels. The Panel supports the Agency's  
30 conclusions that exposure to acrylamide in animals leads to heritable gene mutations and that

1 these results indicate that it may also pose a hazard to humans. In addition, the Panel supports  
2 the Agency's conclusions that the available data are not yet adequate to conduct a robust  
3 assessment of this endpoint at this time. There is still uncertainty about the mode of action of  
4 acrylamide and its metabolite, glycidamide, in the induction of heritable genetic effects. The  
5 potential for DNA adducts of glycidamide to play a role is an attractive hypothesis for the mode  
6 of action. The Panel found the discussion in the document on heritable germ cell effects useful  
7 and presented in a clear, transparent manner reflective of the current science. However, the Panel  
8 suggested that, given the serious consequences of heritable germ cell effects, the considerable  
9 deficiencies of the database should be identified and the significance of this endpoint  
10 emphasized.

11

#### 12 *Physiologically-Based Toxicokinetic (PBTK) modeling*

13 A physiologically-based toxicokinetic (PBTK) model originally developed by Kirman et  
14 al. (2003), and recalibrated by EPA with more recent kinetic and hemoglobin binding data in  
15 rats, mice, and humans, was used in the derivation of the RfD to extrapolate from the animal  
16 dose-response relationship to derive a human equivalent concentration. The Panel commends  
17 EPA for their efforts to adapt the PBTK model of Kirman et al. (2003) for acrylamide and  
18 glycidamide, recognizing that this was a complex and challenging task. The Panel believes,  
19 though, that the documentation is not adequate to determine whether the recalibrated Kirman  
20 model is appropriate for its intended use. While the Panel considered that the model structure  
21 was reasonable, the parameter estimates require greater justification. The Panel was concerned  
22 about the ability of the model to adequately simulate the kinetics of acrylamide and glycidamide.  
23 Several alternatives to the PBTK model have been proposed for making the estimates of internal  
24 dose in rats needed for both the non-cancer and cancer assessments and for calculating the  
25 Human Equivalent Dose (HED).

26

#### 27 *Uncertainty Factors*

28 EPA has proposed to use the default 10X uncertainty factors (UF) to account for  
29 intraspecies (i.e., human) differences. The Panel concurred with this choice, noting that there  
30 were insufficient data on inter-individual differences, based upon lifestage, gender or genetic

1 characteristics, to support departing from the default. Consensus was not achieved on the issue of  
2 the inclusion of an UF to account for deficiencies in the existing database.

3 EPA has suggested that the acrylamide IRIS document include a Table that lists points of  
4 departure for various endpoints to facilitate a Margin of Exposure (MOE) evaluation by EPA's  
5 Regional or Program offices, or by other end users of the assessment. The Panel recommends  
6 the inclusion of such a table, to the extent possible, in all IRIS documents which provides  
7 information that may be used to conduct a variety of MOE analyses for specific endpoints of  
8 interest and/or for other than lifetime durations of exposure and for windows of increased  
9 susceptibility early in the life cycle, in addition to the traditional lifetime focus. Agency risk  
10 assessments would benefit from the inclusion of transparently-developed, peer-reviewed  
11 consensus hazard values.

### 12 13 *Carcinogenicity*

14 The Panel believes that the rationale and justification for acrylamide being a "*likely*  
15 *human carcinogen*" has been well described and the conclusion is scientifically supportable  
16 based on the fact that it produces tumors in rodents in both sexes, that there are multiple tumor  
17 sites, and tumors are induced via multiple routes of exposure. Acrylamide is also clearly and  
18 reproducibly carcinogenic in both rats and mice. Nonetheless, the draft document can be  
19 improved by expanding the discussion of biological plausibility and coherence beyond DNA  
20 adducts. The weight of evidence supports a mutagenic mode of action for carcinogenesis, and  
21 overall the rationale has been clearly and objectively presented. Significant biological support  
22 and data on any putative alternate MOAs are not sufficient for either explaining cancer findings  
23 or quantifying dose response relationships. More than one MOA may operate for a given  
24 carcinogenic chemical, and the likelihood that more than a single MOA is operative increases as  
25 levels of exposure increase.

26 EPA used two chronic drinking water exposure bioassays in Fischer 344 rats (Friedman  
27 et al., 1995; Johnson et al., 1986) to derive the oral cancer slope factor, and to identify the tumors  
28 of interest for the MOA discussion. The Panel agrees that the two chronic bioassays in F344  
29 rats are the main studies to consider in dose response analysis, but the rationale for using only the  
30 Friedman et al. study for derivation of the oral cancer slope factor should be improved with the

1 strengths and limitations of both studies discussed in greater depth The use of the Weibull-in-  
2 time multistage-in-dose analysis is a reasonable and scientifically justifiable way to take into  
3 account the early mortality in the high dose group in the male study. The decision not to employ  
4 this analysis, in the case of the female because mortality across treatment and control groups did  
5 not differ and the overall survival appears to be fairly good, is also reasonable.

6 The draft document used area under the curve (AUC) in the blood for the putative  
7 genotoxic metabolite, glycidamide, as the dose metric for the PBTK model analysis to derive the  
8 human equivalent concentration. The Panel agreed that the AUC for glycidamide is the best  
9 choice for estimating the human equivalent concentration to derive the oral slope factor. One  
10 consideration in using this as the dose metric, however, comes from some of the human studies  
11 in which variability is not accounted for adequately. Consideration of additional human data can  
12 provide an improved basis for adjustments for cross-species differences in pharmacokinetics, as  
13 well as human variability in glycidamide formation from acrylamide.

#### 14 15 *Derivation of the RfC*

16 As with the RfC, EPA concluded that there were insufficient inhalation data to derive an  
17 inhalation unit risk (IUR). The PBTK model was used in a route-to-route extrapolation of the  
18 dose-response relationship from the oral data, and to estimate the human equivalent  
19 concentration for inhalation exposure to acrylamide. The Panel agreed with the use of PBTK  
20 modeling to conduct dose-route extrapolation and commended the EPA for using the PBTK  
21 model to fill the gap resulting from the absence of robust animal toxicology studies investigating  
22 neurotoxicity via the inhalation route that would support the development of an RfC. The Panel  
23 agreed that the absence of evidence for route of entry specific effects would allow route-to-route  
24 extrapolation for deriving an RfC based on using the PBTK model to calculate the human  
25 equivalent concentration (HEC).

26 The Panel agreed that the recommendation to use the age-dependent adjustment factors is  
27 well justified and transparently and objectively described. Additionally the Panel believed that  
28 the discussion of uncertainties is adequate, but that human variability could be more completely  
29 addressed. There is no characterization of sensitive populations, and this should be explored and  
30 discussed to a much greater extent.

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The Panel commends EPA for using the PBTK model for developing the RfD, RfC and

3

Cancer Slope Factors for acrylamide. The Panel notes that the use of internal dose metrics

4

combined with a fairly robust understanding of the mechanism of action may replace the use of

5

the default interspecies factor for toxicokinetic differences (i.e.,  $10^{1/2}$ ), but not the default

6

interspecies factor for pharmacodynamics. This factor is still needed in deriving the RfC and

7

RfD. Further the Panel strongly encourages the Agency to move forward with revising and

8

finalizing their assessment.



## RESPONSES TO THE CHARGE QUESTIONS

**Charge Question 1. *Please comment on the selection of neurotoxicity as the most appropriate choice for the most sensitive endpoint (in contrast to reproductive toxicity, heritable germ cell effects, or other endpoint) based upon the available animal and human data.***

Based on the existing toxicity data base for acrylamide, neurotoxicity does appear to be the most sensitive endpoint, and therefore, the most appropriate for developing the (non-cancer) RfD and RfC. Animal studies report microscopically-detected degeneration in peripheral nerve cells at doses of 1-2 mg/kg day, as compared to levels of 3-13 mg/kg day to detect impaired male reproductive performance. Animal studies provide a clear mechanistic understanding whereby low-dose, subchronic exposure leads to toxicity with concomitant nerve damage. Acrylamide has a direct or indirect effect on the motor protein kinesin or nerve terminals, producing damage in the peripheral and central nervous systems, which leads to sensory and motor disease. Correspondingly, reports of central-peripheral neuropathy, ataxia and muscle weakness in exposed human cohorts have been documented since the early 1950's. Acute occupational exposure to acrylamide can lead to an immediate neurologic response, e.g., sweating, nausea, myalgia, numbness, paresthesia, and weakened legs and hands. Following termination of short term exposure, these acute effects disappear.

There were issues of concern that should be noted:

- 1) As detailed in the response to Question 4, the determination of accurate benchmark doses (e.g., LOAELs, NOAELs, RfDs) from the Friedman et al. (1995) and Johnson et al. (1986) studies may be compromised by their lack of functional testing of neurotoxicity and the use of a relatively insensitive measure, peripheral axonopathy, as the primary index neurotoxicity.
- 2) There was concern that axonal degeneration observed under light microscopy was the endpoint chosen from the Friedman et al. (1995) and Johnson et al. (1986) studies for derivation of the RfD and RfC. Animal studies indicate that nerve terminal degeneration can occur prior to axonal degeneration at some doses. This would suggest that all of the cited

1 studies, including the subchronic Burek study and the 2 year bioassay studies of sciatic nerve  
2 (Friedman et al, 1995) and tibial nerve (Johnson et al, 1986) axons, in looking at axonal  
3 degeneration, may have missed a preceding terminal degeneration at a lower dose,  
4 particularly as no specific mention of terminal degeneration is provided and  
5 functional/behavioral measures of neurotoxicity were not included.

- 6 3) It should be noted that future studies may demonstrate effects of acrylamide exposure on  
7 male reproductive function, as currently evidenced in animal studies by increased pre- and  
8 post-implantation losses and decreased litter sizes, at even lower doses than those currently  
9 associated with neurotoxicity after acrylamide dosing in animal studies. The draft document  
10 states that “associations between human exposure to acrylamide and reproductive effects  
11 have not been reported” (p. 187 and p. 224); rather, these associations *have not been studied*.  
12 The lack of human data is a major limitation in this regard. As noted in the draft document,  
13 data also exist that reveal acrylamide’s capacity to induce heritable germ cell effects at doses  
14 somewhat above those at which neurotoxicity has been observed, but there are as yet no  
15 studies providing an in-depth examination of dose response or identification of credible no-  
16 effect levels. The heritable germ cell effects are very worrisome and deserve even more  
17 consideration, including perhaps the use of this endpoint to generate an independent RfD.
- 18 4) Although still controversial and recognizing that cigarette smoke is a complex mixture made  
19 up of hundreds of compounds, there is growing evidence that supports an association  
20 between cigarette smoking, a known source of acrylamide exposure, and altered semen  
21 parameters, including concentration, morphology, motility, and DNA fragmentation  
22 (Richthoff et al., 2008; Sepaniak et al., 2006; Marinelli et al., 2004). The lack of data  
23 regarding potential interactions between acrylamide and other exposures, including cigarette  
24 smoke, alcohol use, and cosmetics (another source of acrylamide exposure) has been cited as  
25 a major limitation in studies of human acrylamide exposure and adverse health effects (Rice  
26 2005; draft document p.194; p. 224). The investigation of altered semen parameters among  
27 occupationally exposed males, controlling for smoking and alcohol consumption, should be a  
28 high priority.

29  
30 New References

1 Richthoff J, Elzanaty S, Rylander L, Hagmar L, Giwercman A. Association between  
2 tobacco exposure and reproductive parameters in adolescent males. *Int J Androl* 2008; 31:31-9.

3 Sepaniak S, Forges T, Foliguet B, Bene MC, Monnier-Barbarino P. The influence of  
4 cigarette smoking on human sperm quality and DNA fragmentation. *Toxicol* 2006; 223:54-60.

5 Marinelli D, Gaspari L, Pedotti P, Taioli E. Mini-review of studies on the effect of  
6 smoking and drinking habits on semen parameters. *Toxicol* 2004; 207:185-92.

7  
8 **Charge Question 2. *Please comment on the discussion of mode of action for acrylamide-***  
9 ***induced neurotoxicity.***

10  
11 The Panel found the separation of the discussion of MOA(s) for neurotoxicity in two  
12 different sections of the document (Section 4.6.1, pages 123-124; and Section 4.7.3, pages 134-  
13 136) confusing and recommends their incorporation into a single section.

14 Acrylamide is a member of the type-2 alkene chemical class, which includes acrolein,  
15 methylvinyl ketone and methyl acrylate. A weight of evidence evaluation of the current body of  
16 data now suggests that the type-2 alkenes produce toxicity via a common molecular mechanism;  
17 i.e., formation of adducts with essential sulfhydryl thiolate groups on proteins that play  
18 regulatory roles in cellular processes (LoPachin et al., 2007a,b, 2008a; reviewed in LoPachin and  
19 Barber, 2006b; LoPachin et al., 2008b).

20 Currently, there are two hypotheses regarding the mechanism of acrylamide  
21 neurotoxicity: 1) Acrylamide/glycidamide inhibits fast axonal transport by forming adducts with  
22 kinesin, the transport motor (reviewed in Sickles et al., 2002). 2) Acrylamide disrupts nerve  
23 nitric oxide (NO) signaling at the nerve terminal (reviewed in LoPachin et al., 2006a). The Panel  
24 did not attempt to resolve the debate over the MOA of neurotoxicity. It is also possible that both  
25 MOAs may be pertinent, and studies directly comparing the time course of the two proposed  
26 MOAs in a single model have not been carried out. However, the Panel agreed that the further  
27 delineation of MOAs will improve acrylamide risk assessment. Both of the proposed MOAs  
28 suggest that visible axonal degeneration on light microscopy is not likely to be the low-dose  
29 effect in the causal pathway. Regardless, it should also be evident that substantial, detailed

1 molecular information is available regarding mechanisms of acrylamide neurotoxicity and that  
2 these data should be included.

3

4 Thus, the following deficiencies in the draft document were identified by the Panel:

- 5 1) As drafted, the document's coverage of research findings is incomplete and does not  
6 adequately reflect the current molecular understanding of the mechanisms of acrylamide  
7 neurotoxicity. Moreover, information in the document regarding the hypothesized MOAs is  
8 not presented in a sufficiently transparent manner consistent with the Agency's guidance on  
9 identification of the key events leading to the effect of concern, i.e., use of the modified  
10 Bradford Hill criteria with respect to dose-response concordance, temporal relationship(s),  
11 strength, consistency, specificity of association and biological plausibility and coherence, as  
12 is done for carcinogenicity.
- 13 2) There was insufficient discussion of acrylamide adduct chemistry and corresponding  
14 neuronal targets pertinent to understanding the MOAs.
- 15 3) There was lack of a discussion of residual questions surrounding the respective roles of the  
16 parent toxicant, acrylamide, and its epoxide metabolite, glycidamide, in the production of  
17 neurotoxicity.

18

19 The Panel recommends that the Agency expand its discussion of the two MOAs. Panel  
20 members provided more specific text that describes the two proposed MOAs, and the Panel  
21 offers this text to EPA for consideration in revising the acrylamide assessment. The text is given  
22 in Appendix B of this report.

23

24 **Charge Question 3. *Please comment on the qualitative discussion of acrylamide's heritable***  
25 ***germ cell effects and whether the discussion is clear, transparently and objectively described,***  
26 ***and reflective of the current science.***

27

28 Discussion in the document of heritable germ cell effects, consisting of 5 heritable  
29 translocation studies, the 2 specific locus studies, 2 studies on acrylamide transformation to  
30 glycidamide and the importance of this metabolism to toxicity, is relevant and useful, and is

1 presented in a clear, transparent manner reflective of the current science. However, the  
2 discussion is a linear description of germ cell toxicity with little synthesis, analysis and scrutiny.  
3 While some SAB members considered the presentation objective, some expressed concerns over  
4 the lack of inclusion of all potential MOAs. Given the serious consequences of heritable germ  
5 cell effects, the considerable deficiencies of the database should be identified and the  
6 significance of this endpoint emphasized.

7 The entire section is prefaced and summarized with the perspective that DNA adduct  
8 formation and mutagenicity is the only operative mechanism for heritable germ cell effects of  
9 acrylamide. While adducts can certainly lead to the observations, there are alternative  
10 mechanisms for discussion. Clastogenic mechanisms, as well as, mitotic spindle defects are  
11 viable candidates for dominant lethal effects. There is a wealth of acrylamide studies reporting  
12 these alternative mechanisms that should be included in this discussion as well. They were  
13 briefly outlined in the carcinogenicity section, but should also be identified here. In regards to  
14 spindle defects, the effects of acrylamide on kinesin motors involved in cell division should be  
15 added to the document (Sickles et al., 2007).

16 Adequate response data are lacking in the existing heritable germ cell studies such that  
17 the shape of the dose response relationship cannot be ascertained. However, in Tyl et al (2000)  
18 dose responses are identified - a NOAEL of 2 mg/kg/d and a LOAEL of 5 mg/kg/d for a 13 week  
19 exposure. All of the dominant lethal studies were conducted at a dose of 50 mg/kg or higher and  
20 most with multiple exposures. The specific locus studies were conducted at 50 mg/kg/d for 5  
21 days (Russel et al., 1991) or with a single 100-125 mg/kg exposure (Ehling and Neuhauser-  
22 Klaus, 1992). The discrepancy between the negative results of Russel et al. (1991) and the  
23 positive results of Ehling and Neuhauser-Klaus (1992) may be dose-related or due to other  
24 factors. The fact that heritable translocations appeared at high frequency at the lowest doses  
25 tested implies that even lower doses may produce such effects.

26 However, in the absence of these data, the uncertainty should be identified. As a  
27 consequence of these limitations in the database, there is some uncertainty related to the RfD.  
28 The Panel unanimously agreed that this is an extremely serious data gap that should be a top  
29 priority for further study. Additional studies to address the aforementioned database deficiencies  
30 in mechanisms and dose-responses would be desirable.

1 The document requires correction in that the NTP/CERHR report was published in  
2 February 2005, not 2004. Also, there appears to be a discrepancy in the text (Pg 117 indicates the  
3 historical controls were 6%, yet on pg 116 in the discussion of the Adler et al. (1994) study, the  
4 historical controls are listed as 5/9890 which is 0.05%).

5  
6 **Charge Question 4. *Please comment on whether the selection of the Friedman et al, 1995***  
7 ***and Johnson et al, 1986 studies as co-principal studies has been scientifically justified.***  
8 ***Although EPA considers Friedman et al and Johnson et al to be co-principal studies, the final***  
9 ***quantitative RfD value is derived only from the Johnson study. Please comment on this aspect***  
10 ***of the EPA's approach. Please comment on whether this choice is transparently and***  
11 ***objectively described in the document. Please identify and provide the rationale for any other***  
12 ***studies that should be selected as the principal studies.***

13  
14 The Panel afforded considerable discussion to the question of whether the Friedman et al  
15 (1995) and Johnson et al (1986) studies were the best choices for derivation of the quantitative  
16 RfD (and RfC). The main concerns with these studies included the fact that they were primarily  
17 designed as cancer bioassays rather than for evaluation of neurotoxicity. Specifically, the Panel  
18 contended that the endpoint of axonal degeneration visible under light microscopy is an  
19 insensitive measure of neurotoxicity. Alterations visible under electron microscopy or  
20 functional/behavioral alterations would have provided more sensitive endpoints.

21 Nevertheless, the Panel agreed that the selected studies did have some important  
22 strengths, including reasonable statistical power due to the relatively large number of animals,  
23 chronic dosing, and the fact that the NOAELs for the endpoint in the two studies were similar,  
24 implying some precision in the effect estimate measured. The Panel also noted that there are no  
25 studies yet available which include the sensitive functional/behavioral assessments that would be  
26 most desirable. Several Panel members noted that this issue is a significant data gap that should  
27 be considered in the context of setting a database uncertainty factor.

28 With respect to the Burek et al. (1980) study, the Panel notes that while the endpoint in  
29 this study (axolemmal invaginations under electron microscopy) is a highly sensitive one for use  
30 in risk assessment, the study was subchronic. One Panel member proposed that EPA consider

1 generating an RfD based on the data in Burek et al. (1980), but not use a subchronic-to-chronic  
2 uncertainty factor given the existence of the two chronic studies, to compare the resulting RfD to  
3 that based on the less sensitive endpoint of axonal degeneration. Such a comparison might begin  
4 to quantify the degree of potential under-estimate of risk due to the less satisfactory choice of  
5 endpoint in the Johnson and Friedman studies.

6 There was a brief discussion of the report of foot splay at 0.5 mg/kg in F<sub>0</sub> males in the  
7 Tyl et al. (2000a) two-generation reproductive toxicity/dominant lethal mutation study. The use  
8 of this gross functional endpoint could also serve as a point of departure, although it was  
9 considered questionable because: it was only observed in the F<sub>0</sub> generation, was found in control  
10 animals to some degree (raising questions about the methodology used in the lab), and did not  
11 follow a clear dose-response relationship. Overall, the Panel decided that the Tyl study was not a  
12 good choice for derivation of the RfD.

13 The Panel also considered the option of deriving an RfD based on human data. Both the  
14 Calleman et al. (1994) and the Hagmar et al. (2001) studies contain sufficient data to allow the  
15 Agency to calculate an RfC or potentially an RfD. In this regard, the Panel made the following  
16 observations: (1) in general, it is preferable to use human data when available; (2) the Calleman  
17 study included a measure of internal dose (adduct levels) and a fairly sensitive measure of effect,  
18 thereby making it appealing for risk assessment; (3) PBTK modeling could allow dose  
19 extrapolation based on adduct levels, such that an ingested or inhaled dose could be estimated for  
20 purposes of setting either an RfC or an RfD from the data.

21 However, the Panel also cautioned that there are a number of drawbacks to using the  
22 human studies, including the following: (1) the sample sizes are small; (2) the samples mostly  
23 include young adult males; (3) the healthy worker effect would tend to bias these studies  
24 (especially the Calleman study) toward the null, since workers with significant neurological  
25 symptoms would leave the workplace, thus selecting for individuals with lower genetic  
26 susceptibilities; (4) the workers in each study were exposed to other confounding neurotoxicants  
27 (acrylonitrile and *N*-methylolacrylamide (NMA)), but this would tend to generate a more  
28 conservative risk estimate because these other exposures would tend to result in an over-estimate  
29 of the effect; and (5) the exposure duration was relatively short and variable (1 month to 11.5  
30 years in the Calleman study with an average of 3 years, and 55 days in the Hagmar study). In the

1 end, the Panel suggested that EPA undergo the exercise of generating an RfD from the Calleman  
2 study for purposes of comparison with the RfD derived based on the animal data. The Panel  
3 stopped short of recommending that the human RfD be used in place of the one in the draft  
4 document, but instead saw this as a type of sensitivity analysis, to help determine whether the  
5 RfD based on the Johnson study appears to be adequately health-protective despite the  
6 insensitive endpoint used in that study.

7  
8 **Charge Question 5. *Please comment on the benchmark dose methods and the choice of***  
9 ***response level used in the derivation of the RfD, and whether this approach is accurately and***  
10 ***clearly presented. Do these choices represent the most scientifically justifiable approach for***  
11 ***modeling the slope of the dose-response for neurotoxicity? Are there other response levels or***  
12 ***methodologies that EPA should consider? Please provide a rationale for alternative***  
13 ***approaches that should be considered or preferred to the approach presented in the document.***  
14

15 Use of the benchmark dose methodology has become the preferred approach and an  
16 acknowledged improvement over the historically traditional NOAEL  $\div$  UF procedure for the  
17 derivation of RfDs. Its application in this instance is scientifically supported, given the nature  
18 and robustness of the data sets available for the endpoint of interest. The calculations and  
19 choices made were described clearly at an appropriate level of detail.

20 EPA's Benchmark Dose guidance provides default criteria to be used for selecting the  
21 benchmark response (BMR). For quantal data, an excess risk of 10% is the default BMR, since  
22 the 10% response is at or near the limit of sensitivity in most studies. In this case, even though  
23 the BMR at 10% extra risk also was within the range of observation, the BMR<sub>5</sub> was selected for  
24 the point of departure. The choice of a BMR<sub>5</sub> makes sense and is well-justified: (1) the 95%  
25 lower bound of the benchmark dose (BMD), BMDL<sub>5</sub>, remained near the range of observation;  
26 (2) the 5% extra risk level is supportable given the relatively large number of animals used in the  
27 critical studies; and (3) the use of BMDL<sub>5</sub> is consistent with the Agency's technical guidance for  
28 BMD analysis which allows flexibility in making such a choice. One of the strengths of the  
29 Johnson study is that it is sufficiently large (i.e., numbers of animals/group) to allow the lower

1 5% bound to be identified with sufficient stability that it is usable for risk assessment purposes.  
2 Therefore, it is reasonable to use that strength in the underlying data set and choose this number.  
3 Such a choice is appropriately conservative (i.e., public health protective).

4 While alternative approaches such as averaging the BMDLs from each of the four data  
5 sets (Friedman and Johnson, male and female) rather than using just the one for males in the  
6 Johnson study were discussed, the Panel concluded that the steps described by the Agency in the  
7 draft document represented the preferred approach.

8  
9 **Charge Question 6. Please comment on the selection of the uncertainty factors (other than the**  
10 **interspecies uncertainty factor) applied to the point of departure (POD) for the derivation of**  
11 **the RfD. For instance, are they scientifically justified and transparently and objectively**  
12 **described in the document? [Note: This question does not apply to the interspecies uncertainty**  
13 **factor which is addressed in the questions on the use of the PBTK model (see PBTK model**  
14 **questions below)]**

15  
16 The Agency has proposed to use a composite uncertainty factor (UF) of 30: 10X to  
17 represent human variability ( $10_H$ ) and 3X to reflect the toxicodynamic component of the default  
18 interspecies uncertainty factor ( $10_A$ ). The other half of the 10x interspecies UF, i.e., the 3X that  
19 would otherwise account for interspecies differences in toxicokinetics, is subsumed in the PBTK  
20 modeling.

21 Two points were raised about the use of 3X as a default to account for interspecies  
22 toxicodynamic differences. First, it was noted that the rodents are less sensitive to the neurotoxic  
23 effects of acrylamide than humans. The Panel concluded that the application of a UF for  
24 interspecies toxicodynamics was directionally correct. Second, there is insufficient information  
25 available to define a chemical-specific factor and the default factor of 3X UF for interspecies in  
26 pharmacodynamics is therefore appropriate. It was noted that recent International Programme  
27 for Chemical Safety guidelines divide the default  $10_A$  into 2.5X for toxicodynamic differences  
28 and 4.0X for toxicokinetics differences, based primarily upon a review of the literature published  
29 in 1993 -(WHO IPCS 2005. *Guidance Document for the Use of Data in Development of*  
30 *Chemical-specific Adjustment Factors (CSAFs) for Interspecies Differences and Human*

1 *Variability in Dose/Concentration-Response Assessment*). The use of the factor of 3 (or  $\sqrt{10}$ ) is  
2 consistent with current EPA practice: according to the recent EPA (2004) Staff Paper “a default  
3 UF of 10 for interspecies variability that can now be reduced to 3 when animal data are  
4 dosimetrically adjusted to account for toxicokinetics.” The Staff paper cites the EPA (2002)  
5 RfD/RfC methodology document. That document divides UFs “into toxicokinetic and  
6 toxicodynamic components that have assigned default values of 3.16 ( $10^{1/2}$ ) each.”

7 EPA has proposed to use the default 10X UF to account for intraspecies (i.e., human)  
8 differences. The Panel concurred with this choice, noting that there were insufficient data on  
9 interindividual differences, based upon lifestage, gender or genetic characteristics, to support  
10 departing from the default.

11 Consensus was not achieved on the issue of the inclusion on an UF to account for  
12 deficiencies in the existing database that would confound the derivation of the most  
13 scientifically-defensible RfD. EPA concluded that an  $UF_D > 1$  was not necessary, arguing that  
14 the existing database is sufficiently robust, even though they acknowledge there are some  
15 unresolved issues that warrant further research: describing the MOA(s) for neurotoxicity, the  
16 potential for behavioral or functional adverse effects not detected in the assays to date, and the  
17 uncertainty that heritable germ cell effects may occur at lower than previously reported doses.  
18 Some Panel members agreed with EPA’s position. One Panel member noted that additional UFs  
19 were implicitly, if not explicitly, incorporated into the RfD derivation. Using the output of the  
20 log-logistic model applied to the data set for the male rats in the Johnson study resulted in the  
21 lowest set of BMDs/BMDLs. According to one Panel member, it was perhaps conferring an  
22 extra UF of ~2X. In addition, using the BMDL<sub>5</sub> as the POD, rather than the default BMDL<sub>10</sub>,  
23 also could be seen as conferring an extra UF of ~2X.

24 Other Panel members, however, disagreed with the Agency’s position regarding the  
25 database UF, arguing that the remaining uncertainties have major implications that could result  
26 in effects at significantly lower doses and thus a lower RfD. Database deficiencies include the  
27 following:

- 28
- 29 1) EPA had to rely on the observation of axonal degeneration visible by light microscopy,  
30 an endpoint which is not likely to be the most sensitive. EPA is using studies that were

1 not designed to evaluate neurotoxicity robustly, e.g., histopathology coupled with  
2 systematic evaluation of functional or behavioral parameters at multiple time points with  
3 robust numbers of animals/treatment and robust number of treatment groups; these  
4 studies should be done in adult animals and in a developmental neurotoxicity study in  
5 order to determine whether or not critical lifestage differences exist;

6 2) Both existing chronic studies were done in the rat, creating some remaining uncertainty  
7 about interspecies differences that is not addressed by the interspecies UF. Based upon  
8 the comparison of results from the Tyl et al (2000) 2-generation study in rats and the  
9 Chapin et al. (1995) 2-generation study in mice, the NOAEL for (adult) neurotoxicity is  
10 essentially the same (0.5 mg/kg/day in rats vs. 0.8 mg/kg/day in mice), but the difference  
11 could potentially be driven by dose spacing rather than a true difference in response. The  
12 outcomes of long-term exposure in mice hold the possibility of yielding lower  
13 NOAELs/LOAELs/BMDs than observed/calculated from the rat data. If this were to  
14 occur, the RfD/RfC would be lower.

15 3) The germ cell effects have not been fully explored and have major intergenerational  
16 implications if they do occur at dose levels lower than those for neurotoxicity. There is a  
17 lack of adequate data to define the dose response for heritable germ cell effects. While  
18 the existing data describe adverse effects at doses somewhat higher than those at which  
19 neurotoxicity was observed, BMD modeling of robust dose-response data may yield  
20 results competitive with/lower than the neurotoxicity BMDs/BMDLs.

21  
22 **Charge Question 7. *Please provide any other comments on the derivation of the RfD and on***  
23 ***the discussion of uncertainties in the RfD.***

### 24 25 **Acrylamide and Cumulative Risk Assessment**

26 The Food Quality Protection Act (FQPA) of 1996 mandates EPA to consider the  
27 “cumulative effects” of pesticides and other substances that have a “common mechanism of  
28 toxicity” when setting, modifying or revoking tolerances for food use pesticides. Were  
29 acrylamide registered as a food use pesticide, its activity as a type-2 alkene would support a  
30 cumulative risk assessment of it and other chemicals in the class. From a scientific standpoint

1 and particularly from a public health perspective, they should be subjected to a cumulative risk  
2 assessment (e.g., see Wilkinson et al., 2000). Evaluating the cumulative effects of the type-2  
3 alkenes is particularly germane since human exposure is pervasive; i.e. chemicals in this class are  
4 used extensively in the agricultural, chemical and manufacturing industries. Furthermore, they  
5 are well-recognized environmental pollutants (e.g., acrolein, acrylonitrile), food contaminants  
6 (e.g., acrylamide, methyl acrylate) and endogenous mediators of cellular damage (e.g., acrolein,  
7 4-hydroxy-2-nonenal) (see LoPachin et al., 2008b). Thus, the application of standard approaches  
8 may result in RfDs and RfCs which could be associated with risks in the population. At a  
9 minimum, a caveat in this regard should be included in the acrylamide assessment document.

## 11 **Charge Question 8**

### 12 *Use of the PBTK Model*

13 *A physiologically-based toxicokinetic (PBTK) model originally developed by Kirman et*  
14 *al. (2003), and recalibrated by EPA with more recent kinetic and hemoglobin binding data in*  
15 *rats, mice, and humans (Boettcher et al., 2005; Doerge et al., 2005a,b; Fennell et al., 2005)*  
16 *was used in the derivation of the RfD to extrapolate from the animal dose-response*  
17 *relationship (observed in the co-principal oral exposure studies for neurotoxicity) to derive a*  
18 *human equivalent concentration (HEC). The HEC is the external acrylamide exposure level*  
19 *that would produce the same internal level of parent acrylamide (in this case the area under*  
20 *the curve [AUC] of acrylamide in the blood) that was estimated to occur in the rat following*  
21 *an external exposure to acrylamide at the level of the proposed point of departure, and related*  
22 *to a response level of 5% (i.e., the BMDL<sub>5</sub>). The model results were used in lieu of the default*  
23 *interspecies uncertainty factor for toxicokinetics differences of 10<sup>1/2</sup>, which left a factor of*  
24 *10<sup>1/2</sup> (which is rounded to 3) for interspecies differences in toxicodynamics.*

25 *With respect to the RfC, there are presently insufficient human or animal data to*  
26 *directly derive an RfC for acrylamide. The PBTK model was thus used to conduct a route-to-*  
27 *route extrapolation (oral-to-inhalation) to derive an RfC based on the dose-response*  
28 *relationship observed in the co-principal oral exposure studies for neurotoxicity. In this case,*  
29 *the HEC was based on a continuous inhalation exposure to acrylamide in the air that would*  
30 *yield the same AUC for the parent acrylamide in the blood as that estimated for the rat*

1 *following an external oral exposure to acrylamide at the level of the proposed point of*  
2 *departure (i.e., the BMDL<sub>5</sub>).*

3  
4 *Please comment on whether the documentation for the recalibrated Kirman et al. (2003)*  
5 *PBTK model development, evaluation, and use in the assessment is sufficient to determine if*  
6 *the model was adequately developed and adequate for its intended use in the assessment.*

7 *Please comment on the use of the PBTK model in the assessment, e.g., are the model structure*  
8 *and parameter estimates scientifically supportable? Is the dose metric of area-under-the-*  
9 *curve (AUC) for acrylamide in the blood the best choice based upon what is known about the*  
10 *mode of action for neurotoxicity and the available kinetic data? Please provide a rationale for*  
11 *alternative approaches that should be considered or preferred to the approach presented in the*  
12 *document.*

13  
14 The Panel commends EPA for their efforts to adapt the PBTK model of Kirman et al.  
15 (2003) for acrylamide and glycidamide, recognizing that this was a complex and challenging  
16 task. The modified Kirman et al. model was produced by changing the model initially described  
17 for the rat, and adapting it to fit updated data published since the original publication in 2003,  
18 and to describe pharmacokinetics in humans. Three major modifications were described to the  
19 partition coefficients for glycidamide, the metabolic rate constants for oxidation and conjugation,  
20 and the partition coefficients for acrylamide. The simulations of the modified Kirman model  
21 were presented as tables containing comparisons of AUC data, and the extent of metabolism of  
22 acrylamide to glycidamide, and the extent of conjugation of each with glutathione.

23 However, the Panel had a number of concerns about the description of the model, and its  
24 parameterization. The Panel believed that the documentation is not adequate to determine  
25 whether the recalibrated Kirman model is appropriate for its intended use. Among the items that  
26 the Panel would like to see to justify the performance of the model are: the model code;  
27 graphical presentation of the data for time course simulations; and graphical presentation of dose  
28 response simulated by the model. Side by side comparisons of the model parameters for the rat  
29 and human could be accomplished by combining Tables E-4 and E-6.

30

1 The Panel noted that the model with some changes has been described in a manuscript  
2 published in 2007 by Walker et al. If life stage considerations are planned for subsequent work,  
3 PBTK modeling is the recommended tool for dosimetry estimates across life stages. The Panel  
4 would like to see the model used to simulate or show the degree of consistency with data  
5 published since 2005.

6 The Panel also noted that there have been additional studies of acrylamide, its metabolites  
7 and adducts, with varying data quality, and varying understanding of exposures. For example,  
8 exposures in smokers are likely a composite of exposure from diet (oral) and smoke (inhalation).  
9 There are possible ambiguities in assignment of acrylamide and glycidamide metabolites (the  
10 acrylamide mercapturic acid sulfoxide and the glycidamide mercapturic acids are isomeric, and  
11 need to be resolved chromatographically for appropriate quantitation). The Panel suggests that  
12 EPA review these reports for data quality and suitability, and if appropriate use them in  
13 evaluation/refinement of the model.

14 The Panel noted discrepancies between the PBTK predicted and measured critical dose  
15 metrics for the non-cancer (acrylamide AUC) or cancer (glycidamide AUC) PODs following  
16 drinking water exposures in rats (see table below).  
17

			EPA PBTK Model Predictions	Tareke/Doerge Measured Data (2005, 2006)
EGV	BMDL (mg/kg/day)	Critical Dose Metric	Internal dose (uM-hr)	Internal dose (uM-hr)
RfD	0.27	AA_AUC	18.1	4.2
oral cancer	0.3	GA_AUC	15.1	4.7

18  
19  
20 The draft document notes that the data of Doerge et al. (2005 a,b) were available (page E-  
21 5), but it is not clear if the data were actually considered in updating the model.

1           While the Panel concluded that the model structure was reasonable, the parameter  
2 estimates require greater justification. The review notes (Page E-18 last paragraph) that: “In  
3 comparing different versions of the model, it was also noted that the model parameters were  
4 underdetermined, that is, there is just not enough basic pharmacokinetic data to derive a unique  
5 set of optimal parameter values, given the number of “adjustable” parameters in the current  
6 model.”

7           The Panel was concerned about the ability of the model to adequately simulate the  
8 kinetics of acrylamide and glycidamide. There is little justification presented for the adjustment  
9 of parameters from the original Kirman model. The method of optimization was not well  
10 described. The comparisons provided between observed data and model simulations are largely  
11 for AUC in tables. Thus it is difficult to determine how the model would perform under the kind  
12 of tests usually applied to a model, including the ability to fit kinetic data. Table E-4 indicates  
13 that while AUC for acrylamide and glycidamide can be simulated reasonably well with the  
14 revised rat model, and AM-GSH is reasonably close, the extent of metabolism to GA-GSH is  
15 overestimated by 3 fold by the model. Approximately 40% of the urinary metabolites were  
16 reported as GA-GSH (Fennell et al., 2005), but the model simulates that 70% would be derived  
17 from GA-GSH.

18           Table E-9 indicates that almost 50% of acrylamide is converted to glycidamide in  
19 humans. The data reported in Fennell et al. (2005) indicate approximately 13.5 % of the  
20 urinary metabolites were derived from glycidamide. Some recent studies indicate a higher degree  
21 of glycidamide formation from acrylamide, and substantial variation among individuals in this  
22 formation (Vesper et al. 2008; Hartmann et al. 2008). The model simulations are based on the  
23 assumption that all of the acrylamide not accounted for by excretion in urine by 24 hours is  
24 converted to glycidamide. As noted above, there are data not modeled that could greatly  
25 improve the model parameter estimates, using human urine kinetic data for acrylamide,  
26 glycidamide and urinary metabolites (e.g., Fennell et al. 2006; Hartmann et al. 2008; Vesper et al.  
27 2006, 2008). Table E-7 cites the Ratio of GA-GSH to AA-GSH metabolite excretion at low  
28 doses reported by Boettcher et al. (2005) as 0.206 as a data point used for calibration. Yet the  
29 model simulation reports a value of 0.733 (Table E-9). The half-life estimated for acrylamide in  
30 the model is approximately 5.8 hours and the half-life estimated for glycidamide is

1 approximately 6.1 hours. The half life calculated from urinary excretion rate for acrylamide in  
2 humans by Fennell et al. (2006), who studied small groups of healthy infertile adult men, was  
3 approximately half this, ranging from 3.13-3.49 hours. The issue of adjusting the parameters for  
4 partition coefficients and the rates of glutathione conjugation and oxidation is a serious one. It is  
5 possible to simulate the same AUC in blood with different model parameters, but with wildly  
6 different extents of metabolism and dose to the tissues for acrylamide or glycidamide, by  
7 adjusting partition coefficients, and metabolic rate constants. In other words, there may not be  
8 unique solutions unless the full body of reported data can be used in model verification. It is  
9 exceedingly important to carefully consider the extent of metabolism as a key piece of  
10 information in making parameter selections.

11 The description of the parameters and calibration for the human Kirman model are  
12 generally presented clearly on pages E-17 and E-18. A possible exception is the very general  
13 description of the “iterative process” that was used to evaluate physiologically feasible options to  
14 best fit the Fennell et al. (2005b) and Boettcher (2005) human data on adult adduct levels and  
15 urinary metabolites. A rough comparison of the final rat and human values suggests increased  
16 values for a number of tissue binding and metabolic parameters in the human model. Many of  
17 these parameters that changed from rat to human increased roughly by a factor of 2 with the  
18 exception of the Cytochrome P-450 oxidation rate that decreased by a factor of almost 2.1. It is  
19 not clear from the description of the iterative process used to calibrate these values whether the  
20 process was designed to force these parameters to move as groups or exactly what logic was  
21 employed to adjust these multiple parameters. The general logic behind the iterative testing of  
22 permutations of values could be clarified here without going into extreme detail.

23 An alternative approach that should be considered is a re-evaluation of the revised PBPK  
24 model of Kirman et al. (2003). Determining how well it simulates the more recent data and  
25 adjusting the metabolic parameters as necessary is one approach. The Panel had an extensive  
26 discussion as to whether the dose metric of area-under-the-curve (AUC) for acrylamide in the  
27 blood was the best choice based upon what is known about the mode of action for neurotoxicity  
28 and the available kinetic data. A variety of opinions were expressed, ranging from the assertion  
29 that AUC for acrylamide in blood was a suitable dose metric, to the fact that it may not be the best  
30 choice, but may be expedient. The best choice would be to have compartments for the tissues of

1 interest, and to model the amount of acrylamide and/or glycidamide reaching the tissues. The  
2 Kirman model and the modified Kirman model are both limited by the tissue descriptions: liver,  
3 lung, blood and a single compartment for remaining tissues.

4 There was extensive discussion among the Panel members about whether the  
5 neurotoxicity of acrylamide could clearly be attributed to acrylamide alone, to glycidamide, or to  
6 a mixed mode of action. This question was raised in the review document (Page 136, last full  
7 paragraph). Therefore the choice of acrylamide in blood as the dose metric may need to be  
8 revisited as this question is clarified.

9 Several alternatives to the PBTK model exist for making the estimates of internal dose in  
10 rats needed for both the non-cancer and cancer assessments and for calculating the Human  
11 Equivalent Dose (HED). The data available in Doerge et al. (2005) and Tareke et al. (2006)  
12 provide measured serum acrylamide and glycidamide AUCs in rats exposed at drinking water  
13 concentrations and resulting doses near the PODs. Simple linear extrapolation could be used to  
14 calculate the critical internal dose metrics. The hemoglobin adduct and other data available in  
15 several recent publications (Fennell et al. 2005; Vesper et al. 2006, 2008; Hartmann et al. 2008)  
16 together provide a robust means of estimating HEDs. The Panel also discussed the alternative  
17 approach of using pharmacokinetic principles to interpret measurements of hemoglobin adducts  
18 of acrylamide and glycidamide and thereby model glycidamide formation.

19 The Panel also raised concerns about the population variability in the metabolism and  
20 pharmacokinetics of acrylamide, and how that could be incorporated in the model. It was  
21 recognized that there are some high quality human data sets that could be used for PBPK model  
22 development (e.g. Fennell et al., 2005, 2006). However, there are limitations with the small  
23 number of selected subjects compared with the general population, in describing the population  
24 variation. The Panel has identified some studies that suggest variation in the extent of  
25 metabolism of acrylamide to glycidamide (Vesper et al. 2006, 2008; Hartmann et al 2008), and  
26 differences in extent of conversion of acrylamide to glycidamide in children (Heudorf et al.,  
27 2008). There is a need for a better understanding of exposure route differences, inter-individual  
28 variation and life stage differences in the metabolism of acrylamide to glycidamide, and their  
29 clearance. The Panel encourages an evaluation of the available literature, and if possible,  
30 simulation of human variability within the PBPK model.

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**Charge Question 9. *Is the Young et al model adequately discussed relative to structure, parameter values and data sets used in the model?***

The Young et al. paper does not provide citations or values for many of its physiological model parameters. This is an unusual situation for a PBTK modeling paper. For chemical specific model parameter values, the authors fitted the chemical specific model parameter values for each administered dose, creating a model that is calibrated for each dose. This results in an unwieldy model for use in risk assessment. The preferred approach is to use all the administered dose groups and create a model with one set of chemical specific model parameters that describes all the pharmacokinetic data sets. The model was based on the use of linear terms to describe chemical specific reactions (e.g., binding, DNA adducts, and metabolism). This approach may not hold (and non-linear terms will be needed) when developing one set of chemical specific model parameters to describe the kinetics over a range of doses.

***Do you agree with the conclusion that the recalibrated Kirman et al. 2003 model is the best for deriving toxicity values?***

In the opinion of the Panel, the recalibrated Kirman model was superior to the Young et al. PBTK model. However, the Panel noted that the recalibrated model requires updating to include new data sets in the rat and human. The concerns described in Charge Question 8 need to be addressed to use the recalibrated Kirman et al 2003 model. The Panel also noted that an approach to calculating internal doses at the non-cancer and cancer PODs is available that relies on measured data (and minimal linear extrapolation in a dose range that has been shown to be linear) instead of the PBTK model. This approach also affords the ability to calculate the HED corresponding with the critical internal dose metrics associated with the PODs (see response to question 8). If life stages are considered, the PBTK modeling or another pharmacokinetic approach is the preferred approach for determining a HED or HEC.

1 **Charge Question 10.** *According to US EPA's RfC Methodology (1994), the use of PBTK*  
2 *models is assumed to account for uncertainty associated with the toxicokinetic component of*  
3 *the interspecies uncertainty factor across routes of administration. Does the use of the PBTK*  
4 *model for acrylamide objectively predict internal dose differences between the F344 rat and*  
5 *humans, is the use of the model scientifically justified, and does the use of the PBTK reduce*  
6 *the overall uncertainty in this estimate compared to the use of the default factor? Are there*  
7 *sufficient scientific data and support for use of this PBTK model to estimate interspecies*  
8 *toxicokinetic differences and to replace the default interspecies factor for toxicokinetic*  
9 *differences (i.e., 10<sup>1/2</sup>)? Is the remaining uncertainty factor for toxicodynamic differences*  
10 *scientifically justified, appropriate and correctly used?*

11  
12 The Panel commends EPA for using the PBTK model for developing the RfD, RfC and  
13 Cancer Slope Factors for acrylamide. The kinetics of acrylamide are well characterized and thus  
14 the use of internal dose metrics that are thought to represent the critical dose metrics for non-  
15 cancer (neurotoxicity) and cancer (various tumor types) is a preferred approach for extrapolating  
16 across species. The Panel agrees that the use of internal dose metrics (calculated using the  
17 PBTK model or other pharmacokinetic approaches alluded to above) combined with a fairly  
18 robust understanding of the mechanism of action and thus the critical dose metric replaces the  
19 use of the default interspecies factor for toxicokinetic differences (i.e., 10<sup>1/2</sup>).

20 The Panel agreed with the use of the remaining UFs representing interspecies differences  
21 in pharmacodynamics and intraspecies variability in both pharmacokinetics and  
22 pharmacodynamics.

23  
24 **Charge Question 11.** *Please comment on whether the PBTK model is adequate for use to*  
25 *conduct a route-to-route extrapolation for acrylamide to derive an RfC in the absence of*  
26 *adequate inhalation animal or human dose-response data to derive the RfC directly. Was the*  
27 *extrapolation correctly performed and sufficiently well documented?*

28  
29 The Panel discussed the lack of inhalation toxicology and PK studies. One Panel  
30 member who has conducted inhalation PK exposure studies noted the difficulty with conducting

1 controlled rodent exposure studies and the difficulty in maintaining stable exposure  
2 concentrations because of the low volatility of acrylamide and its propensity to sublime. The  
3 Panel agreed with the use of PBTK modeling to conduct dose-route extrapolation. Additionally,  
4 the Panel commends the EPA for using the PBTK model to fill the gap resulting from the  
5 absence of robust animal toxicology studies investigating neurotoxicity via the inhalation route  
6 that would support the development of an RfC. The Panel agreed that the absence of evidence  
7 for route of entry specific effects would allow route-to-route extrapolation for deriving an RfC  
8 by using the PBTK model to calculate the human equivalent concentration (HEC). This would  
9 yield an equivalent internal dose (Acrylamide AUC) associated with those achieved at the POD  
10 from the oral sentinel (Johnson et al.) studies. The Panel noted that few inhalation PK studies  
11 exist to allow a robust parameterization of the inhalation component of the PBTK model for  
12 either rats or humans. Despite this, the Panel noted that acrylamide is very water soluble and  
13 non-volatile, and the compound has a relatively long half-life. Therefore, the absorption of  
14 acrylamide via inhalation should be nearly complete, and first pass effects are negligible, thereby  
15 making the pharmacokinetics of acrylamide via inhalation easy to extrapolate from the oral case,  
16 using simple principles of pharmacokinetics. The Panel agreed that the application of  
17 pharmacokinetic approaches (e.g., the use of the PBTK model) reduces uncertainty associated  
18 with animal to human extrapolation and thus warrants replacing the default UF associated with  
19 interspecies extrapolation for pharmacokinetic differences as was done for deriving the RfD.

20 The Panel noted that the air concentration one would derive using the default approach  
21 (multiply the HED by body weight [70 kg] and dividing by daily inhalation rate [20 m<sup>3</sup>/day]  
22 yielding 0.266 µg/m<sup>3</sup>) is very similar to the HEC derived using the PBTK model (0.25 µg/m<sup>3</sup>).  
23 Therefore, if the EPA also decides to provide an extrapolation based on measured data (as  
24 described in the response to charge question 8), the default approach of extrapolating from an  
25 absorbed oral dose to an equivalent intake from the inhalation route (multiplying by 70 kg and  
26 dividing by 20 m<sup>3</sup>/day) can be used with confidence to calculate the RfC.

27

28 **Charge Question 12. *Please provide any other comments on the derivation of the RfC and on***  
29 ***the discussion of uncertainties in the RfC.***

30

1           The Panel has no further comments beyond those already discussed above.

2

3           **Charge Question 13. *Would you suggest that EPA include a Table that lists points of***  
4 ***departure (e.g., NOAELs, BMDs, etc.) for various endpoints that could be used, in***  
5 ***conjunction with exposure assessments, to conduct a MOE analysis?***

6

7           To the extent permitted by the available data, the Panel supports the concept of the  
8 inclusion of a table in the IRIS acrylamide document which provides information that could be  
9 used to conduct a variety of MOE analyses for specific endpoints of interest and/or for other than  
10 lifetime durations of exposure, in addition to the traditional lifetime focus. In doing so the  
11 magnitude of the MOE that represents a negligible risk should be reported for each point of  
12 departure tabulated.

13           Currently, for those environmental agents for which sufficient data exist, IRIS documents  
14 will present the derivation of a Reference Dose (RfD) and a Reference Concentration (RfC), as  
15 traditionally defined, to be used in the assessment of scenarios which assume that long-term or  
16 lifetime exposures are occurring to non-carcinogenic hazards. Additionally, in those cases where  
17 the agent of interest has been shown to have carcinogenic potential, an oral cancer slope factor  
18 (CSF) and/or an inhalation unit risk (IUR) may be derived, in order to estimate lifetime cancer  
19 risks. Whether or not this step is included is determined by a weight-of-evidence evaluation of  
20 the body of evidence supporting carcinogenic potential and an understanding, or lack thereof, of  
21 the mode(s) of action by which the carcinogenic responses are mediated. These four values (the  
22 RfD, RfC, CSF and IUR) are applicable in situations where the assessment is focused on the  
23 general population exposed over a lifetime, and may have more limited utility in the assessment  
24 of specific subpopulations and/or less-than-lifetime exposure durations.

25           EPA Program and Regional offices and other end-users of IRIS documents often must  
26 develop risk assessments for specific populations and/or less-than-lifetime exposure scenarios in  
27 order to carry out their respective legislative and regulatory mandates. These risk assessments  
28 would benefit from the inclusion of transparently-developed, peer-reviewed consensus hazard  
29 values.

1 A comprehensive table would, for example, include NOAELs, LOAELs, BMDs and  
2 BMDLs at the 1%, 5% and 10% risk levels (as the default) for those studies deemed the most  
3 appropriate for the assessment of specific endpoints and for acute, intermediate and long-term  
4 exposure scenarios, data permitting. It is recognized that it will typically not be possible to fill in  
5 every cell for every endpoint and all exposure durations of interest and that a different  
6 BMD<sub>R</sub>/BMDL<sub>R</sub> may better reflect the study's results. Some EPA program offices have extensive  
7 experience in the selection of study types and durations that best lend themselves to the  
8 assessment of specific endpoints, exposure durations and subpopulations.

9 For this draft acrylamide assessment, such a table would display the relevant outcomes of  
10 a review of the reliable and well-performed studies which evaluated the potential for  
11 neurotoxicity in the adult and developing organism, reproductive toxicity including heritable  
12 germ effects, developmental toxicity, and general systemic toxicity following acute, intermediate  
13 and long-term exposure, as appropriate.

14  
15 **Charge Question 14. *Please comment on the discussion of methods to quantitate the dose-***  
16 ***response for heritable germ cell effects as to whether it is appropriate, clear and objective, and***  
17 ***reflective of the current science. Has the uncertainty in the quantitative characterization of***  
18 ***the heritable germ cell effects been accurately and objectively described?***

19 *[It should be noted that the section under review is 5.5 rather than 5.4. In addition, page 215*  
20 *which includes figures 5-2 and 5-2a, was inadvertently omitted in the draft EPA report and thus*  
21 *not available for review by the Panel. Correction of this error, however, is not expected to*  
22 *impact the recommendations of the Panel on this question as outlined below.]*

23  
24 Although reservations were expressed about the lack of data to quantify dose-response, it  
25 was the consensus of the Panel that the discussion of the methods should be retained in the  
26 report. The report adequately characterizes the current science, reflects historical attempts to  
27 estimate these risks and notes that the quantitation methods are based only on the Dearfield et al.  
28 (1995) publication. Concerns about the validity of the data and methods are given throughout  
29 the section and it is appropriately noted on page 217, “ these uncertainties in the assumptions and

1 data gaps warrant further research to improve the usefulness of the following quantitative  
2 estimates of risk of acrylamide-induced heritable effects.”

3 Some specific observations/recommendations/concerns are outlined below:

- 4 • The parallelogram models were clearly described and the rationale for the decision to use  
5 the modified direct and doubling dose approach appears appropriate.
- 6 • Clearly, there is considerable uncertainty regarding the validity of the underlying  
7 assumptions for these methods and these methods may underestimate risk since they do not take  
8 into account all elements that may contribute to the risk.
- 9 • The extrapolation of exposure is based on animal studies using high dosages (50 to 100  
10 mg/kg or even higher)
- 11 • The risk extrapolation factors (REFs; pg. 217) should be explained in more detail and  
12 information included on how each number is derived (range, etc).
- 13 • In agreement with the report, given the differences in glycidamide production in different  
14 species, an REF of 1 for the metabolic and dose rate variability is likely incorrect. There appear  
15 to be significant dose-rate and species-dependent variations in acrylamide metabolism to  
16 glycidamide (e.g., see Barber et al., 2001; Fennell and Friedman, 2005).
- 17 • An REF for uncertainty in the mode of action was recommended since the doubling dose  
18 is dramatically higher when generated using specific locus studies which are clearly point  
19 mutations (53.1 mg/kg using Ehling and Neuhauser-Klaus, 1992) versus using heritable  
20 translocation data that could be based on clastogenic mechanisms (1.8, 3.3, 0.39 mg/kg for  
21 Shelby et al., 1987, Adler et al., 1994 and Adler, 1990).
- 22 • The implementation of the modified direct approach was difficult to understand when, in  
23 the absence of the number of human loci capable of mutating to dominantly expressed disease  
24 alleles, it was assumed to be 1000. Clarification of how this number was derived would be  
25 helpful (i.e. how do we know the number of mutable genes?).
- 26 • In the doubling dose approach it was not clear how the four data sets, each of which used  
27 high acrylamide dosing rates without significant dose ranges, could accurately predict the  
28 number of new diseases in the offspring at low doses.

1 Lack of current research in this area is a major concern and little has been done to update the  
2 research and data collection based on the Dearfield et al. (1995) methods. The Panel is in  
3 agreement with the report that recommends further research and data to fill the critical data gaps  
4 and reduce uncertainties including gaps in interspecies extrapolation factors, the quantitative  
5 relationship between genetic alterations in germ cells and heritable disease, and the shape of the  
6 low-dose response relationship. Research might include multiple dose studies, including dose  
7 selection comparable to that employed in the repeated dose studies which identified  
8 neurotoxicity as a critical effect. It is also recommended that impacts on different cell types be  
9 determined and that biomonitoring data be utilized in any models developed.

10  
11 **Charge Question 15. *Please comment on the scientific support for the hypothesis that***  
12 ***heritable germ cell effects are likely to occur at doses lower than those for neurotoxicity?***  
13 ***What on-going or future research might help resolve this issue?***

14  
15 The Panel unanimously agreed that germ cell-induced effects should be taken very  
16 seriously, as their implications are highly significant from a public health perspective. There is  
17 an absence of data on these effects in lower dose ranges, making it very difficult to speculate  
18 about the relevance of this endpoint at or below the dose levels that cause neurotoxicity.  
19 Panelists did point out that heritable translocations appeared with very high frequency at the  
20 lowest doses tested (i.e., 5 x 40 mg/kg resulted in 24% translocation carriers, Shelby et al.,  
21 1987). The high frequency of germ cell effects at these doses implies that these studies were far  
22 from identifying a LOAEL or NOAEL, and that there would likely be germ cell effects at much  
23 lower doses. However, the combination of lack of testing at lower doses, and the narrow dose  
24 range in which testing has been done, makes it very difficult to extrapolate down to a low dose  
25 range. The Panel agreed that it is a high priority to extend the heritable translocation studies  
26 down into lower dose ranges, and that this information would be very useful for risk assessment  
27 once it is completed.

28  
29 **Charge Question 16. *The risks of heritable germ cell effects (i.e., number of induced genetic***  
30 ***diseases per million offspring) for some estimated exposure in workers and the population are***

1 ***presented in Table 5-11, and are based on the quantitative methods and parameter estimates***  
2 ***discussed in Section 5.4 of the Toxicological Review. Please comment on whether or not the***  
3 ***quantitation of heritable germ effects should be conducted, the level of uncertainty in the***  
4 ***results, if Table 5-11 is useful for risk assessment purposes, and if the RfD should be included***  
5 ***in the Table as one of the exposure levels.***  
6

7 The Panel supports the Agency's conclusions that exposure to acrylamide in animals  
8 leads to heritable gene mutations and that these results indicate that it may also pose a hazard to  
9 humans. In addition, the Panel supports the Agency's conclusions that the available data are not  
10 adequate to conduct a robust assessment of this endpoint at this time.

11 The Panel's deliberations regarding quantifying heritable germ cell mutations centered on  
12 the importance of including data such as those presented in Table 5-14 (not Table 5-11, as noted  
13 in the final question), the potential significance of these endpoints to human risk assessment, and  
14 the paucity of new data developed since the Dearfield et al. (1995) review upon which this  
15 section relied heavily (including Table 5-14). A majority of Panel members were supportive of  
16 the inclusion of this table in the document and for including the RfD and RfC among the  
17 concentrations in the table as this would facilitate comparison with the neurological endpoints.  
18 Suggestions also included adding more information into the review regarding the role of CYP  
19 2E1 in the dominant lethal effects of acrylamide, which indicated a requirement for metabolism  
20 to glycidamide. While the caveats from the Dearfield et al. (1995) review were recapitulated in  
21 the document, the Panel discussed the need to further elaborate the limitations in the underlying  
22 data and to include reference to the new relevant studies that pertain to uncertainty and dose-  
23 response.  
24

25 ***Charge Question 17. Do you know of any additional data or analyses that would improve the***  
26 ***quantitative characterization of the dose-response for acrylamide-induced heritable germ cell***  
27 ***effects? Would these data also support the quantitative characterization of "total" male-***  
28 ***mediated reproduction risks to offspring (i.e., lethality + heritable defect)? If data are not***  
29 ***available, do you have any recommendations for specific needed studies?***  
30

1 A concern raised by the Panel was that there is a lack of a suitable data set for dose  
2 response assessment for acrylamide-induced heritable germ cell effects. The majority of the  
3 studies reported have been conducted in mice, using relatively high doses.

4 Using wild type and Cyp 2E1 knockout mice, it has been demonstrated that oxidation of  
5 acrylamide to glycidamide is required for the dominant lethal effect (Ghanayem et al., 2005a)  
6 and for the induction of erythrocyte micronuclei and DNA strand breaks in lymphocytes, liver  
7 and lung using the Comet assay (Ghanayem et al., 2005b). The greater incidence of heritable  
8 translocation carriers in mice administered glycidamide (Generoso et al., 1996) compared with  
9 acrylamide (Adler et al., 1994) suggests that glycidamide plays a key role in the mode of action  
10 for heritable genetic effects.

11 The risk equivalent factors (REFs, page 217) need to be updated. There are profound  
12 differences between rats, mice and humans in the extent of metabolism of acrylamide to  
13 glycidamide, and the relative internal dose of acrylamide and glycidamide differs markedly  
14 between mice, rats and humans. The extension of the physiologically-based pharmacokinetic  
15 modeling approach to include the mouse should be a priority. The blood-testis barrier is thought  
16 to contribute to the reduction of internal dose in the testis compared with other tissues for  
17 ethylene oxide (Fennell et al., 2001). Testis should be included as a compartment in the model.  
18 Data permitting, including the testis as a compartment in the model could potentially improve the  
19 dose response characterization for this endpoint.

20 In reviewing data needs (page 220), it is noted that “The estimates do not take into  
21 account other potential genotoxic mechanisms such as effects in spermatogonia stem cells,  
22 effects in female germ cells, or induction of recessive mutations that would not appear in the first  
23 generation, but could lead to additional adverse effects in subsequent generations.” Studies to  
24 examine the dose response for heritable genetic effects, and the effect of long-term exposure to  
25 acrylamide are needed.

26 There is still uncertainty about the mode of action of acrylamide and glycidamide in the  
27 induction of heritable genetic effects. The potential for DNA adducts of glycidamide to play a  
28 role is an attractive hypothesis for the mode of action. With respect to the possible role for  
29 protamine modification in the generation of effects, there was extensive Panel discussion  
30 concerning the potential of glycidamide to form adducts with cysteine in proteins and peptides.

1 Adducts to protamine from acrylamide have been identified in late stage spermatids and  
2 suggested to mediate the dominant lethal effects (Sega et al., 1989). Whether glycidamide will  
3 form similar protamine adducts has not been determined. Kinesin motor proteins associated with  
4 cell division are an additional site of potential action leading to heritable germ defects (Sickles et  
5 al., 2007) that requires future consideration. Both AA and GA inhibit two kinesin motor  
6 associated with spindle formation and maintenance as well as separation of chromosomes. Loss  
7 of fidelity of chromosomal separation is related to aneuploidy, micronuclei formation and  
8 instability of the genome. The motor protein inhibitions occur at concentrations well below the  
9 occurrence of all heritable germ cell effects. Furthermore, glycidamide is more potent than  
10 acrylamide. Surveying populations occupationally exposed to acrylamide in manufacturing  
11 plants was suggested as an approach for evaluation in humans.

12

13 **Charge Question 18. *Have the rationale and justification for the cancer designation for***  
14 ***acrylamide been clearly described? Is the conclusion that acrylamide is a likely human***  
15 ***carcinogen scientifically supportable?***

16

17 Yes, the rationale and justification has been clearly described, although it should be  
18 further expanded (see below), and the conclusion is scientifically supportable. Acrylamide is  
19 clearly and reproducibly carcinogenic in both rats and mice. As outlined in the draft document, it  
20 produced tumors at multiple sites in the rat in multiple chronic studies, and was a skin tumor  
21 initiator in mice by multiple routes. To paraphrase the International Agency for Research on  
22 Cancer (IARC) Monographs Preamble, in the absence of tumor data in humans it is both  
23 reasonable and prudent to regard evidence of carcinogenicity in experimental animals as  
24 evidence for a probable cancer hazard to humans. This conclusion is consistent with both  
25 national and international guidelines for carcinogenic hazard identification. The U.S. National  
26 Toxicology Program (NTP) has long emphasized that chemicals that cause tumors at multiple  
27 sites or in more than a single species are reasonably anticipated to be human carcinogens. Both  
28 the NTP and IARC have placed acrylamide in cancer classifications similar to that of EPA's  
29 "likely human carcinogen" (This could be noted in the Toxicological Review).

1           When experimental exposure of rats or mice to known human carcinogens is via diet or  
2 drinking water, tumor sites observed in those species do not necessarily correspond to the same  
3 tumor sites in humans. Exposure to chemicals that cause tumors of the mammary gland or the  
4 liver in mice or rats, for example, does not necessarily correspond to increased cancer risk  
5 specifically for female breast or liver in humans. The essential point to be considered is that in  
6 any given case a tumor at these or any other site(s) results from an MOA known to operate in  
7 humans, such as somatic cell mutagenicity.

8           Primary CNS tumors as a group, which are discussed at considerable length in the draft  
9 document, should be restored to the list of experimental tumors produced by acrylamide and that  
10 are of interest for the MOA discussion. The Panel cautions that the viruses that can cause  
11 primary CNS tumors in hamsters and other non-human species are not relevant to this  
12 discussion.

13           It should be emphasized that the spectrum of tumors consistently seen in acrylamide-  
14 exposed rats is completely consistent with a DNA-reactive MOA, based on published data about  
15 other substances that induce or initiate the same kinds of neoplasms. The only agents known  
16 conclusively to induce tumors of the brain and peritesticular mesothelium in rats are all DNA-  
17 reactive, and in fact a single exposure to a direct-acting mutagenic carcinogen has been observed  
18 to suffice for tumor induction at either site. The concept that acrylamide acts by a mutagenic  
19 MOA is thus supported by the spectrum of acrylamide-associated tumors that occur in exposed  
20 rats and mice, as well as by the biotransformation pathway of acrylamide *in vivo*.

21           Tumor initiation – promotion data for mouse skin are perhaps not sufficiently emphasized  
22 in the draft document. First, only DNA-reactive chemicals or chemicals biotransformed to  
23 DNA-reactive metabolites are established tumor initiators. As acrylamide is an initiator, and by  
24 multiple routes of administration, it is a permissible inference that acrylamide is also acting by a  
25 DNA-reactive MOA in mouse skin, as do other initiators. It is most striking that, in mice,  
26 systemic exposure to acrylamide is more effective for skin tumor initiation than direct  
27 application to the skin. The order of efficiency, oral > ip > dermal application, for initiation of  
28 TPA-promotable squamous cell papillomas and carcinomas on mouse skin strongly supports the  
29 importance of systemic exposure and post-hepatic distribution of a reactive metabolite in the  
30 MOA for carcinogenicity at this site.

1

2 **Charge Question 19.** *Do you agree that weight of the available evidence supports a*  
3 *mutagenic mode of carcinogenic action, primarily for the acrylamide epoxide metabolite,*  
4 *glycidamide (GA)? Has the rationale for this MOA been clearly and objectively presented,*  
5 *and is it reflective of the current science?*

6

7 A sound rationale and justification already supports the mutagenic MOA, and this  
8 evidence is further supported by additional new data as described below. The weight of evidence  
9 supports a mutagenic mode of action, and overall the rationale for this mode of action has been  
10 clearly and objectively presented. Some improvements to the presentation are as follows. The  
11 discussion of biological plausibility and coherence could be expanded beyond DNA adducts and  
12 the human relevance section could be somewhat more expansive without being repetitive. The  
13 argument on page 145 regarding the lack of relationship of cytogenetic damage to a mutagenic  
14 MOA should be carefully re-considered, as the literature is full of these correlations. Evidence  
15 for and against the arguments set out should be carefully evaluated, and much better referencing  
16 included. Reports from Bonassi and Hagmar are cited as supportive, yet contradictory findings  
17 from the same authors supporting an alternative argument could just as easily have been cited.  
18 The discussion includes strong generalizations that may not hold up to close scrutiny.

19 There has been one published study to date that has examined biomarkers of acrylamide  
20 exposure and human cancer risk. Olesen et al (2008) characterized hemoglobin adducts of  
21 acrylamide and glycidamide in a case-control study of post-menopausal breast cancer. The  
22 authors found no association between levels of glycidamide hemoglobin adducts and breast  
23 cancer risk. Moreover, they found no overall association between acrylamide adducts and risk.  
24 Upon adjustment for smoking status, however, they observed a 2.7-fold (1.1-6.6) increased risk  
25 restricted to ER+ breast cancer per 10-fold increase in acrylamide-hemoglobin level. With  
26 respect to this study design, the authors did not match or restrict the cases and controls on  
27 smoking status, which raises concern given the very strong link between smoking and  
28 acrylamide adducts. Interpretability of the Olesen study with respect to supporting the mode of  
29 carcinogenic action should be taken cautiously.

1 For very high levels of acrylamide exposure, the animal and other experimental data do  
2 support a mutagenic effect of acrylamide. It has been questioned whether such a mechanism  
3 might also apply to lower doses (and indeed, at the lowest doses to which humans are exposed),  
4 because of uncertainty about whether the compensatory mechanisms are in place to detoxify  
5 acrylamide. But data clearly indicate that glycidamide is formed. There are the consistent  
6 observations in humans of glycidamide-hemoglobin adducts (Bjellaas et al., 2007; Chevolleau et  
7 al., 2007; Vesper et al., 2006, 2007) or glycidamide urinary metabolites (Urban et al., 2006) ,  
8 including children (Heudorf et al. 2008), thus demonstrating the widespread internal exposure to  
9 the putative mutagenic metabolite of acrylamide at ongoing low levels of exposure in the general  
10 population.

11 The Panel did not consider the carcinogenicity to be hormonally-related. The existing  
12 short-term mouse studies in SENCAR, ICR (skin) and A/J (lung) show no such selectivity of  
13 carcinogenicity for hormonally regulated tissues. Also, the Panel discussed the fact  
14 acrylamide/glycidamide is not unique among DNA-reactive epoxides for carcinogenic action in  
15 thyroid, peritesticular mesothelium, and mammary tissue (e.g., glycidol, ethylene oxide). In  
16 addition, this argument does not consider the CNS tumors observed in both chronic acrylamide  
17 cancer bioassays, a site that was discussed by the Panel as representing strong evidence for a  
18 DNA-damaging mechanism (cf. Rice, 2005). Finally, a recent publication considered by the  
19 Panel of short-term exposures to high doses of acrylamide in male F344 rats found essentially no  
20 evidence for hormonal dysregulation in the hypothalamus-pituitary-thyroid axis based on  
21 measurements of gene expression, neurotransmitters, hormones, and histopathology (Bowyer et  
22 al., 2008). Some studies of chronic low dose exposure, such as the cohort study of acrylamide  
23 and ovarian/endometrial cancers (Hogervorst et al., 2007) and others (Khan et al., 1999) have  
24 shown positive associations with hormones. The Panel encourages the Agency to review all  
25 relevant new data that has been published since their completion of the current draft assessment  
26 as the revise and finalize this IRIS document

27

28 **Charge Question 20. *Are there other MOAs that should be considered? Is there significant***  
29 ***biological support for alternative MOAs for tumor formation, or for alternative MOAs to be***  
30 ***considered to occur in conjunction with a mutagenic MOA? Please specifically comment on***

1 ***the support for hormonal pathway disruption. Are data available on alternate MOAs sufficient***  
2 ***to quantitate a dose-response relationship?***

3  
4 No, there is not significant biological support for MOA alternatives to the mutagenic  
5 MOA, and data on any putative alternate MOAs are not sufficient to quantify dose response  
6 relationships. It must be emphasized that more than one MOA may operate for a given  
7 carcinogenic chemical, and the likelihood that more than a single MOA is operative increases as  
8 levels of exposure increase. Some well-documented non-DNA reactive MOAs appear to be  
9 high-dose phenomena. These are often important for understanding bioassay results in  
10 experimental animals, and sometimes for high-exposure situations in human experience, but they  
11 are usually less important because they represent negligible risks when cumulative human  
12 exposures to these and similarly acting compounds fall considerably below bioassay dosage  
13 levels. MOAs that can occur both in experimental rodents and in humans and that operate both  
14 at bioassay dosage levels in experimental animals and at lower levels as well, into the human  
15 exposure range, are most significant for humans. In general, for chemicals such as acrylamide  
16 where there is a compelling body of data to support a DNA-reactive MOA via biotransformation  
17 to glycidamide, the evidence for alternative or additional high-dose MOAs would have to be  
18 convincing to explore alternative approaches to dose response and risk assessment. One caveat  
19 that should be mentioned is that mutations induced by acrylamide are observed following high  
20 doses. There are similarly acting agents, such as methylmethanesulfonate (MMS) that create N7-  
21 Guanine, the same DNA adducts, as does glycidamide yet show a threshold for mutations. These  
22 data are consistent with robust repair mechanisms for the specific type of DNA adducts produced  
23 by glycidamide and MMS. However, it should also be noted that low dose exposures have not  
24 been tested in animal mutation studies and NOAELs have not yet been established. Therefore  
25 future research should include dose response analyses to stringently test the relationship between  
26 DNA adducts and mutations and gain a better understanding of the effects at lower doses. The  
27 Agency should mention the finding of inhibition of kinesin motor proteins as a newly-identified  
28 and potential site of action of AA or GA in the production of carcinogenicity (Sickles et al,  
29 2007).

1 Occasionally high-dose or “unique rodent-specific” MOAs may be invoked or postulated  
2 to discredit bioassay results as irrelevant to humans, especially when such putative MOAs are  
3 observed uniquely in non-human species. Such a postulated MOA needs to be very precisely  
4 defined and its relevance thoroughly investigated and critically tested before the postulated MOA  
5 is accepted by the biomedical and risk assessment communities. Any MOA developed for a  
6 single substance is at best speculative until a general pattern can be rigorously demonstrated for a  
7 family of substances that operate via the same MOA. The hormonal disruption MOAs proposed  
8 for acrylamide as tissue-specific alternatives to a DNA-reactive MOA are highly speculative, are  
9 supported by at most limited evidence, and do not meet this standard as noted in response to  
10 charge question 19. The data are insufficient for characterizing dose-response relationships for  
11 any of these proposed alternatives.

12

13 **Charge Question 21. *Two chronic drinking water exposure bioassays in Fischer 344 rats***  
14 ***(Friedman et al., 1995; Johnson et al., 1986) were used to derive the oral slope factor, and to***  
15 ***identify the tumors of interest for the MOA discussion. Are the choices for the studies,***  
16 ***tumors, and methods to quantify risk transparent, objective, and reflective of the current***  
17 ***science? Do you have any suggestions that would improve the presentation or further reduce***  
18 ***the uncertainty in the derived values?***

19

20 The two chronic studies bioassays in F344 rats are the main studies to consider in dose  
21 response analysis. Overall the document does a good job discussing these studies, but the  
22 rationale for using only the Friedman et al. study for derivation of the oral slope factor is  
23 problematic, and the strengths and limitations of both studies should be discussed in greater  
24 depth. The text describes the Friedman et al. study as “superior” and “larger and better  
25 designed” but the Panel does not agree that this is the case, and recommends that both studies  
26 should be subjected to modeling for the purposes of deriving oral slope factors. The two studies  
27 may have fairly similar oral slope factors. At a minimum, estimates for the second study should  
28 also be presented to clarify the impact of study selection in the uncertainty discussion.

29 The methods to quantify risk are transparently presented and reflective of current science,  
30 with the exception that a factor to scale for pharmacodynamic differences in potency between

1 humans and animals has not been applied. The development of unit risk based on HEC accounts  
2 for the pharmacokinetic but not pharmacodynamic differences, and in such situations EPA's  
3 2005 *Guidelines for Carcinogen Risk Assessment* (p. 3-7) indicates inclusion of a  
4 pharmacodynamic factor be considered. The potential human variability in cancer response  
5 attributable to human pharmacokinetic variability in handling acrylamide should be discussed  
6 qualitatively and analyzed quantitatively. Hemoglobin adduct data could provide the basis for  
7 such an analysis. The assumption underlying the modeling is that each and every individual of  
8 the same age exposed to the same external dose faces the same risk of cancer is inconsistent with  
9 these data.

10 With respect to study selection, one of the reasons for not using the Johnson study had to  
11 do with the rates of CNS tumors in this study, particularly in the controls. The Friedman et al.  
12 study was designed "to investigate whether glial tumors in the Johnson et al. study were  
13 significant." But, as Rice (2005) points out, the histopathological examination for glial tumors  
14 was incomplete. Only one-fifth of the 1.0 mg/kg-day dose females' spinal cords were subjected  
15 to histopathological examination, even though one-third of the glial tumors in the Johnson et al.  
16 study were seen in the spinal cord. The approach to the evaluation of CNS tumors in Friedman et  
17 al. was seen by the Panel as a significant study limitation.

18 Another improvement over the Johnson study noted in the document for the Friedman et  
19 al. study was different, presumably better dose spacing. The doses for males in the Friedman et  
20 al. and Johnson et al. studies were the same, except Johnson et al. had one additional lower dose  
21 group. The doses in Friedman for females were 1.0 and 3.0 mg/kg-day compared to 0.01, 0.1,  
22 0.5 and 2.0 mg/kg-day for the Johnson study. The Friedman study did extend the high end of the  
23 dose response range for females and did offer a more complete dose response function for  
24 thyroid tumors, employed somewhat larger dose groups (100 per group and two control groups).  
25 But Johnson et al. did have 60 animals per dose group, did provide a complete histopathological  
26 evaluation, and had more dose groups than a standard bioassay.

27 Another limitation of the Friedman et al. study is that the degree of histopathological  
28 examination of oral tissue is unclear. The Friedman study does not tabulate findings for certain  
29 tumor sites seen in the Johnson study, so quantitative comparisons are not possible and the reader  
30 is not able to consider these sites or perform independent evaluations regarding the significance

1 of the findings. It appears EPA may have the data needed to do the analysis since it was able to  
2 do a time-dependent analysis for slope estimation using the Tegeris Lab report. EPA could then  
3 look at the data and analyze as appropriate the data for these sites.

4 A criticism about the possible impact of a sialodacryoadenitis virus on tumor findings  
5 had been raised and was another reason given for using the Friedman study. On the other hand,  
6 US FDA had raised some issues in auditing the Friedman et al. study regarding environmental  
7 controls at the lab facility and the possibility of some under-dosing of animals. Ultimately both  
8 studies have strengths and weaknesses and on balance neither seems clearly superior. Both are  
9 reasonably strong studies, and thus oral slope estimates should be presented for both studies.

10 Some comments regarding details on tumor data presentation and analysis in the EPA  
11 draft document follow:

12 Tests for dose-related trends should be conducted and presented for the all tabulated sites.  
13 By Fisher's exact test, the mammary tumors in the 0.5 mg/kg-d group in the Friedman et al.  
14 study are significant ( $p < 0.05$ ). The statistics used in the draft document that correct for  
15 intercurrent mortality should be re-checked. It appears this group has a treatment-related finding  
16 and this should be noted and the discussion that this group is devoid of treatment-related tumors  
17 (page 75) changed. The clitoral gland findings in the Johnson et al. study stand out because  
18 histology was done only on clitoral tissues observed with gross masses. This is worth an  
19 explanatory footnote. Also given the approach taken to collecting this tissue, the clitoral tumors  
20 in the 0.5 mg/kg dose group also appear worthy of note. All four masses analyzed indicated  
21 tumor compared to none in controls ( $p < 0.1$ ). In the Friedman et al. study, CNS tumors of glial  
22 origin should be combined for analysis as was done by WHO 2006. Considering the findings of  
23 glial tumors in females in the Johnson study, the dose related trend for both sexes in the  
24 Friedman study, although falling a hair short of statistical significance at the  $p \leq 0.05$  level,  
25 provide some evidence of a CNS glial cell effect in the Friedman study. This should be  
26 discussed. Also, the extent of examination of oral tissue in the Friedman study is unclear.  
27 Finally, the Friedman study employed two control groups for the male rats that do not differ  
28 from one another. For the statistical treatments, there is no apparent reason why these groups  
29 should not be combined. The Toxicological Review did this for the dose response analysis but  
30 may not have done the same for the pairwise comparisons.

1           The data choice for modeling to address the discrepancy between the Friedman et al. and  
2 the Tegeris laboratory reporting of thyroid tumors for the male noted in Appendix D of EPA's  
3 draft document was appropriate. A final minor point, in the discussion of the confidence in dose  
4 response analysis in chapter 6 (page 229), issues are raised that seem better placed in the  
5 discussion of the hazard characterization.

6  
7 **Charge Question 22.** *The cancer slope factor (CSF) derivation includes an adjustment for*  
8 *early mortality (i.e., time-to-tumor analysis). Is this adjustment scientifically supported in*  
9 *estimating the risk from the 2-year bioassay data for increased incidence of tumors in the*  
10 *rats?*

11  
12           The use of the Weibull-in-time multistage-in-dose analysis is a reasonable and  
13 scientifically justifiable way to take into account the early mortality in the high dose group in the  
14 male study. The decision not to employ this analysis in the case of the females is also reasonable  
15 since mortality across treatment and control groups did not differ and the overall survival appears  
16 to be fairly good.

17  
18 **Charge Question 23.** *Please comment on whether AUC for glycidamide is the best choice of*  
19 *the dose metric in estimating human equivalent concentration to derive the oral slope factor.*

20  
21           The Panel agreed that using the AUC for glycidamide is the best choice for estimating the  
22 human equivalent concentration to derive the oral slope factor. This decision was based on the  
23 strong evidence from experimental results that the AUC was linearly correlated with adduct  
24 levels in single/repeat dosing studies. There was agreement that glycidamide is the more  
25 mutagenic metabolite based on experimental studies. The Panel felt there was good  
26 documentation in the report regarding the correlation between levels of DNA adducts and extent  
27 of mutations *in vivo*. Moreover, the metabolic conversion of acrylamide to glycidamide supports  
28 the MOA.

29           One consideration in using this as the dose metric, however, comes from some of the  
30 human studies in which variability is not accounted for adequately, specifically, inter-individual

1 variation is not assessed and that the value used for cross-species comparisons is based on small  
2 numbers of healthy adult male humans. This is discussed at greater length in response to  
3 Question 8. Consideration of additional human data (e.g., Vesper et al., 2006) to evaluate the  
4 degree humans form glycidamide from acrylamide is clearly warranted. Such data may provide  
5 the basis for comparing human acrylamide and glycidamide AUCs, using methodology of  
6 Calleman, Bergmark and colleagues (Bergman et al., 1991). This in turn can provide an  
7 improved basis for adjustments for cross-species differences in pharmacokinetics, as well as  
8 human variability in glycidamide formation from acrylamide.

9  
10 **Charge Question 24.** *As with the RfC, there were insufficient cancer inhalation data to derive*  
11 *an inhalation unit risk (IUR). The PBTK model was used in a route-to-route extrapolation of*  
12 *the dose-response relationship from the oral data, and to estimate the human equivalent*  
13 *concentration for inhalation exposure to acrylamide. Please comment on whether this*  
14 *extrapolation to derive the inhalation unit risk was correctly performed and sufficiently well*  
15 *documented.*

16  
17 The response to this question is nearly identical to the response to charge question #11.  
18 The Panel agreed with the use of PBTK modeling to conduct dose-route extrapolation and  
19 commended the EPA for using the PBTK model to fill the gap resulting from the absence of  
20 robust animal toxicology studies investigating neurotoxicity via the inhalation route that would  
21 support the development of an RfC. The Panel agreed that the absence of evidence for route of  
22 entry specific effects would allow route-to-route extrapolation for deriving an RfC based on  
23 using the PBTK model to calculate the human equivalent concentration (HEC). This would yield  
24 an equivalent internal dose (Glycidamide- AUC) associated with those achieved at the point of  
25 departure from the oral sentinel (Johnson et al.) studies. The Panel noted that few inhalation PK  
26 studies exist to allow a robust parameterization of the inhalation component of the PBTK model  
27 for either rats or humans. Despite this, the Panel noted that acrylamide is very water soluble and  
28 non-volatile, and the compound has a relatively long half life. Therefore, the absorption of  
29 acrylamide via inhalation should be nearly complete, and first pass effects are negligible, thereby  
30 making the pharmacokinetics of acrylamide via inhalation easy to extrapolate from simple

1 principles of pharmacokinetics. The Panel agreed that the application of pharmacokinetic  
2 approaches (e.g., the use of the PBTK model) reduces uncertainty associated with animal to  
3 human extrapolation and thus warrants replacing the default uncertainty factor associated with  
4 interspecies extrapolation for pharmacokinetic differences as was done for deriving the RfD.  
5 The use of the PBTK model however does not address cross-species differences in  
6 pharmacodynamics, which should be considered, following the Agency's 2005 Guidelines for  
7 Carcinogen Risk Assessment.

8 The Panel noted that the air concentration one would derive using the default approach  
9 (multiply the HED by body weight [70 kg] and dividing by daily inhalation rate [20 m<sup>3</sup>/day]  
10 yielding 0.266 µg/m<sup>3</sup>) is very similar to the HEC derived using the PBTK model (0.25 µg/m<sup>3</sup>).  
11 Therefore, if the EPA decides to also provide an extrapolation based on measured data (as  
12 described in the response to charge question 8), the default approach of extrapolating from an  
13 absorbed oral dose to an equivalent intake from the inhalation route (multiplying by 70 kg and  
14 dividing by 20 m<sup>3</sup>/day) can be used with confidence to calculate the RfC.

15

16 **Charge Question 25. *The recommendation to use the age-dependent adjustment factors***  
17 ***(ADAFs) is based on the determination of a mutagenic MOA for carcinogenicity. Is this***  
18 ***recommendation scientifically justifiable and transparently and objectively described?***

19

20 The recommendation to use the age-dependent adjustment factors is well justified and  
21 transparently and objectively described. The Panel's deliberations regarding quantitating age-  
22 dependent adjustment factors (Section 5.4.6) followed from discussions of a mutagenic mode of  
23 action for acrylamide and the typically enhanced sensitivity of fetal and neonatal animals from  
24 exposure to such agents in accordance with EPA's Supplemental Guidance for Assessing  
25 Susceptibility from Early Life Exposure to Carcinogens (2005b). The Panel also discussed the  
26 value of using the PBTK model to evaluate the effect of lifestage on CYP 2E1 and glutathione  
27 levels that could modify internal exposure to glycidamide. Such modeling results could be used  
28 to reduce the uncertainty associated with lifestage extrapolations and the derivation of age-  
29 dependent adjustment factors. Such efforts would be directed at pharmacokinetic aspects of the

1 age-dependent adjustment factors. Uncertainty regarding pharmacodynamics would remain to  
2 be addressed by the age-dependent adjustment factors.

3  
4 **Charge Question 26. *Please provide any other comments on the CSF or IUR, and on the***  
5 ***discussion of uncertainties in the cancer assessment.***

6  
7 The discussion of uncertainties is good, but human variability could be addressed in  
8 greater length. It is unclear why in Table 5-13 the consideration/approach is “Method used to  
9 protect sensitive populations.” There is no characterization of sensitive populations, and this  
10 could be explored and discussed to a much greater extent.

11 Specifically, not enough attention was paid to consequences of individual differences in  
12 metabolism and cancer risk. Both the CYP2E1 polymorphisms and glutathione transferase(s)  
13 (even though rodent data suggests no role for this pathway) polymorphisms could be looked at in  
14 human populations. The degree to which increased activity influences the risk should be  
15 considered, including whether this might be tumor site dependent. Also, much weight is put on  
16 the two chronic studies in the Fischer344 rat. The limitations of not having another rodent  
17 species should be discussed in more detail with respect to other carcinogens where 2 species  
18 were evaluated and similar or different results were found.

19 A factor to scale for toxicodynamic differences between humans and animals was not  
20 included in the derivation of the CSF and IUR. The 2005 EPA Carcinogenic Risk Assessment  
21 Guidelines (see e.g., Guidelines pp 1-13 and 3-7) discusses how toxicodynamics can be  
22 addressed by such a factor. The development of unit risk-based on HEC accounts for the  
23 toxicokinetic but not toxicodynamic interspecies differences.

## ABREVIATIONS

1		
2		
3	ADAF	age-dependent adjustment factor
4	AM-GSH	Acrylamide-Glutathione
5	AUC	area under the curve
6	BMD	benchmark dose
7	BMDL	benchmark dose level
8	BMR	benchmark response
9	CNS	Central Nervous System
10	CSAF	Chemical-specific Adjustment Factors
11	CSF	Cancer slope factor
12	DNA	Deoxyribonucleic Acid
13	EPA	Environmental Protection Agency
14	FQPA	Food Quality Protection Act
15	GA or Gly	Glycidamide
16	GA-GSH	Glycidamide-Glutathione
17	HEC	Human Equivalent Concentration
18	IARC	International Agency for Research on Cancer ()
19	IRIS	Integrated Risk Information System
20	IUR	inhalation unit risk
21	LOAEL	Lowest Adverse Effect Level
22	MMS	Methylmethanesulfonate
23	MOA	mode of action
24	MOE	Margin of Exposure
25	NMA	N-Methylolacrylamide
26	NO	Nitric Oxide
27	NOAEL	No Adverse Effect Level
28	NTP/CERHR	National Toxicology Program
29	PBPK	physiologically-based pharmacokinetic
30	PBTK	physiologically-based toxicokinetic
31	PK	Pharmacokinetic
32	POD	point of departure
33	RfC	reference concentration
34	RfD	reference dose
35	TP	tumor promoter
36	UF	uncertainty Factor
37	WHO	World Health Organization
38		
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10/1/08 Draft

DO NOT CITE OR QUOTE

This draft SAB panel report has been prepared for quality review and approval of the chartered SAB.

This report does not represent EPA policy

1 APPENDIX A CHARGE QUESTIONS



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

OFFICE OF  
RESEARCH AND DEVELOPMENT

February 4, 2008

**MEMORANDUM**

**SUBJECT:** Request for SAB review of the Draft IRIS Assessment for Acrylamide

**FROM:** Ila Cote, Ph.D., Acting Director  
National Center for Environmental Assessment, Research Triangle Park (B243-01)  
Office of Research and Development

**TO:** Sue Shallal, Ph.D.  
Designated Federal Officer  
EPA Science Advisory Board Staff Office (1400F)

This is to request a review by the Science Advisory Board of the draft document entitled "Toxicological Review of Acrylamide (CAS No. 79-06-1)" in support of summary information on the Integrated Risk Information System (IRIS). This document is an assessment of the potential for cancer and noncancer effects following exposure to acrylamide. The Toxicological Review of Acrylamide was prepared by the National Center for Environmental Assessment (NCEA), which is the health risk assessment program in the Office of Research and Development. The document has been made available for public comment on the Agency's NCEA web site at the following URL:  
<http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=187729>

The Toxicological Review of Acrylamide broadly supports activities authorized in the 1990 Clean Air Act and is applicable to the information and regulatory needs of all program Offices and Regions in evaluating the cancer and noncancer effects following exposure to acrylamide. EPA last published an assessment of the potential hazardous effect of acrylamide in 1988. The current assessment reviews more recent data and applies more recent methodology for deriving toxicity values.

Attached are the charge questions to the Science Advisory Board that provide background information as well as the questions and issues that are to be the focus of the Science Advisory Board's consultation on this assessment.

Attachment: Charge for EPA's Science Advisory Board (SAB) - IRIS Toxicological Review of Acrylamide

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11

1 **Charge Questions**

2

3 **Selection of Studies and Endpoints for the Oral Reference Dose (RfD)**

4

5 In the draft document, the proposed most sensitive noncancer effect from exposure to acrylamide  
6 is neurotoxicity. This endpoint is based on an extensive database of animal and human studies.

7 The next most sensitive effect is reproductive toxicity, which was in the 3-5 fold higher exposure  
8 range for a no effect response in animal studies. No human data were identified for acrylamide  
9 related reproductive effects. Heritable germ cell effects, a potentially serious noncancer effect,  
10 have been observed in male mice, however, the lowest dose levels tested are considerably higher  
11 (two orders of magnitude) than the doses where neurotoxicity were observed, and there is  
12 uncertainty about the shape of the low-dose-response relationship.

13

14 1. Please comment on the selection of neurotoxicity as the most appropriate choice for the most  
15 sensitive endpoint (in contrast to reproductive toxicity, heritable germ cell effects, or other  
16 endpoint) based upon the available animal and human data.

17 2. Please comment on the discussion of mode of action for acrylamide-induced neurotoxicity.  
18 Is the discussion clear, transparently and objectively described, and accurately reflective of  
19 the current scientific understanding?

20 3. Please comment on the qualitative discussion of acrylamide's heritable germ cell effects and  
21 whether the discussion is clear, transparently and objectively described, and reflective of the  
22 current science.

23

24 **Derivation of the Reference Dose (RfD)**

25

26 The proposed RfD (0.003 mg/kg-day) for acrylamide is based on a benchmark dose analysis of  
27 the dose-response relationship for neurotoxicity in two chronic drinking water exposure  
28 bioassays using Fischer 344 rats. Uncertainty factors and a PBPK model are used to extrapolate  
29 the animal dose-response to a human equivalent dose-response in the derivation of the RfD.

30

- 1 4. Please comment on whether the selection of the Friedman et al., (1995) and Johnson et al.,  
2 (1986) studies as co-principal studies has been scientifically justified. Although EPA  
3 considers Friedman et al. and Johnson et al. to be co-principal studies, the final quantitative  
4 RfD value is derived only from the Johnson study. Please comment on this aspect of EPA's  
5 approach. Please also comment on whether this choice is transparently and objectively  
6 described in the document. Please identify and provide the rationale for any other studies  
7 that should be selected as the principal study(s).
- 8 5. Please comment on the benchmark dose methods and the choice of response level used in the  
9 derivation of the RfD, and whether this approach is accurately and clearly presented. Do  
10 these choices represent the most scientifically justifiable approach for modeling the slope of  
11 the dose-response for neurotoxicity? Are there other response levels or methodologies that  
12 EPA should consider? Please provide a rationale for alternative approaches that should be  
13 considered or preferred to the approach presented in the document.
- 14 6. Please comment on the selection of the uncertainty factors (other than the interspecies  
15 uncertainty factor) applied to the point of departure (POD) for the derivation of the RfD. For  
16 instance, are they scientifically justified and transparently and objectively described in the  
17 document? [Note: This question does not apply to the interspecies uncertainty factor which is  
18 addressed in the questions on the use of the PBPK model (see PBPK model questions  
19 below)]
- 20 7. Please provide any other comments on the derivation of the RfD and on the discussion of  
21 uncertainties in the RfD.

22  
23 **Use of a PBPK Model in the Derivation of the RfD and the Inhalation Reference**  
24 **Concentration (RfC)**

25  
26 A physiologically-based toxicokinetic (PBTK) model originally developed by Kirman et al.  
27 (2003), and recalibrated by EPA with more recent kinetic and hemoglobin binding data in rats,  
28 mice, and humans (Boettcher et al., 2005; Doerge et al., 2005a,b; Fennell et al., 2005) was used  
29 in the derivation of the RfD to extrapolate from the animal dose-response relationship (observed  
30 in the co-principal oral exposure studies for neurotoxicity) to derive a human equivalent

1 concentration (HEC). The HEC is the external acrylamide exposure level that would produce the  
2 same internal level of parent acrylamide (in this case the area under the curve [AUC] of  
3 acrylamide in the blood) that was estimated to occur in the rat following an external exposure to  
4 acrylamide at the level of the proposed point of departure, and related to a response level of 5%  
5 (i.e., the BMDL<sub>5</sub>). The model results were used in lieu of the default interspecies uncertainty  
6 factor for toxicokinetics differences of 10<sup>1/2</sup>, which left a factor of 10<sup>1/2</sup> (which is rounded to 3)  
7 for interspecies differences in toxicodynamics.

8  
9 With respect to the RfC, there are presently insufficient human or animal data to directly derive  
10 an RfC for acrylamide. The PBPK model was thus used to conduct a route-to-route extrapolation  
11 (oral-to-inhalation) to derive an RfC based on the dose-response relationship observed in the co-  
12 principal oral exposure studies for neurotoxicity. In this case, the HEC was based on a  
13 continuous inhalation exposure to acrylamide in the air that would yield the same AUC for the  
14 parent acrylamide in the blood as that estimated for the rat following an external oral exposure to  
15 acrylamide at the level of the proposed point of departure (i.e., the BMDL<sub>5</sub>).

- 16  
17 8. Please comment on whether the documentation for the recalibrated Kirman et al. (2003)  
18 PBTK model development, evaluation, and use in the assessment is sufficient to determine if  
19 the model was adequately developed and adequate for its intended use in the assessment.  
20 Please comment on the use of the PBTK model in the assessment, e.g., are the model  
21 structure and parameter estimates scientifically supportable? Is the dose metric of area-  
22 under-the-curve (AUC) for acrylamide in the blood the best choice based upon what is  
23 known about the mode of action for neurotoxicity and the available kinetic data? Please  
24 provide a rationale for alternative approaches that should be considered or preferred to the  
25 approach presented in the document.
- 26 9. Is the Young et al. (2007) PBTK model adequately discussed in the assessment with respect  
27 to model structure, parameter values, and data sets used to develop the model? Do you agree  
28 with the conclusion (and supporting rationale) that the recalibrated Kirman et al. (2003)  
29 model (model structure and parameter values presented in the Toxicological Review)  
30 currently represents the best model to use in the derivation of the toxicity values?

- 1 10. According to US EPA's RfC Methodology (1994), the use of PBTK models is assumed to  
2 account for uncertainty associated with the toxicokinetic component of the interspecies  
3 uncertainty factor across routes of administration. Does the use of the PBTK model for  
4 acrylamide objectively predict internal dose differences between the F344 rat and humans, is  
5 the use of the model scientifically justified, and does the use of the PBTK reduce the overall  
6 uncertainty in this estimate compared to the use of the default factor? Are there sufficient  
7 scientific data and support for use of this PBTK model to estimate interspecies toxicokinetic  
8 differences and to replace the default interspecies factor for toxicokinetic differences (i.e.,  
9  $10^{1/2}$ )? Is the remaining uncertainty factor for toxicodynamic differences scientifically  
10 justified, appropriate and correctly used?
- 11 11. Please comment on whether the PBTK model is adequate for use to conduct a route-to-route  
12 extrapolation for acrylamide to derive an RfC in the absence of adequate inhalation animal or  
13 human dose-response data to derive the RfC directly. Was the extrapolation correctly  
14 performed and sufficiently well documented?
- 15 12. Please provide any other comments on the derivation of the RfC and on the discussion of  
16 uncertainties in the RfC.

17

### 18 **Margin of Exposure (MOE) Analysis**

19

20 IRIS documents do not include exposure assessments, which precludes the ability to conduct a  
21 Margin of Exposure (MOE) analysis. It has been suggested, however, that the acrylamide  
22 assessment include a Table that lists points of departure for various endpoints to facilitate a MOE  
23 evaluation by EPA's Regional or Program offices, or by other end users of the assessment.

24

- 25 13. Would you suggest that EPA include a Table that lists points of departure (e.g., NOAELs,  
26 BMDs, etc.) for various endpoints that could be used, in conjunction with exposure  
27 assessments, to conduct a MOE analysis?

28

### 29 **Quantitating Heritable Germ Cell Effects**

1 The Toxicological Review includes a discussion of methods to quantitate the risk for heritable  
2 germ cell effects (Section 5.4). The questions below address the uncertainty and utility of the  
3 quantitative results.

4  
5 14. Please comment on the discussion of methods to quantitate the dose-response for heritable  
6 germ cell effects as to whether it is appropriate, clear and objective, and reflective of the  
7 current science. Has the uncertainty in the quantitative characterization of the heritable germ  
8 cell effects been accurately and objectively described?

9 15. Please comment on the scientific support for the hypothesis that heritable germ cell effects  
10 are likely to occur at doses lower than those seen for neurotoxicity? What on-going or future  
11 research might help resolve this issue?

12 16. The risks of heritable germ cell effects (i.e., number of induced genetic diseases per million  
13 offspring) for some estimated exposure in workers and the population are presented in Table  
14 5-11, and are based on the quantitative methods and parameter estimates discussed in Section  
15 5.4 of the Toxicological Review. Please comment on whether or not the quantitation of  
16 heritable germ effects should be conducted, the level of uncertainty in the results, if Table 5-  
17 11 is useful for risk assessment purposes, and if the RfD should be included in the Table as  
18 one of the exposure levels.

19 17. Do you know of any additional data or analyses that would improve the quantitative  
20 characterization of the dose-response for acrylamide-induced heritable germ cell effects?  
21 Would these data also support the quantitative characterization of “total” male-mediated  
22 reproduction risks to offspring (i.e., lethality + heritable defect)? If data are not available, do  
23 you have any recommendations for specific needed studies?

## 24 25 **Carcinogenicity of Acrylamide**

26  
27 In accordance with EPA’s 2005 *Guidelines for Carcinogen Risk Assessment*

28 ([www.epa.gov/iris/backgr-d.htm](http://www.epa.gov/iris/backgr-d.htm)), acrylamide is described as *likely to be carcinogenic to humans*  
29 based on: (1) significant increased incidences of thyroid tumors in male and female rats, scrotal  
30 sac mesotheliomas in male rats, and mammary gland tumors in female rats in two drinking water

1 bioassays; (2) initiation of skin tumors following oral, intraperitoneal, or dermal exposure to  
2 acrylamide and the tumor promoter, TPA, in two strains of mice; and (3) increased incidence of  
3 lung adenomas in another mouse strain following intraperitoneal injection of acrylamide.  
4 Evidence from available human studies is judged to be limited to inadequate.

5  
6 The mechanisms by which acrylamide may cause cancer are poorly understood, but EPA has  
7 determined that the weight of the available evidence supports a mutagenic mode of carcinogenic  
8 action, primarily for the acrylamide epoxide metabolite, glycidamide (GA). Other mode(s) of  
9 action (MOA) have been proposed for the carcinogenicity of acrylamide, but there is less  
10 support.

11  
12 18. Have the rationale and justification for the cancer designation for acrylamide been clearly  
13 described? Is the conclusion that acrylamide is a likely human carcinogen scientifically  
14 supportable?

15 19. Do you agree that weight of the available evidence supports a mutagenic mode of  
16 carcinogenic action, primarily for the acrylamide epoxide metabolite, glycidamide (GA)?  
17 Has the rationale for this MOA been clearly and objectively presented, and is it reflective of  
18 the current science?

19 20. Are there other MOAs that should be considered? Is there significant biological support for  
20 alternative MOAs for tumor formation, or for alternative MOAs to be considered to occur in  
21 conjunction with a mutagenic MOA? Please specifically comment on the support for  
22 hormonal pathway disruption. Are data available on alternate MOAs sufficient to quantitate a  
23 dose-response relationship?

24 21. Two chronic drinking water exposure bioassays in Fischer 344 rats (Friedman et al., 1995;  
25 Johnson et al., 1986) were used to derive the oral slope factor, and to identify the tumors of  
26 interest for the MOA discussion. Are the choices for the studies, tumors, and methods to  
27 quantify risk transparent, objective, and reflective of the current science? Do you have any  
28 suggestions that would improve the presentation or further reduce the uncertainty in the  
29 derived values?

- 1 22. The cancer slope factor (CSF) derivation includes an adjustment for early mortality (i.e.,  
2 time-to-tumor analysis). Is this adjustment scientifically supported in estimating the risk from  
3 the 2-year bioassay data for increased incidence of tumors in the rats?
- 4 23. The dose metric used in the PBTK model analysis to derive the human equivalent  
5 concentration was area under the curve (AUC) in the blood for the putative genotoxic  
6 metabolite, glycidamide. Please comment on whether AUC for glycidamide is the best  
7 choice of the dose metric in estimating the human equivalent concentration to derive the oral  
8 slope factor. If other dose metrics are preferable, please provide the scientific rationale for  
9 their selection.
- 10 24. As with the RfC, there were insufficient cancer inhalation data to derive an inhalation unit  
11 risk (IUR). The PBTK model was used in a route-to-route extrapolation of the dose-response  
12 relationship from the oral data, and to estimate the human equivalent concentration for  
13 inhalation exposure to acrylamide. Please comment on whether this extrapolation to derive  
14 the inhalation unit risk was correctly performed and sufficiently well documented.
- 15 25. The recommendation to use the age-dependent adjustment factors (ADAFs) is based on the  
16 determination of a mutagenic MOA for carcinogenicity. Is this recommendation scientifically  
17 justifiable and transparently and objectively described
- 18 26. Please provide any other comments on the CSF or IUR, and on the discussion of  
19 uncertainties in the cancer assessment.
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1   **APPENDIX B           Proposed MOAs for Acrylamide Neurotoxicity**  
2

3           The following text on the two proposed MOAs for acrylamide neurotoxicity was written  
4 by one panel member. It is offered for the Agency’s consideration in writing the revised version  
5 of the acrylamide IRIS document:  
6

7           1. Disruption of Nitric Oxide (NO) Signaling at the Nerve Terminal Hypothesis

8           Acrylamide is a conjugated  $\alpha,\beta$ -unsaturated carbonyl derivative in the type-2 alkene  
9 chemical class. Because electrons in pi orbitals of a conjugated system are mobile, the  $\alpha,\beta$ -  
10 unsaturated carbonyl structure of acrylamide is characterized as a soft electrophile according to  
11 the hard-soft, acid-base principle (reviewed in Pearson, 1967). Characteristically, soft  
12 electrophiles will preferentially form Michael-type adducts with soft nucleophiles, which in  
13 biological systems are primarily sulfhydryl groups on cysteine residues (Hinson and Roberts,  
14 1992; LoPachin and DeCaprio, 2005). Free sulfhydryl groups can exist in the reduced thiol-state  
15 or in the anionic thiolate-state and recent research indicates that the highly nucleophilic thiolate  
16 is the preferential adduct target for acrylamide (LoPachin et al., 2007b; see also Friedman et al.,  
17 1995). Based on the pKa of cysteine (pH 8.5), at physiological pH (7.4) the thiolate state exists  
18 only in unique protein motifs called catalytic triads, where proton shuttling through an acid-base  
19 pairing of proximal amino acids (e.g., aspartic acid and lysine) regulates the protonation and  
20 deprotonation of the cysteine sulfhydryl group. Indeed, both mass spectrometric and kinetic data  
21 have demonstrated the selective adduction of cysteine residues on many neuronal proteins  
22 (Barber and LoPachin, 2004; Barber et al., 2007). Furthermore, it is now recognized that the  
23 redox state or nucleophilicity of cysteine sulfhydryl groups within catalytic triads can determine  
24 the functionality of these proteins (reviewed in LoPachin and Barber, 2006; Stamler et al., 2001).  
25 In contrast to acrylamide, the epoxide metabolite glycidamide (Gly), is a hard electrophile that  
26 preferentially forms adducts with hard nucleophiles such as nitrogen, carbon and oxygen.  
27 Nucleotide residues of DNA contain abundant hard nucleophilic targets, which is consistent with  
28 the formation of glycidamide adducts on adenine and guanine bases in acrylamide-intoxicated  
29 animals (Doerge et al., 2005; reviewed in Besaratinia and Pfeifer, 2007).

1           Based on the observation that the processes affected (e.g., neurotransmitter release and  
2 storage) and corresponding kinetics ( $K_m$ ,  $V_{max}$ ) were similar in synaptosomes exposed in vitro to  
3 acrylamide and those isolated from acrylamide-intoxicated rats (Barber and LoPachin, 2004;  
4 LoPachin et al., 2004, 2006), LoPachin and colleagues have reasoned that the parent compound,  
5 acrylamide, is responsible for neurotoxicity. Moreover, cysteine thiolate groups have clear  
6 regulatory functions in many critical neuronal processes (LoPachin and Barber, 2006), whereas  
7 protein valine, lysine and histidine residues, which are the likely hard nucleophilic targets for a  
8 hard electrophile such as Gly, have unclear functional and therefore toxicological relevance.  
9 Quantitative morphometric and silver stain analyses of PNS and CNS of acrylamide-intoxicated  
10 animals have shown that axon degeneration was an epiphenomenon related to dose-rate; i.e.,  
11 degeneration occurred at lower but not higher dose-rates. In contrast, nerve terminal  
12 degeneration occurred regardless of dose-rate and in correspondence with the onset and  
13 development of neurological deficits (Crofton et al., 1996; Lehning et al., 1998, 2002a,b, 2003;  
14 reviewed in LoPachin et al., 1994, 2002, 2003), suggesting the nerve terminals as a primary site  
15 of action. Subsequent neurochemical studies showed that both in vitro and in vivo acrylamide  
16 exposure produced early disruptions of neurotransmitter release, reuptake and vesicular storage  
17 (Barber and LoPachin, 2004; LoPachin et al., 2004, 2006, 2007a). Further, proteomic analyses  
18 indicated that the inhibition of presynaptic function was due to the formation of cysteine adducts  
19 on proteins that regulate neurotransmitter handling; e.g., Cys 264 of *N*-ethylmaleimide sensitive  
20 factor, Cys 254 of v-ATPase (see Barber and LoPachin, 2004; Barber et al., 2007; Feng and  
21 Forgac, 1992; LoPachin et al., 2007a,b, 2008b; reviewed in LoPachin and Barber, 2006). The  
22 anionic sulfhydryl state, which is only found in the catalytic triads of regulatory proteins, is an  
23 acceptor for nitric oxide (NO) and, therefore, has lead to the proposal that acrylamide-induced  
24 neurotoxicity results from disruption of neuronal NO signaling (LoPachin and Barber, 2006;  
25 LoPachin et al., 2008a).

26

## 27           2. Fast Axonal Transport Disruption Hypothesis

28           Another proposed MOA is that both acrylamide and Gly inhibit the movement of  
29 materials in fast axonal transport (Sickles et al., 2002). According to the “kinesin/axonal  
30 transport” hypothesis, toxicant inhibition of kinesin could lead to reductions in the axonal

1 delivery of macromolecules that would eventually produce a deficiency of essential proteins  
2 required to maintain axon structure and/or function. Distal axons and nerve terminals are  
3 particularly vulnerable to transport defects based upon an exceptionally large axonal volume (as  
4 much as 1000 times the volume of the neuron cell body) and the dependence of these distal  
5 regions on long distance transport (100 fold longer length than diameter of the cell body). This  
6 regional sensitivity is consistent with the previously identified distal spatial distribution of  
7 toxicant-induced damage (Cavanagh, 1964).

8         Microtubule motility assays using purified kinesin from bovine brain identified a dose-  
9 dependent inhibition of kinesin as well as a less sensitive effect on microtubules (Sickles *et al.*,  
10 1996). Preincubation of either kinesin or taxol-stabilized microtubules produced a reduction in  
11 the affinity between kinesin and microtubules, recognized as a reduced number of microtubules  
12 bound or locomoting on an adsorbed bed of kinesin. Microtubules that were locomoting did so in  
13 a less directed or staggering type of progression. The inhibitions were due to covalent adduction,  
14 presumably through sulfhydryl alkylation, although adduction of other amino acid residues such  
15 as valine was possible. The non-neurotoxic analogue, propionamide had no effect. Other  
16 investigators have identified kinesin inhibition by sulfhydryl reagents such as N-ethylmaleimide  
17 and ethacrynic acid (Walker *et al.*, 1997). As with acrylamide, inhibition by these sulfhydryl  
18 reagents produced the characteristic staggering movement of microtubules. The reaction was  
19 slow and temperature dependent suggesting a sterically hindered cysteine residue as an important  
20 adduct target. Additional studies have demonstrated a comparable effect of glycidamide on  
21 kinesin (Sickles, unpublished data). The predicted outcome of such an effect would be reduced  
22 quantity of flow, precisely the outcome from several experiments where rate of transport versus  
23 quantity could be discriminated (Sickles, 1989a; Sickles, 1989b; Stone et al., 1999).

24         Fast axonal transport has been studied in a variety of model systems using diverse  
25 techniques. A comprehensive survey of acrylamide effects on fast anterograde and retrograde  
26 axonal transport (Sickles et al., 2002) revealed that all studies measuring fast transport within 24  
27 hours of acrylamide exposure demonstrated significant reductions, whereas longer postexposure  
28 delay was not associated with changes in transport. Furthermore, a reduction in transport  
29 quantity (but not rate) has been reported within 20 minutes of exposure. The duration of this  
30 effect was 16 hours, with full recovery at 24 hours (Sickles, 1991). Quantitation of transport

1 after multiple dosings (i.e. 4, 7 or 10 doses) had a similar effect on transport in the proximal  
2 sciatic nerve (Sickles, 1991). The changes in transport were not due to an effect on protein  
3 synthesis and exposure of only the axons confirmed that the target was axonal (Sickles, 1989a;  
4 Sickles, 1992). Collectively, these results suggested action on a target that is replaced via the fast  
5 transport system, consistent with kinesin. The actions of acrylamide on fast axonal transport  
6 were independent of effects on axonal neurofilaments, as similar reductions were observed in  
7 wild-type and transgenic mice lacking axonal neurofilaments (Stone *et al.*, 1999; Stone *et al.*,  
8 2000). The same results were observed using radiolabelling of proteins in mouse optic nerves  
9 and differential interference microscopy of isolated sciatic nerve axons. Other recent studies  
10 have identified a parallel inhibition of retrograde axonal transport by acrylamide (Sabri and  
11 Spencer, 1990), although it is unclear whether this effect is due to inhibition of cytoplasmic  
12 dynein, the retrograde axonal transport motor, or whether this is a result of indirect effects of  
13 kinesin motor inhibition (Brady *et al.*, 1990).

14 The predicted outcome from axonal transport compromise is a reduction in vital  
15 macromolecules in the distal axons and an accumulation of transported material within the axon.  
16 Morphological studies have consistently identified accumulations of tubulovesicular profiles and  
17 neurofilaments in axons of acrylamide-intoxicated animals (Spencer and Schaumburg, 1991),  
18 which are morphological elements transported via kinesin along microtubules. Other studies  
19 have identified reduced synaptic vesicles in neuromuscular junctions (DeGrandchamp and  
20 Lowndes, 1990; DeGrandchamp *et al.*, 1990). A reduction in GAP-43 in the terminal neurites of  
21 cultured primary spinal cord neurons following acrylamide exposure has been observed (Clarke  
22 and Sickles, 1996). Future studies are required to quantitate reductions in specific axonal  
23 compartments using a variety of neurotoxic and non-neurotoxic dosing regimens *in vivo* to  
24 confirm the loss of physiologically or structurally important macromolecules.

25 Additional supportive data for the axonal transport hypothesis comes from several studies  
26 of kinesin knockouts as well as similarity to human diseases. While most knockouts are lethal,  
27 low level mutations of kinesin motors in *Drosophila* have identified an identical spatial pattern of  
28 dysfunction and morphological similarity in axonal pathology (Gho *et al.*, 1992; Hurd and  
29 Saxton, 1996) as with acrylamide intoxication. The group of neurological disorders classified as  
30 hereditary spastic paraplegias has a spatial pattern of ataxia, spasticity and muscle weakness as

1 observed with acrylamide intoxication. Some of these types have been associated with mutations  
2 in kinesin motors (Reid *et al.*, 2002), while others are the result of either axonal or glial protein  
3 mutations. However, the common theme is alteration in axonal transport (Reid, 2003; Gould and  
4 Brady, 2004).

5

#### 6 Role of Acrylamide vs. Glycidamide

7         The respective adduct chemistries of acrylamide and glycidamide are well understood  
8 and could have fundamental implications for neurotoxicity regardless of the proposed  
9 mechanism; i.e., kinesin inhibition (Sickles *et al.*, 2002) or blockade of NO signaling (LoPachin  
10 and Barber, 2006; LoPachin *et al.*, 2008). Accordingly, an obvious data gap in the current  
11 mechanistic understanding of acrylamide neurotoxicity, is the relative roles of the parent  
12 compound and Gly. Thus, although early research suggested that Gly produced neurotoxicity  
13 both in whole animal (Abou-Donia *et al.*, 1993) and in vitro (Harris *et al.*, 1994) model systems,  
14 other studies using similar models failed to find neurotoxic effects associated with this  
15 metabolite (Brat and Brimijoin, 1993; Costa *et al.*, 1992, 1995; Moser *et al.*, 1992). Clearly,  
16 resolving the relative roles of acrylamide vs. glycidamide is an important issue that will require  
17 more research. Although the adduct chemistry of these toxicants has been reasonably defined,  
18 the precise molecular mechanisms and sites of neurotoxicity are unknown.

19

20

## **SAB Comments on the Draft Acrylamide Panel Report - UPDATE**

### **1. Dr. Meryl Karol**

a) *Are the original charge questions to the SAB Panel adequately addressed in the draft report?*

The draft report does an excellent job of addressing the 26 charge questions in a clear and concise manner. In addition, the cover letter provides an excellent bulleted summary of the panel's key points and recommendations, and the Executive Summary clearly states the most important findings and recommendations.

b) *Is the draft report clear and logical?*

The report is clear and logical. Especially helpful was the list of 35 abbreviations. The occasional lengthy sentences require editing, specifically:

p. 10 lines 6-9

p. 11 lines 19-22

p. 15 lines 1-5

p. 20 lines 1-5

p. 25 line 1, it is unclear what "they" refers to

p. 46 line 18 is unclear

c) *Are the conclusions drawn, and/or the recommendations made supported by information in the body of the report?*

Yes.

d) *Errors/omissions*

Grammar: the occasional misuse of which/that should be corrected.

p. 8 line 26 delete "also"

p. 9 line 2 delete "yet"

p.10 line 16 replace "in" with "of"

p. 45 line 20 move "bioassays" to precede "studies"

p.45 line 23 change line to ....problematic. ~~and~~ The strengths and .....

p.45 line 26 delete "should"

p.46 lines 7-9 add commas as follows ... modeling, is that each .....of cancer, is

p.50 line 1 correct spelling of "pharmacokinetics"

### **2. Dr. George Lambert:**

a) Dr. George Lambert:

Comments on the letter to the "Toxicological Review of Acrylamide"

Over all the document is very well done and is thorough, but the degree of concern the panel had about the review can only be realized in the Responses to the Charge questions. I have only a few broad comments.

In the letter to the the Administrator The section on the RfD and RfC does not seem to reflect the executive summary. In particular the panel thought the RfD did not include the most sensitive measures of neurotoxicity and several members noted that this was therefore a significant data gap. As a reviewer, this seems to be a serious concern that is not reflected in the letter to the Administrator and may place serious doubt as the true usefulness of the proposed RfD.

The executive summary seems to capture more of the uncertainty of the data and conclusions and the summary is not much longer than the letter -- could most of the summary be part of the letter? Several critical issues were brought up by the panel which do not come through clearly in the letter. One example is the panel felt that there is no characterization of sensitive populations, and this should be explored and discussed at a much greater extent (line 28-30, page 11) when discussing the derivation of the RfC.

The Panel's recommendation on how to improve the review are also not highlighted in the letter, such as including transparently developed; peer-reviewed consensus hazard values (page 10) and other recommendations.

The Panel felt that the document need to be revised and up-dated prior to finalizing the assessment. This is not clear in the letter and needs to be forcefully stated in the letter.

The letter should indicate the panel had 26 charge questions and the letter and executive summary does not discuss each of the charge questions, so the reader must read the entire document to be informed about the Panel's review of the 26 charge questions.

The response to the charge questions also seems to contain more concern than the executive summary and particularly the letter reflect.

### **3. Steve Roberts:**

The Panel should be commended on an excellent job responding to numerous charge questions on the draft Toxicological Review of Acrylamide. It is clear from the comments that the panel members were conscientious in their review of the report and in crafting comments and recommendations. The report is well organized and, in general, the responses to the questions and rationale are clearly articulated. There are a few areas in which the comments could be improved, in my opinion. These are outlined in the points below:

- a) pg 15, lines 6-9, "It should be noted that future studies may demonstrate effects of acrylamide exposure on male reproductive function ... at even lower doses than those currently associated with neurotoxicity ..." This should be accompanied by an explanation of the basis for the comment.

- b) pg 15, lines 16 – 17, “The heritable germ cell effects are very worrisome and deserve even more consideration, including perhaps the use of this endpoint to generate an independent RfD.” The recommendation of perhaps generating an RfD for heritable germ cells effects appears to be contrary to the conclusion expressed in the cover letter (third bullet): “... the Panel supported the Agency’s conclusions that the available data on heritable gene mutations are not adequate to conduct a robust assessment of this endpoint at this time.”
- c) pg 17, lines 9-12. It is unclear to me how or why the MOA discussion should be presented in the context of Hill criteria.
- d) pg 19, Charge Question 4 asks for comment on derivation of the final RfD value using only data from the Johnson study. The response that follows doesn’t explicitly address this issue.
- e) pg 24, Charge Question 7 asks for other comments on the derivation of the RfD and uncertainties associated with it. The response recommends a cumulative risk assessment for acrylamide, which doesn’t seem germane to question asked.
- f) pg 30, lines 7-8, “Therefore the choice of acrylamide in blood as the dose metric may need to be revisited as this question is clarified.” The question to be clarified is not apparent. In fact, it’s not clear from reading this section exactly where the Panel stood on the question posed whether the AUC for acrylamide in blood is the best choice as the dose metric. The response to Charge Question 8 is somewhat long, but contains good discussion. It could benefit from some concise statements up front or at the end summarizing the Panel’s response to the specific questions.
- g) pg 32, lines 16-19, “The Panel agrees that the use of internal dose metrics ... combined with a fairly robust understanding of the mechanisms of action and thus the critical dose metric replaces the use of the default interspecies factor for toxicokinetic differences (i.e.,  $10^{1/2}$ ).” It’s not clear [to me] from the response to Charge Question 8 that we have a robust understanding of the critical dose metric. Perhaps with some clarification in the response to Charge Question 8, and a stronger endorsement of the dose metric chosen, this would be resolved.
- h) pg 40, lines 17-29: In describing why the cancer designation chosen for acrylamide is appropriate, this section refers to the IARC and NTP classification schemes, but makes no mention of why the definition fits according to EPA guidelines, which are presumably the most relevant.
- i) pg 44, Charge Question 20: This question asks specifically for comments on support for hormonal pathway disruption as a possible MOA. Nothing in the response that follows addresses this. The subject is covered, however, in the response to the previous charge question. That text should be moved to the response to this charge question. Alternatively, a reference to that text (a “shout out”, in the parlance of aspirants to high office) could be placed here.
- j) pg 49, Charge Question 24, “The response to this question is nearly identical to the response to charge question #11.” It’s too identical. This question is in

regard to the IUR, while the response talks about the RfC and air concentration comparisons. Part of the first paragraph and all of the second paragraph need to be revised to address the IUR.

### Minor edits:

#### Panel Report

- a) pg 7, line 9: "... a PBTK model, and the derivation ..."
- b) pg 8, line 9: replace PBPK with PBTK
- c) pg 9, lines 13-16: This sentence is awkward and too long.
- d) pg 9, line 23, "... have been proposed ...": By whom? Need citations or more information.
- e) pg 10, lines 5-9: This sentence is much too long and convoluted.
- f) pg 11, line 11, "Consideration of additional human data ...": A little vague. An example would help.
- g) pg 11, line 16, "As with the RfC ...": The topic of this section is the RfC, not the IUR. The best fix is probably to just delete this sentence. Also, the last two sentences of this paragraph are partially redundant.
- h) pg 11, line 26: A new heading on age-dependent adjustment factors is needed here.
- i) pg 13, line 9: Delete "(s)" after Review – the sentence is referring specifically to the review on acrylamide.
- j) pg 15, line 30: These references should be moved to the reference section.
- k) pg 16, line 28: Suggest "... visible axonal degeneration seen with light microscopy ..."
- l) pg 17, lines 28 and 28: I believe that convention is to spell out these numbers, e.g., "... five heritable translocation studies, two specific locus studies ...".
- m) pg 18, lines 9: "... these observations ..." ?
- n) pg 19, lines 3 and 4: 6% and 0.05% what? What is the endpoint?
- o) pg 23, line 11: "... inclusion of an UF ..." The meaning of this sentence isn't clear to me. Perhaps just end the sentence at "... database."
- p) pg 24, lines 7-11: The NOAELs are "essentially the same" but the "difference could potentially be driven by dose spacing ..." This seems contradictory. I suggest replacing "but" with "and" [and maybe describing as the "small difference"].
- q) pg 26, line 18, "Three major modifications ...": Were there three modifications to each of the three parameters (partition coefficients for glycidamide, etc.) or just modifications to the three parameters? It's not clear the way the sentence is structured.
- r) pg 26, line 21: Delete "and"
- s) pg 26, line 23: Delete "However" and remove the comma after "model"
- t) pg 29, line 23: Replace PBPK with PBTK
- u) pg 29, line 29: Replace "fact" with "belief" or "opinion". The topic (best choice) is inherently subjective.

- v) pg 30, lines 21 and 30: Replace PBPK with PBTK
- w) pg 34, line 15: Replace “which” with “that”
- x) pg 36, line 7: Comma after “methods”
- y) pg 40, line 5: “motors”
- z) pg 40, line 17: Replace “has” with “have”
- aa) pg 42, line 9: Replace “mode of action” with “MOA”
- bb) pg 43, line 26: Replace “the” with “they” and put period at the end of the sentence.

Letter to the Administrator

- a) First bullet, second line: Suggest “... neurotoxicity appears to be ...”
- b) Second bullet, second line: Delete “deemed”
- c) Third bullet, third line: Replace “In addition” with “However”
- d) Sixth and seventh bullets: Both cover the use of PBTK and should be combined.

#### **4. Dr. James Sanders**

Are the charge questions adequately addressed?

Yes, the Panel clearly and carefully addressed each charge question. Given the number of questions, the Panel is to be commended for their responses, and for the layout of the report.

Is the report clear and logical?

Yes. While the report is very specific and provides detail about many of the charge questions, the end result is a readable report for the general reader, as well as for the expert.

Are the conclusions supported?

The report provides back up and support for its comments and recommendations. The Panel has done a very good job of ensuring that their recommendations can be evaluated in their context.

#### **5. Dr. Thomas Wallsten**

I have read the three draft reviews. It appeared to me that all three adequately addressed the charge questions, were logically laid out, and provided supporting information for their conclusions and recommendations. I have three comments on the reports:

- a) The review of the White Paper on "Aquatic Life Criteria for Contaminants of Concern" mentioned the use of expert panels to provide professional judgment during criteria development (Section 4.1.6). I concur that such panels can be very useful. My question is whether EPA has, or has not considered, guidelines for how such panels should operate to assure careful, unbiased judgmental extrapolations from available data to end points of concern?

- b) The same white paper urges that attention be paid to the possible effects of mixtures of contaminants, not just contaminants acting alone. This point would seem to apply to the "SAB Advisory on EPA's Third Drinking Contaminant Candidate List," yet I did not see it mentioned there (although I may have missed it).
- c) Finally, only the review of "Toxicological Review of Acrylamide" included a list of abbreviations. While some acronyms are common (e.g., LOEL, NOEL, DNA), others may be unique to specific fields or topics (e.g., CEC, ROPC, WBDO). It would be helpful for all reports to have a list of acronyms.

## **6. Dr. Terry Daniel**

*The original charge questions to the SAB Panel are adequately addressed in the draft report, the report is clear and logical, and the conclusions and recommendations are supported by the information in the body of the report.*

Both the initial document and the SAB Panel review appeared very thorough and carefully considered. Given my level of expertise relevant to the substantive issues addressed, it seems most prudent in this case for me to vote "present."

## **7. Dr. Rogene Henderson**

I found this to be a thorough, well-thought-out and clearly stated discussion of the draft IRIS assessment for acrylamide. It was a job well done! The charge questions were answered in detail. Responses were logical and well-supported by the text. I had only one small editorial note. I agree with the use of the term "toxicokinetic" instead of "pharmacokinetic" in discussing the kinetics of a toxin rather than a pharmaceutical agent. However I think we need to be consistent. I suggest changing "PBPK" on page 8, line 9 to "PBTk." On page 11, line 12, I suggest changing "pharmacokinetics" to "toxicokinetics."

I like having possible modes of action described in an Appendix.

## **8. Dr. David Allen**

-Page 10, line 2: A discussion of the range of panel views on the range of UF that might be recommended due to data deficiencies would be useful; I could not find this discussion in the subsequent sections of the report.

## **9. Dr. John Balbus**

a) Are the original charge questions to the SAB Panel adequately addressed in the draft report?

*Yes; the report is well organized according to the original charge questions, and the text does address the questions.*

b) Is the draft report clear and logical?

*Yes; the report is well organized and understandable.*

c) Are the conclusions drawn, and/or recommendations made, supported by information in the body of the report?

*Yes; the body of the report supports the conclusions and recommendations.*

### **10. Dr. Duncan Patten**

**General Comment.** In all three cases, the SAB review committees have offered excellent review and advice to EPA. The reviews are comprehensive and in sufficient detail to allow EPA staff to reconsider their positions on topics of concern and to rewrite or rework the materials presented in the white papers.

**Specifically on Acrylamide:** This is an area that is very distant from the experiences. However, the committee's response to the use of non-cancer endpoints seems appropriate as long as it continues to point out the continued use and importance of the cancer descriptor. The recommendation of continued use of pharmacological models also seems appropriate.

Other than these general comments, I find I am not expert enough to fully understand the commentary of the committee and therefore may make inappropriate comments or recommendations.

### **11. Dr. Bernd Kahn:**

I have read the three draft Reviews and consider them to be well written. I have the following two minor questions concerning the Toxicological Review of Acrylamide:

p.4, l. 12 and 18: what is the distinction between "SAB Members" and "Other SAB Members"?

p.12, l.5: Should not "uncertainty" be inserted before "factor"? In subsequent discussions of the UF, use of UF every time would clarify the discussions.

### **12. Dr. Agnes Kane:**

The SAB review panel's assessment of the "Toxicologic Review of Acrylamide" is outstanding. The panel members had significant expertise on this topic and provided appropriate, up-to-date feedback on various technical aspects of this report. Appendix B and the updated references provided an excellent discussion of possible modes of action for acrylamide that serves as a model for future IRIS assessments. Congratulations to the panel members for their hard work!

### **13. Dr. McMullen:**

I have read the documents and have found them to be well organized and easy to follow. I believe they answer the charge questions that were provided to the committee. These documents are not in my area of expertise and as such I have little to add on their technical merit.

### **14. Dr. Timothy Buckley:**

This looks to me to be very well done. I identified just a couple of issues for your consideration:

- a) On page 11: The document, on lines 1-5 states: “The use of the Weibull-in-time multistage-in-dose analysis is a reasonable and scientifically justifiable way to take into account the early mortality in the high dose group in the male study. The decision not to employ this analysis, in the case of the female because mortality across treatment and control groups did not differ and the overall survival appears to be fairly good, is also reasonable.” **This underlined sentence seems to be cumbersome and unclear.**
- b) On page 13: Lines 12-14 state: “The SAB was asked to comment on (1) whether the document is logical, clear and concise, (2) if the discussion is objectively and transparently represented, and (3) if it presents an accurate synthesis of the scientific evidence for non-cancer and cancer hazard. **I don’t see a response to these questions. There may be a need to include a paragraph up front in the ES to address these global issues.**
- c) On page 20-21, Lines 1-2 state: “In the end, the Panel suggested that EPA undergo the exercise of generating an RfD from the Calleman study for purposes of comparison with the RfD derived based on the animal data. **This strikes me as an important recommendation that should be captured in the Executive Summary.**

## **Attachment I**

**EMAIL TO: T.O. Miller, 10/22/2008:**

Attached is a transmittal letter and manuscript on TVM's being submitted on behalf of the North American Polyelectrolyte Producers Association. We would greatly appreciate your providing this material for the SAB members to review. At the same time since the manuscript is not yet published, we request that the SAB limit broad scale distribution so as not to adversely impact the ability to get the manuscript published.

I further note that NAPPA will be submitting additional comments for the SAB's review, unfortunately a little later than I had hoped.

Please contact me if I can clarify any information.

Bob Fensterheim  
North American Polyelectrolyte Producers Association  
1250 Connecticut Avenue NW, Suite 700  
Washington, DC 20036  
202-419-1500

## NORTH AMERICAN POLYELECTROLYTE PRODUCERS ASSOCIATION

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October 22, 2008

National Center for Environmental Assessment  
US Environmental Thomas Miller  
Designated Federal Officer (DFO)  
EPA SAB Staff Office (1400F)  
U.S. EPA  
1200 Pennsylvania Avenue, NW.  
Washington, DC 20460

**Re:** Induction of Tunica Vaginalis Mesotheliomas in Rats by Xenobiotics

Dear Dr. Miller:

On behalf of the North American Polyelectrolyte Producers Association (NAPPA), I hereby submit the attached manuscript entitled Induction of Tunica Vaginalis Mesotheliomas in Rats by Xenobiotics. This manuscript, which was prepared by a series of prominent researchers lead by Dr. Robert Maronpot of Experimental Pathology Laboratories (EPL), was recently submitted to Critical Reviews in Toxicology. An early draft of this manuscript was provided to the SAB/EPA in July of this year.

NAPPA wishes to bring this new manuscript to the SAB's attention in the context of the Acrylamide Review Panel (ARP) report on acrylamide that will be reviewed on October 28. NAPPA sponsored this effort at EPL primarily to further expound on issues associated with the relevance of tunica vaginalis mesotheliomas (TVM) to human cancer risk, as well as to evaluate the suggestion in the ARP report that all chemicals that cause TVM tumors are mutagenic. As noted in response to question #18:

*The only agents known conclusively to induce tumors of the brain and peritesticular mesothelium in rats are all DNA-reactive, and in fact a single exposure to a direct-acting mutagenic carcinogen has been observed to suffice for tumor induction at either site.*

As discussed in the attached manuscript, compounds that were found to exhibit robust TVM responses tended to be mutagenic in Salmonella but not in all cases. More importantly, only 2 of the 7 compounds with non-significant to marginal TVM responses (which includes acrylamide) were found to be Ames test positive.

Maronpot et al. examined the nature of TVM responses in 21 published rat cancer bioassays. The manuscript also highlights the lack of relevance that these rodent tumors have to man. The assessment explains that TVMs are seen most frequently in F344 male rats, as opposed to other rat strains, and are causally associated with the high background incidence of Leydig cell tumors of the testes of these rats. Hormone imbalance brought about by

Thomas Miller  
October 22, 2008  
Page 3 of 3

perturbations of the endocrine system is proposed as a key factor leading to both spontaneous and treatment-associated TVM.

NAPPA maintains that TVMs in rodents should not be considered germane to a human health risk assessment associated with acrylamide exposure. It is significant to note that the draft IRIS assessment acknowledged this view noting that “there is some evidence to suggest that acrylamide can promote or enhance age-related decreases in serum prolactin and testosterone in older male F344 rats (Friedman et al., 1999b; Khan et al., 1999; Ali et al., 1983; Uphouse et al., 1982) and that this enhancement may lead to the development of tunica vaginalis mesotheliomas due to larger adjacent Leydig cell tumors (Iatropoulos et al., 1998).” However, EPA stated that before concluding that TVM’s are not relevant to man, there was a need for additional information in other animal species. The draft IRIS assessment states:

*Additional support for this proposal, such as the lack of mesotheliomas in other rat strains or other animal species exposed chronically to AA, however, is not available.*

NAPPA believes that the ARP should have more seriously considered these issues in its review of the draft acrylamide IRIS assessment. There is no indication in the draft ARP report that the information and analysis by EPL was considered. NAPPA further maintains that the SAB should be recommending to EPA that the ongoing mouse chronic bioassay being conducted by NCTR should address the limitation highlighted by the Agency by providing information on another animal species.

Please let me know if we can clarify any of this mention. Dr. Al Wiedow, a member of NAPPA will be presenting on this topic at the SAB meeting. If desired, we can arrange for Dr. Maronpot to be available by phone.

Sincerely,



Robert J. Fensterheim  
Executive Director

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## Induction of Tunica Vaginalis Mesotheliomas in Rats by Xenobiotics

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## Glossary of Abbreviations Used in This Review

Ah – aryl hydrocarbon receptor  
AKT – a family of genes that encode protein kinases  
AP-1 – activator protein 1  
ARNT – aryl hydrocarbon receptor nuclear translocator  
BrdU - bromodeoxyuridine  
CDKN2A/ARF – cyclin dependent kinase inhibitor 2A/ADP ribosylation factor  
EGF – epidermal growth factor  
EGFR – epidermal growth factor receptor  
Flt-1 – a tyrosine-protein kinase  
HGF – hepatocyte growth factor  
IGF2 – insulin-like growth factor 2  
IL-6 – interleukin 6  
KDR – kinase insert domain receptor  
LH – leutinizing hormone  
LHRH – leutinizing hormone releasing hormone  
LOH – loss of heterozygosity  
Mdr1 – multiple drug resistance gene 1  
Nf2 – neurofibromatosis type 2  
NOEL- no observed effect level  
NTP – National Toxicology Program  
p16 – a cyclin-dependent kinase inhibitor gene  
p16(INK4a) – a p16 gene that regulates cell cycle  
p19(ARF) – a tumor suppressor that attenuates degradation of p53  
p38MAPkinase – p38 mitogen-activated protein kinase  
PAS – Periodic Acid Schiff stain  
PDGF – platelet-derived growth factor  
TGF-beta – transforming growth factor beta  
TSG – tumor suppressor gene  
TVM – tunica vaginalis mesothelioma  
VEGF – vascular endothelial growth factor  
Wnt/beta-catenin – wingless-type gene that is the homolog of the mouse int-1 oncogene  
WT-1 – Wilm’s tumor 1

## **ABSTRACT**

To better understand the relevance of tunica vaginalis mesotheliomas (TVM) to human cancer risk, we examined the nature of TVM responses in 21 published rat cancer bioassays against the backdrop of the biology and molecular biology of mesothelium, and of spontaneous and treatment-induced TVM. Although relatively rare in all species including humans, TVM are seen most frequently in F344 male rats, as opposed to other rat strains, and are causally associated with the high background incidence of Leydig cell tumors of the testes of these rats. Hormone imbalance brought about by perturbations of the endocrine system is proposed as a key factor leading to both spontaneous and treatment-associated TVM. Of 21 F344 rat studies with a treatment-associated TVM response, 7 were judged to have a non-significant to marginal response, 11 had a robust TVM response, and 3 were non-informative due to early mortality from other induced tumors. Of the 11 chemicals with robust responses, 8 were directly mutagenic in Salmonella and 3 are known to be mutagenic after metabolism. Only 2 of the 7 with non-significant to marginal responses were Ames test positive. TVM responses are F344 rat-specific, their incidence can be exacerbated by treatment, and their causal association with F344 rat Leydig cell tumors indicates that when this rat bioassay tumor response is not robust, it is not relevant to humans and does not pose a risk for human cancer.

## **INTRODUCTION**

Spontaneously occurring mesotheliomas have been documented in a wide range of animals but are relatively rare. They have been observed in humans, lower vertebrates, domesticated and laboratory reared mammals, avian species, and marsupials, and occur in the thoracic and abdominal cavities (Ilgren, 1993; Crosby, 2000; Crosby et al., 2000) with rare reports of atriocaval mesotheliomas in cardiac chambers (Hoch-Ligeti et al., 1986; Peano et al., 1998; Chandra et al., 1993). Spontaneous mesotheliomas, which occur primarily in the scrotal sac and peritoneal cavity, have been documented in various rat strains, with the highest frequency occurring in male Fischer 344 rats (Solleveld et al., 1984; Deerberg and Rehm, 1981; Pelfrene and Garcia, 1975; Gould, 1977). These peritoneal mesotheliomas occur in rats 20 to 24 months of age or older and arise in the mesothelium investing the testis, epididymis, and scrotal sac, and may extend or seed into the peritoneal cavity.

Mesotheliomas can be induced by a wide variety of agents including various forms of asbestos, other natural and man-made fibers, metals, viruses, synthetic estrogens, and individual chemicals (Ilgren, 1993; Ilgren and Wagner 1991; Pelnar, 1988). Recently, multi-walled nanotubes injected intraperitoneally have been shown to induce peritoneal mesotheliomas in mice (Takagi et al., 2008). Depending upon the route of exposure, induced mesotheliomas also can occur in the thoracic or peritoneal cavity.

While the diagnostic terms for mesotheliomas used in the studies reviewed in this document include testicular mesothelioma, epididymal mesothelioma, peritoneal mesothelioma, and malignant mesothelioma, all are considered to have arisen in the tunica vaginalis mesothelium. Morphologically, tunica vaginalis mesotheliomas are typically less invasive and have fewer stromal components than the more familiar asbestos-induced pleural mesotheliomas. Tunica vaginalis mesotheliomas in rats rarely metastasize, and are confined to the scrotal sac and abdominal cavity.

This review provides a brief overview of basic biology, key events, mode of action, and examples of xenobiotics that have been associated with tunica vaginalis mesotheliomas in F344 rats based on the National Toxicology Program (NTP) database and a search of literature. This review was undertaken to understand and evaluate the relevance of this unique F344 rat tumor to human health risk assessment. Because tumors initiated by direct DNA interaction (genotoxic mechanisms) are regulated in a different fashion from those that arise from non-DNA reactive modes of action, it is important to understand the etiology of these tumors and whether they are relevant to humans. We postulate that the high incidence of Leydig cell tumors in the F344 rat is causally linked to development of tunica vaginalis mesotheliomas.

## **EMBRYOLOGY**

During early embryogenesis the coelom is a common cavity of mesodermal origin that will ultimately form the pleural, peritoneal, and cardiac cavities and mesothelial linings. This mesoderm forms two types of epithelial cells, viz., *mesothelium* which is a

squamous cell that forms from mesoderm and lines body cavities, and *endothelium*, which is a squamous cell that lines vascular and lymphatic channels (Banks, 1993). During development, the septum transversum, which will become the future diaphragm, separates the pleuropericardial membranes from the peritoneal membranes to form the separate pleural and peritoneal cavities (Hall, 1990; Arey, 1954). The peritoneal cavity and its contained abdominal organs are lined by a single layer of flattened mesothelial cells supported by delicate fibrous connective tissue. Peritoneal mesothelium extends into the scrotum and lines the surfaces of the testes, epididymis, and mesorchium where it is referred to as the tunica vaginalis. Since mesothelial linings in the pleural, peritoneal and scrotal cavities all derive embryologically from the same coelomic mesoderm, it is reasonable to expect the biology and pathobiology of thoracic and peritoneal mesothelium to have common attributes. Furthermore, since mesothelium embryologically derives from mesoderm, it is not surprising that new mesothelium can arise from existing adjacent mesenchyme during wound healing in serous cavities (Lewis, 1923).

### **FEATURES OF SPONTANEOUS AND TREATMENT-INDUCED MESOTHELIOMAS OF RATS**

Spontaneously occurring mesotheliomas are rare and, in general, are more commonly seen in males. A comprehensive listing of mesotheliomas in animals and humans can be found in the publications by Ilgren (Ilgren and Wagner, 1991; Ilgren, 1993). The highest background incidences of spontaneous mesotheliomas occurs in rats, and range from 0.2 to 5%. With rare exceptions, rat mesotheliomas occur in aged males, originate in the tunica vaginalis, and may spread by extension or seeding into the peritoneal cavity. Spontaneous mesotheliomas have been seen in Wistar, Sprague-Dawley, and other rat strains (Pelfrene and Garcia, 1975; Deerberg and Rehm, 1981) but most reports and descriptions in the literature are based on examples in Fischer 344 males (Goelz et al., 1993; Shibuya et al., 1993; Shibuya et al., 1990; Tanigawa et al., 1987; Gould, 1977; Hall, 1990; Mitsumori and Elwell, 1988).

Chemical exposure-associated tunica vaginalis mesotheliomas in rats have been identified in several cancer bioassays conducted for safety assessment or hazard identification, as well as in specific research studies. With the exception of reduced latency and an increased tendency to extend into the peritoneal cavity, the pathological features of these treatment-associated tumors are indistinguishable from those in concurrent controls and spontaneous cases reported in the literature.

The abundant literature dealing with pleural mesotheliomas associated with human exposure to asbestos and other fibers will not be covered in detail in this review, other than to compare and contrast the fiber-induced tumors with the chemical-induced tumors, where appropriate. Nonpleural mesotheliomas, including tunica vaginalis mesotheliomas, have been reported in humans (Hassan and Alexander, 2005). Spontaneous tunica vaginalis mesotheliomas are rare in humans, with fewer than 100 cases reported in the literature in the last 36 years (Guney et al., 2007; Winstanley et al., 2006; Carp et al., 1990; Jones et al., 1995; Plas et al., 1998; Gupta et al., 1999; Antman et

al., 1984). In contrast to the rat, the tunica vaginalis in the adult human does not directly connect to the peritoneal cavity. Consequently, tunica vaginalis mesotheliomas in humans are typically confined to the scrotal vaginal tunics, are locally invasive in about 50% of the cases and, when metastatic, typically spread via the lymphatics (Guney et al., 2007). In a review of 74 human cases, lymph node metastases occurred in approximately 15% of the cases of tunica vaginalis mesothelioma (Plas et al., 1998). A correlation of asbestos exposure with some cases of human tunica vaginalis mesothelioma has been suggested (Guney et al., 2007; Plas et al., 1998).

The histomorphology of tunica vaginalis and peritoneal mesotheliomas in rats is similar in spontaneous and chemically induced lesions, and is histologically indistinguishable from tunica vaginalis and peritoneal mesotheliomas in other species, including humans. Mesotheliomas vary from complex papillary to sessile nodular growths with a sarcomatous component. Smaller papillary lesions consist of a fibrovascular stroma lined by a single layer of flattened to cuboidal mesothelial cells, while larger papillary structures may have areas covered by multiple irregular layers of mesothelial cells forming a pavement or stratified pattern. Tumor cells are cuboidal to polygonal with round to oval nuclei and a prominent nucleolus, and may be arranged in solid sheets, nests, or in glandular and tubular structures. They can form cystic structures in which connective tissue cyst walls are lined by flattened to cuboidal mesothelial cells. The tumor cells may occasionally contain iron-positive material, are mucicarmine positive, and are typically positive for hyaluronic acid. Intracellular keratin and vimentin, and WT-1, can be detected by immunohistochemistry. Mesotheliomas in rats can be classified as epitheliomatous, sarcomatous, or mixed. This classification scheme is consistent with classification of mesotheliomas in humans. As in humans, rat peritoneal mesotheliomas arising in the tunica vaginalis may have features of malignancy, including pleomorphism, cytological atypia, and local invasiveness. Ultrastructurally, mesothelioma cells rest on a distinct basal lamina, have microvilli, junctional complexes, abundant cytofilaments, pinocytotic vesicles, dilated RER cisternae and a prominent Golgi apparatus (Damjanov and Friedman, 1998).

As with most well-studied cancers, a spectrum of lesion severity ranging from hyperplasia to benign neoplasia and ultimately to malignant neoplasia is characteristic of tunica vaginalis and peritoneal mesotheliomas in rats. Mesothelial hyperplasia ranges from a focal or multifocal increased density of usually plump to cuboidal mesothelial cells arranged as a single layer lining a serosal surface, to a blunt but small papillary projection lacking a fibrovascular stalk but sometimes associated with a small amount of connective tissue. Benign mesothelioma typically forms as a papillary structure with single and stratified layering of mesothelial cells lining a fibrovascular stalk. Non-papillary growth patterns of stratified mesothelial cells on a fibrous tissue base may also be considered benign. The mesothelial cells in benign mesotheliomas are generally cuboidal to polygonal and uniform. It is easy to appreciate that benign mesothelial lesions represent a morphological continuum with hyperplasia, and differences of opinion relative to diagnoses between the two would not be surprising. Malignant mesotheliomas have a spectrum of easily recognized morphological features including cellular and nuclear atypia, a pleomorphic growth pattern, and invasion through the serosa, and typically involve multiple sites throughout the peritoneal cavity. Because malignant

mesotheliomas can form glandular and tubular structures, they must be distinguished from metastatic adenocarcinoma. The lack of a primary adenocarcinoma elsewhere in the body, plus use of immunohistochemical staining, are used to support a diagnosis of malignant mesothelioma. Organizations such as the NTP do not subclassify mesotheliomas, but rather consider all mesotheliomas to be potentially malignant. In contrast, literature reports often consider mesotheliomas confined to the scrotal tunics, and without localized invasion, to be benign, while those that spread to the peritoneal cavity and are pleomorphic with cellular atypia and invasive features are generally considered malignant.

Distinguishing mesotheliomas from adenocarcinomas is an important consideration in diagnosis of human cases, especially for lesions in the thoracic cavity. Consequently, a large battery of stains, including immunohistochemical stains, has been used to assist in the diagnosis (Ordonez, 2003) (Table 1). While these stains can help in differential diagnosis, they do not distinguish benign from malignant mesotheliomas (Friedman et al., 1996). Several of these staining methods work well with rodent tissues although not all have been applied to rodent mesotheliomas as yet. Before the advent of immunohistochemistry staining batteries, staining for the acid mucopolysaccharide, hyaluronic acid, was commonly used to distinguish mesotheliomas from adenocarcinomas. Hyaluronic acid can be identified by Alcian blue (pH 2.5) staining with and without hyaluronidase. Mesotheliomas are generally Periodic Acid Schiff (PAS) negative after diastase treatment.

## **BIOLOGY AND MOLECULAR BIOLOGY OF THE MESOTHELIUM AND MESOTHELIOMAS**

Mesothelial cells are relatively easy to culture *in vitro* where they can undergo spontaneous as well as treatment-induced transformation and gain malignant phenotypes (Kobliakov et al., 2006). Consequently, much of the literature on the biology of normal and transformed mesothelium derives from *in vitro* studies. Similarly, cells derived from spontaneously occurring and induced mesotheliomas have been studied in *in vitro* test systems. Based on the similar embryological origin of the pleural and peritoneal mesothelium, it is reasonable to assume a similar biology in cell cultures derived from either of these tissue sites.

Normal and spontaneously transformed rat mesothelial cells studied *in vitro* express CYP1A1 and CYP1B1 mRNAs, which are decreased in transformed cells and in asbestos-induced mesothelioma cells from Wistar rats (Kobliakov et al., 2006). P-Glycoprotein, the *mdr1* gene product, was not detected in normal mesothelial cells. Furthermore, mRNA for the Ah receptor and ARNT, proteins that regulate induction of CYP enzymes via signal transduction in the cell nucleus, did not differ among the various cultured cells. The relevance of these *in vitro* findings relates to the biological functions of the studied proteins. The CYP enzymes potentially oxidize xenobiotics in some cases to metabolites which can induce cellular toxicity and carcinogenicity unless eliminated from the organisms by conjugation with glutathione or other cell substances, and in other cases detoxify xenobiotics to polar less toxic substances. P-Glycoprotein is a

transmembrane pump that functions to eliminate xenobiotics from cells. Its absence in mesothelial cells suggests that the cells are not able to eliminate potentially harmful xenobiotics by this specific mechanism.

Insulin-like growth factors (IGFs) are polypeptides that are associated with cell proliferation and differentiation. Cell lines from normal rat mesothelium and from spontaneous rat peritoneal mesotheliomas express RNA transcripts for IGF2, but cell lines from asbestos-induced rat mesotheliomas do not (Rutten et al., 1995). Since all 3 cell types have receptors for IGF2, as well as for IGF1 and insulin, the expression of IGF2 in the normal rat mesothelium and in the spontaneous mesothelioma indicates the probability that IGF2 is functioning as an autocrine growth factor, and suggests that asbestos-induced mesotheliomas arise through a different transformation pathway than do spontaneous mesotheliomas.

The basic immunobiology associated with mesotheliomas is poorly understood. Using a mouse model of malignant mesothelioma, Bielefeldt-Ohmann et al., (1994) showed significant production of the cytokines TGF-beta, interleukin-6 (IL-6), IL1, and tumor necrosis factor (TNF), by the mesothelioma cells. The authors suggested that the elaboration of these factors by the mesothelioma cells is contributory to sabotaging antitumor host defenses, and can induce perturbations in immune surveillance.

#### Oncogenes.

Oncogenes appear to play a minor role in the pathogenesis of mesotheliomas. Nishiyama et al. (1995) found no point mutations in H-, K- or N-*ras* proto-oncogenes, or the *p53* tumor suppressor gene, in three ferric nitrilotriacetate-induced peritoneal mesotheliomas in Wistar rats. In an analysis of 17 human and 22 rat asbestos-induced mesotheliomas, no mutations in exons 12, 13, or 61 of the K-*ras* proto-oncogene were identified by direct DNA sequence analysis (Ni et al., 2000). There is some evidence, however, that the early response gene pathway leading to chronic stimulation of cell proliferation is involved in asbestos-induced rat mesotheliomas. A dose-dependent induction of *c-fos* and *c-jun* mRNA in rat mesothelial cells by asbestos leads to persistent induction of AP-1 transcription factors which drive the cell proliferation process (Heintz et al., 1993). Thus, this early response gene pathway involved in asbestos-induced rat mesotheliomas leads to chronic stimulation of cell proliferation. The fibrous geometry of the particulates appears to be critical in induction of *c-fos* and *c-jun* in rat pleural mesothelial cells, with crocidolite and chrysotile asbestos causing a more dramatic increase in these early response genes than nonfibrous particles (Janssen et al., 1994). There is also some evidence that this induction of *c-fos* and *c-jun* in rat mesothelial cells by asbestos is not directly triggered by active oxygen species generation. The initial response of rat mesothelial cells to active oxygen species is an increase in antioxidant enzymes followed by induction of *c-fos* and *c-jun*, secondary to a redox-sensitive component in the signaling cascade influenced by intracellular thiol (glutathione) levels. (Janssen et al., 1995). Although there have been a number of studies of the role of *c-fos* and *c-jun* in asbestos-induced mesotheliomas, there have been no similar studies of chemically induced tumors.

#### Tumor suppressor genes.

In contrast to oncogenes, tumor suppressor genes (TSG) appear to play a more important role in mesothelial tumorigenesis. Alterations in tumor suppressor genes are characteristic of human malignant mesotheliomas (Apostolou et al., 2005) and are also seen in murine mesothelioma animal models (Kane 2006). In general, TSG are important regulators of cell cycle machinery. In human malignant mesotheliomas there is frequent inactivation of *Nf2* and loss of p16(INK4a) secondary to deletion of the CDKN2A/ARF locus. There are also indications of alterations in p19(ARF), AKT, and WT-1. Genetic alterations in *p16* and *Nf2*, both of which are important regulators of the cell cycle, have been identified in human malignant pleural mesothelioma and in asbestos-exposed, *Nf2*-deficient mice (Jaurand & Fleury-Feith, 2005). These studies show a similar profile of TSG alteration in asbestos-induced mesotheliomas in mice and humans. Inactivation of *Nf2* is typically associated with tumors of neuroectodermal origin. P16/CDKN2A, as a tumor suppressor gene, is an important inhibitory protein that maintains the necessary balance between cyclin activation of cell proliferation and inhibition of the uncontrolled cell division that is characteristic of cancer cells. It is also potentially important in cell motility and invasiveness (Kane, 2006).

In another study, alterations of p16, 85% of which were homozygous deletions, were present in all 40 human malignant mesothelioma cell lines examined, and homozygous deletions were present in 5 of 23 (22%) primary malignant mesotheliomas (Cheng et al., 1994). *Nf2* mutations were detected in 8 of 15 (53%) human malignant mesothelioma cell lines, nearly all of which were confirmed in matched primary tumor DNAs (Bianchi et al., 1995). Asbestos-exposed *Nf2*(+/-) knockout mice had significantly accelerated mesothelioma development compared with similarly exposed wild type littermates (Altomare et al., 2005a). Biallelic inactivation secondary to loss of the wild type allele occurred in all the knockout mice and in 50% of the wild type mice. Alterations in p19/Arf and p15/Cdkn2b were frequent in asbestos-treated mice hemizygous for *Nf2*, with similar alterations in human mesothelioma cell cultures (Lecomte et al., 2005). These same authors also noted loss of heterozygosity for *Nf2*, as was noted by Altomare et al., (2005a).

No *p53* mutations were detected in an analysis of 17 human and 22 rat asbestos-induced mesothelioma tissue samples (Ni et al., 2000), and neither spontaneous rat mesotheliomas nor erionite-induced mesotheliomas in rats were found to have *p53* alterations (Kleymenova et al., 1999). On the other hand, there was a low rate of *p53* mutations in mesothelioma cells from asbestos-treated *Nf2* hemizygous mice (Lecomte et al., 2005). While *p53* does not appear to play a major role in malignant mesotheliomas, there is an accelerated development of asbestos-induced mesotheliomas in heterozygous *p53* +/- mice (Vaslet et al., 2002). As the tumors develop in these mice there is loss of heterozygosity accompanied by genetic instability, decreased apoptosis, and accelerated tumor growth and invasiveness. The murine *Nf2*+/- model of environmental carcinogenesis is remarkably similar to human malignant mesothelioma and recapitulates many molecular features of the human tumor (Altomare et al., 2005b).

The WT-1 suppressor gene is expressed in normal and neoplastic mesothelial cells in rats and humans (Walker et al., 1994), and immunohistochemical staining for

WT-1 is useful in distinguishing mesotheliomas from adenocarcinomas and other neoplasms.

From these findings regarding tumor suppressor genes, it is apparent that there is considerable genetic instability in both human cases and mouse models of mesothelioma, and that multiple TSG are involved in mesothelial tumorigenesis. It is likely that vasmultiple molecular events, interacting either sequentially or in the aggregate, are involved in the development of mesotheliomas.

#### Other molecular factors.

AKT is a protein kinase that is important in mammalian cell signaling. It plays an important role in tumorigenesis and therapeutic resistance and is frequently inactivated in human malignant mesotheliomas, as well as in Nf2(+/-) mice (Altomare et al., 2005a,b).

#### Growth factors and cytokines.

A number of different growth factors associated with proliferation of normal and neoplastic mesothelial cells have been documented and much of what has been learned about these factors was generated from *in vitro* cell culture studies.

Normal rat pleural mesothelial cells exposed *in vitro* to long carcinogenic mineral fibers upregulate epidermal growth factor receptor (EGFR), with increases in EGFR protein occurring 24 hours prior to initiation of the protein kinase mitogenic signaling cascade leading to increased cellular proliferation (Faux et al., 2001). Furthermore, fibers with greater potential to cause mesothelioma induce a more marked upregulation of EGFR than less carcinogenic fibers. The EGFR response is linked to phagocytosis of the mineral fibers by the rat mesothelial cells.

The bioactivity of TGF-beta in two mesothelioma cell lines established from spontaneous rat mesotheliomas was 30 to 70 times higher than in normal rat mesothelium (Kuwahara et al., 2001). Based upon application of exogenous TGF-beta to the mesothelioma cell lines and normal rat mesothelial cells, the authors suggested that rat mesothelioma cells produced TGF-beta through an autocrine mechanism that stimulates their growth.

Using asbestos-induced murine mesothelioma models, it was noted that TGF-beta production by mesothelioma cells may permit their escape from immune surveillance based on down-regulation of lymphocyte surface markers (Bielefeldt-Ohmann et al., 1994). TGF-beta 1 and 2 isoforms are expressed by both human and murine malignant mesothelial cells, and inhibition of TGF-beta by antisense RNA reduces the anchorage-independent growth of malignant mesothelial cells *in vitro* and their tumorigenicity *in vivo* (Fitzpatrick et al., 1994). Inhibition of TGF-beta also led to increased T-lymphocyte infiltration into tumors. Thus, it appears that TGF-beta has tumor enhancing effects in mesothelial tumorigenesis.

Altered expression of platelet-derived growth factor (PDGF) is characteristic of human mesotheliomas. There is no expression of PDGF in asbestos-induced rat mesotheliomas, although the PDGF receptors are highly expressed (Walker et al., 1992).

The species differences between human and rat mesothelioma cells suggest that expression of PDGF may be species-specific, at least for asbestos-induced mesotheliomas.

The growth factors TGF-beta, EGF, and PDGF all independently stimulate a round of cell proliferation in serum-deprived, quiescent, primary normal human mesothelial cells (Gabrielson et al., 1988). When the growth medium is supplemented with chemically denatured serum, these same growth factors can sustain continuous replication of mesothelial cells. Based on the responses to PDGF and TGF-beta, the authors concluded that mesothelial cells have growth regulatory properties similar to connective tissue cells. Normal human mesothelial cells secrete more TGF-beta than mesothelioma cell lines. In contrast mesothelioma cell lines secrete more PDGF than normal human mesothelioma cells (Gerwin et al., 1987).

TGF-alpha is expressed in asbestos-transformed rat mesothelial cells but not in spontaneously transformed mesothelial cells, while both cell types express functional EGF receptors (Walker et al., 1995). Although TGF-alpha inhibits the growth of spontaneously transformed mesothelial cells, it also functions in an autocrine growth control fashion to stimulate growth of asbestos-transformed mesothelial cells (Walker et al., 1995). The implication of this study is that differences in mesothelioma etiology may be responsible for differences in the molecular biology of these neoplasms.

Based upon VEGF expression levels and VEGF blocking by neutralizing antibodies in 4 human malignant mesothelioma cell lines, as well as in biopsies of malignant mesothelioma, VEGF appears to be a key regulator of malignant mesothelioma cell growth (Strizzi et al., 2001). Since malignant mesothelioma cells also express the tyrosine kinase-related VEGF receptors Flt-1 and KDR, VEGF is believed to function as an autocrine growth factor in human malignant mesothelioma.

Cell lines from normal rat mesothelium, as well as spontaneous and asbestos-induced mesothelioma cell lines, all express IGF1, IGF2, and insulin receptors. However, there is ubiquitous expression of IGF2 (important in cell proliferation and differentiation) by normal rat mesothelium and spontaneous mesothelioma cell lines but not by asbestos-induced mesothelioma cell lines (Rutten et al., 1995). Hence, IGF2 appears to function as an autocrine or paracrine growth factor in normal and spontaneously altered rat mesothelial cells. The authors suggested that changes in growth factor expression may be a consequence of different pathways of cell transformation.

Immunostaining of human malignant mesothelioma tissue specimens shows elevated expression of phosphorylated/activated AKT kinases which are protein kinases important in mammalian cell signaling (Altomare et al., 2005b). Hepatocyte growth factor HGF/met receptor signaling in human and murine malignant mesothelioma cell lines is associated with HGF-inducible AKT activity, and suggests that this pathway may be amenable to targeted pharmacological therapy (Altomare et al., 2005b).

In a study of the gene expression profile of rat peritoneal mesotheliomas induced by *o*-nitrotoluene or bromochloroacetic acid, Kim et al., (2006) utilized Ingenuity Analysis Pathway software to identify 169 cancer-related genes. They identified activated

IGF-1, p38 MAPkinase, Wnt/beta-catenin and integrin signaling pathways in these tumors. The authors concluded that the mesotheliomas induced by these two agents were similar to human mesotheliomas with respect to their cellular and molecular features.

In summary, based on several *in vitro* studies effects on cell signaling and cell proliferative responses in normal and transformed mesothelium are influenced by several growth factors and cytokines functioning in an autocrine fashion.

## **EXPERIMENTAL MODELS OF MESOTHELIOMA**

### *In vitro*/cell culture models.

Much of our knowledge of the molecular biology of mesotheliomas has been derived from studies using primary and established cultures of normal and transformed mesothelium, as well as cell lines derived from human and rodent mesotheliomas (see Biology/Molecular Biology section of this review). New cell lines are being continually established and described (e.g., Orengo et al., 1999; Veldwijk et al., 2008; Davis et al., 1992; Marsella et al., 1997; Kane, 2005).

### *In vivo* animal models.

In a recent review, Kane (2006) briefly discussed animal models of mesothelioma, including genetically modified mouse models. Intraperitoneal and intrapleural injections of rodents with asbestos results in malignant mesotheliomas which are similar to human mesotheliomas with regard to latency, patterns of growth, and development of ascites (Engelbrecht and Burger, 1975; Wagner et al., 1973; Adachi et al., 1994; Schurkes et al., 2004, Davis et al., 1992). Lymphatic metastasis and invasion of abdominal adipose tissue and diaphragm muscle resemble cases of diffuse malignant mesothelioma in humans (Altomare et al., 2005a). Murine peritoneal mesotheliomas have histopathological growth patterns and phenotypic markers including cytokeratins, *N*-cadherin, and WT1 which are seen in human diffuse malignant mesotheliomas (Kane, 1998).

While only a minority of human malignant mesotheliomas carry *p53* mutations (Kane, 2006), heterozygous *p53*-deficient mice have accelerated development of asbestos-induced peritoneal mesothelioma (Vaslet, 2002). Heterozygous *Nf2*-deficient mice also show accelerated development and increased invasiveness of peritoneal mesotheliomas following exposure to crocidolite asbestos (Fleury-Feith et al., 2003; Altomare et al., 2005a). The relevance of this model relates to common occurrence of molecular alterations in *Nf2* in human malignant mesothelioma. A subset of asbestos-exposed heterozygous *Nf2* – deficient mice develop mesotheliomas with loss of *p53*, possibly due to the colocalization of *Nf2* and *p53* on mouse chromosome 11 (Kane, 2006). The reported cooperativity between *Nf2* and *p53* would be expected to increase the invasive and metastatic potential of the induced mesotheliomas (McClatchey et al., 1998; McClatchey, 2000). In asbestos-induced murine mesotheliomas in heterozygous *Nf2*-deficient mice, there is constitutive activation of the *Akt* pathway (Altomare et al., 2005b), a pathway frequently upregulated in human mesotheliomas and a key pathway in cell growth and proliferation. It is also noteworthy that the majority of mesotheliomas

induced in heterozygous *Nf2*-deficient mice exhibit codeletion of *p16(Ink4a)* and *p19(arf)* (Kane, 2006), which is frequently observed alterations in human malignant mesotheliomas (Altomare et al., 2005b).

While simian virus 40 has been shown to induce a high incidence of mesotheliomas in hamsters (Cicala et al., 1993), implication of SV40 as a cofactor in asbestos-induced human mesothelioma development is based on identification of SV40 viral sequences in asbestos-associated mesotheliomas, and a causative role for SV40 in human mesotheliomagenesis remains controversial (Gazdar et al., 2002; Toyooka et al., 2002; Klein et al., 2002; Terracini, 2006; Emri et al., 2000). Genetically engineered mice with SV40 T-antigen under control of regulatory elements of the cytokeratin 19 gene develop several epithelial neoplasms in addition to a moderate frequency of mesotheliomas, but due to fertility problems this model is not readily available (Grippo and Sandgren, 2000).

## **TREATMENT-ASSOCIATED TUNICA VAGINALIS MESOTHELIOMAS IN RATS**

### Proposed Modes of Action

Hormone imbalance brought about by perturbations of the endocrine system has been proposed as a key event ultimately leading to both spontaneous and treatment-associated tunica vaginalis mesotheliomas in rats (Turek and Desjardins, 1979; Tanigawa et al., 1987, Shipp et al., 2006). The feasibility of a hormonally driven process was originally appreciated based on the observation that diethylstilbestrol induced mesotheliomas on the genital organs in both sexes of dogs (O'Shea and Jabara, 1971). Decreased testosterone in aging rats leads to Leydig cell hyperplasia and ultimately Leydig cell tumors (Turek and Desjardins, 1979). This aging change is especially dramatic in the F344 rat which has a high spontaneous incidence of Leydig cell tumors (range 88 to 96%), in contrast to other rat stocks used in chronic studies (Boorman et al., 1990; Maekawa and Hayashi, 1992; Takaki et al., 1989; Solleveld et al., 1984). For example, based on Leydig cell hyperplasia, it has been proposed that testicular aging changes seen at 12 months in F344 rats (Kanno et al., 1987) are equivalent to testicular aging changes in 2-year old Wistar rats. The occurrence of Leydig cell tumors, in turn, is causally linked to development of tunica vaginalis mesotheliomas in F344 rats (Turek and Desjardins, 1979).

In the sexually mature rat, both leutinizing hormone (LH) and leutinizing hormone releasing hormone (LHRH) stimulate Leydig cells to produce testosterone (Capen, 1996; Prentice and Meikle, 1995). The testicular LH receptors and the serum testosterone levels decrease in rats between ages 4 and 18 months. In this age range, the testicular LH receptors and testosterone levels are correlated and balanced. As the testosterone levels decline with age, there is a compensatory increase in circulating LH to increase the level of testosterone. The compensatory action results in an increase (hyperplasia) of Leydig cells to increase testosterone levels. Ultimately the compensation is inadequate to maintain youthful levels of testosterone and the testicular-LH interaction strikes a new balance at a lower level (Amador et al., 1985). The ratio of the two is the same as before, but the levels are lower. LH continues to stimulate the Leydig cells to

divide in an attempt to reach youthful levels of testosterone, resulting in progression of the proliferating Leydig cells from hyperplasia to Leydig cell tumors. The testosterone-LH ratio changes once Leydig cell tumors are formed. Leydig cell tumors produce less testosterone than normal Leydig cells. Thus, an age-associated hormonal imbalance persists in older rats bearing Leydig cell tumors. In addition to decreased testosterone, there is an increase in Leydig cell LH receptors, an increase in serum progesterone, decreased prolactin, and decreased LH. In other words, the balance between testicular LH receptor levels and serum testosterone that was present during the 4 to 18 month age interval changes, and the levels of the different hormones become unbalanced in the presence of Leydig cell tumors.

Perturbations in the hypothalamic-pituitary-testis axis lead to Leydig cell proliferation, based on circulating levels of both LH and LHRH and the number of their cognate receptors on Leydig cells. While it may at first seem counter-intuitive, increases as well as decreases in prolactin levels can affect the hypothalamic-pituitary-testis axis and lead to Leydig cell hyperplasia and Leydig cell tumors.

The decrease in testosterone that ultimately leads to Leydig cell proliferation can also be brought about by an age-related increase in prolactin production in rats (Mahoney and Hodgen, 1995; Esquifino et al., 2004; Capen et al., 2002; Turek and Desjardins, 1979). The increased prolactin leads to decreased gonadotrophin releasing hormone (LHRH) as well as decreased LH secretion. Since rat Leydig cells have LHRH receptors that are responsive to LH and LHRH, the hormonal cascade initiated by increased prolactin leads to reduced testosterone production, as is reflected by the decreased serum testosterone levels seen in the aging rat (Mahoney and Hodgen, 1995). It is important to note that while rat Leydig cells have LHRH receptors, human Leydig cells do not (Prentice and Meikle, 1995).

Alternatively, decreased prolactin production may occur secondary to the action of dopamine agonists on the hypothalamus (Prentice and Meikle, 1995; Cook et al., 1999). The decreased prolactin leads to a decrease in LH receptors on the Leydig cells and thereby results in reduced testosterone production. This then causes a compensatory increase in circulating LH and a sustained increase in circulating LH results in Leydig cell hyperplasia and Leydig cell tumors (Prentice and Meikle, 1995).

The proof that age-related hormonal perturbation leads to Leydig cell tumors in the rat is supported by experiments in which Leydig cell hyperplasia and Leydig cell tumors are prevented by testosterone supplementation (Chatani et al., 1990; Fort et al., 1995). Similarly, the hormonal effects leading to Leydig cell tumorigenesis can be mimicked by different classes of chemicals that act through the hypothalamic-pituitary-gonadal axis to ultimately affect LH and testosterone, and lead to Leydig cell hyperplasia and Leydig cell tumors (Shipp et al., 2006). In addition, GnRH receptor agonists cause development of Leydig cell tumors by binding to LHRH receptors on Leydig cells (Prentice and Meikle, 1995; Donaubaue et al., 1987). This latter mechanism is unique to the rat since human Leydig cells do not have LHRH receptors (Prentice and Meikle, 1995).

Leydig cell tumors and their accompanying alterations in systemic hormonal levels have pleiotropic effects on the tissues of the genital system, including decreased spermatogenesis, seminiferous tubule atrophy, and atrophy of seminal vesicles (Kanno et al., 1987; Bartke et al., 1985). Intratesticular androgen levels are significantly higher than circulating levels (Foster, 2007). The alterations in androgen levels that accompany Leydig cell tumors are reflected as a transudate in the interstitial fluid within the testes as well as in the tunica vaginalis fluid compartment. The mesothelium bathed by the tunica vaginalis fluid is exposed to a higher concentration of the altered hormonal levels, probably by diffusion, than would occur following exposure via the circulatory system (Karpe et al., 1982; Gerris and Schoysman, 1984). Exposure of tunical vaginalis mesothelium to altered levels of androgens may trigger mitogenesis via mesothelial cell production of growth hormones that operate in an autocrine fashion, as occurs in other male reproductive system tissues (McKeehan et al., 1984; Kyprianou and Isaacs, 1988). The growth hormones released from the stimulated tunica vaginalis mesothelium include TGF-beta, PDGF, IGF2, and EGF, all of which stimulate mitogenesis. Continued enhanced proliferation of the tunica vaginalis mesothelium will lead to hyperplasia, with a subsequent increased probability for development of genetic damage and subsequent mesotheliomas.

An alternative hypothesis for induction of tunica vaginalis mesotheliomas secondary to Leydig cell tumors in rats relates to the physical pressure or mechanical stress placed on the mesothelial cells lining the scrotal tunics by the enlarged testes (Tanigawa et al., 1987). Based on the idea that pleural mesotheliomas may, in part, be a consequence of physical stimulus from asbestos fibers (Shabad et al., 1974; Stanton and Wrench, 1972), and because of it is known that transformed mesothelium expresses growth factors that stimulate its own mitogenesis (Gerwin et al., 1987; Versnel et al., 1988), it is reasonable to expect that physical pressure from testes enlarged by Leydig cell tumors could lead to transformation and/or growth factor secretion by tunica vaginalis mesothelium. This possible mode of action is further supported by the observation that visceral pleural mesothelial cells release significant levels of the growth factor PDGF in response to mechanical forces (Waters et al., 1997). As is the case with virtually all studies of carcinogenesis, alternative modes of action are not necessarily mutually exclusive, and more than one may act in concert to produce an adverse effect.

While hormone imbalance and mechanical force represent most likely key events for induction of both spontaneous as well as treatment-associated increases in tunica vaginalis mesotheliomas in rats, and especially in the F344 rat, alternative pathways for exacerbation of tumor development from exposure to xenobiotics are certainly plausible. Assuming that a xenobiotic agent or its metabolite can reach the tunica vaginalis mesothelium, both direct genotoxic action or indirect DNA damage via reactive oxygen species could also explain an exacerbation of the low spontaneous background incidence of this tumor. Similarly, enhanced cell proliferation, possibly secondary to irritation, inflammation, or mechanical stress, could contribute to an exacerbation of this low incidence spontaneous tumor. An association between chronic inflammation and both human pleural and rat peritoneal mesothelioma induction has been reported (Hillerdal and Berg, 1985; Grimm et al., 2002).

Evidence for an oxidative stress mode of action is supported by intraperitoneal injection of xenobiotics such as ferric saccharate or ferric nitrilotriacetate (Okada et al., 1989; Nishiyama et al., 1995) as well as by oral exposure to potassium bromate (Kurokawa et al., 1983 ; DeAngelo et al., 1998 ; Wolf et al., 1998) which produce reactive oxygen species (ROS) that can potentially have direct action on tunica vaginalis mesothelium. ROS are also considered important mediators in asbestos-induced mesotheliomas (Attanoos and Gibbs, 1997; Schurkes et al., 2004; Adachi et al., 1994). Alternatively, increases in replicative DNA synthesis in mesothelium that could lead to mesothelioma development either by directly affecting cell cycle machinery or secondary to gene alterations in cell cycle machinery has been shown in testicular mesothelium following subchronic exposure to acrylamide (Lafferty et al., 2004).

From a review of several agents associated with increases in tunica vaginalis mesotheliomas in F344 rats and occasionally in other rat stocks, one or more of the above described key events may be operating in the genesis of tunica vaginalis mesotheliomas. Likely modes of action for mesothelioma induction will be addressed for the specific xenobiotics associated with increases in this tumor and are described in the following sections. Twenty-one substances that were associated with increased incidences of tunica vaginalis mesothelioma in chronic rat carcinogenicity studies were identified in the National Toxicology Program database (Table 2) and in an extensive review of published literature, and their effects are described below.

#### Cancer Bioassays Associated with Increases in Tunica Vaginalis Mesotheliomas in Rats

Most rat cancer bioassays with some evidence of mesothelioma induction reported by NTP or in the literature were conducted using F344 rats. The NTP studies utilized F344 rats from a closed colony, and the other studies used F344 rats from different commercial sources. Consequently, the sensitivity of F344 rats to spontaneous and induced mesotheliomas extends to different colonies of these rats. The specific studies presented below are arranged in order, by route of administration.

#### Specific Chemicals - Intraperitoneal Route of Administration

Various forms of asbestos and a variety of other durable fibers and agents, including ceramic fibers, silicon carbide, stone wool, slag wool, glass wool, erionite, and cellulose, induce peritoneal cavity mesotheliomas in rats by i.p. injection (Wagner et al., 1973; Davis et al., 1986; Mast et al., 1994; McConnell, 1995; Miller et al., 1999; Kamstrup et al., 2002; Kleymenova et al., 1999). These same agents have been shown to induce pleural cavity mesotheliomas in experimental animals injected by the intrapleural route. The various intraperitoneal injection studies have been carried out in different strains such as Osborne-Mendel, Wistar, and F344, sometimes in females rather than males, and typically have used a single intraperitoneal injection. Adhesions and chronic inflammation generally accompanied the induced mesotheliomas which occurred several months after treatment. These studies are not summarized or discussed in detail, below.

Three non-fibrous chemical agents, when introduced into the peritoneal cavity of rats, led to development of tunica vaginalis mesotheliomas. Based on the anatomy of the rat, fluid injected into the abdominal cavity can easily get into the scrotal sac and lead to

exposure of the tunica vaginalis mesothelium. Direct acting carcinogens such as nitrosamines or agents that bring about oxidative stress, either as a primary effect or secondary to peritoneal inflammation, can also cause tunica vaginalis mesothelioma when injected into the peritoneal cavity of rats.

*Methyl(acetoxymethyl)nitrosamine.*

In a comparison of three different rat strains, Berman and Rice, 1979, reported on induction of testicular mesotheliomas following a single intraperitoneal injection of methyl(acetoxymethyl)nitrosamine (DMN-OAc), a short-lived, direct acting carcinogen (Table 3). In addition to the mesotheliomas, atypical mesothelial hyperplasia was noted in rats that didn't develop the tumor. The authors offered the opinion that testicular mesothelium has properties that are distinct from mesothelium elsewhere, and that the ability of mesothelium to respond to chemical carcinogens is an almost exclusive property of testicular mesothelium. In another publication, the authors showed that the spectrum of tumors induced by DMN-OAc in rats is dependent upon the route of administration (Berman et al., 1979).

The average age of death for the treated rats ranged from 14.8 to 16 months, while the average for control rats ranged from 17.2 to 20.9 months. Although DMN-OAc is a direct-acting carcinogen and does not require metabolic activation, it is clear that genetics can influence the susceptibility to mesothelioma formation. Furthermore, the highest incidence of mesothelioma (46%) occurred in the Buffalo rat which did not have any Leydig cell tumors, suggesting that hormonal effects were not driving the response in this particular study. It is noted that even gavage administration of nitrosamines causes peritoneal mesotheliomas (Lijinsky et al., 1985), suggesting that mesothelium may be especially sensitive to nitrosamine carcinogenesis. Methyl(acetoxymethyl)nitrosamine is mutagenic in the Ames test (Table 29).

*Ferric Saccharate.*

Daily intraperitoneal injections of ferric saccharate, which is a colloidal iron, and ferric saccharate plus nitrilotriacetic acid (NTA) for 3 months resulted in a high incidence of mesotheliomas in male Wistar rats (Table 4) (Okada et al., 1989). NTA stabilizes the iron which allows it to more efficiently induce ROS which then promote lipid peroxidation, enhancing the carcinogenic action of iron.

The mesotheliomas were confined to the tunica vaginalis in the ferric saccharate group. Six of the 14 mesotheliomas in the ferric saccharate-NTA group were disseminated throughout the abdominal cavity.

Intramuscular injection site neoplasms have been induced by iron dextran complex (Richmond, 1959) indicating that injected iron can cause cancer at the site of injection. In the Okada et al., 1989 study, the mesotheliomas appeared to arise in the tunica vaginalis, presumably because the injection iron became localized in the testicular sac following intraperitoneal injection. The authors suggest free radical production with localized enhancement of the carcinogenic action of iron by NTA as the likely mode of

action for mesothelioma induction. NTA is not mutagenic in the Ames or mouse lymphoma mutation tests, or produce chromosome damage in mammalian cells in vitro; there are no reported mutagenicity studies of its combination with ferric saccharate.

#### Cytembena.

In an NTP bioassay of cytembena, a cytostatic agent, F344 rats and B6C3F1 mice received intraperitoneal injections 3 times a week for 104 weeks (NTP TR 207). Cytembena produced a strong mesothelioma response and was the only tumor induced in male rats in this study (Table 5). Female rats had an increase only in mammary fibroadenomas, and had 2 malignant abdominal mesotheliomas at the high dose. No induced tumors were seen in mice of either sex.

There was significant, drug-related chronic inflammation in the peritoneal cavity in both sexes of rats, and the inflammation occurred at a greater frequency and severity in the females. While mesotheliomas occurred in 2/50 high dose females, the significantly more robust response was seen in the males. There was no dose response; a maximum response was seen at both doses, and the mesotheliomas were present throughout the abdominal cavity, inclusive of the testis and epididymis. The induction of mesotheliomas in this study is most probably a consequence of inflammation, in combination with the sex predilection for tumor induction in the tunica vaginalis of male F344 rats. The mice in this study received higher doses than the rats, did not have chronic peritoneal inflammation, and did not have mesotheliomas. This observation serves to reinforce the commonly accepted observation that mice in cancer bioassays do not develop mesotheliomas, even following multiple direct intraperitoneal injections for 2 years, and that rats are more sensitive to mesothelial tumorigenesis. Cytembena is mutagenic in the Ames test and produces chromosome damage in cultured mammalian cells, but did not induce chromosome damage in mouse bone marrow cells following i.p. injection. (Table 29).

#### Specific Chemicals - Inhalation Route of Administration

Three inhalation 2-year cancer bioassays resulted in induction of tunica vaginalis and associated peritoneal mesotheliomas in male F344 rats.

#### Ethylene oxide.

Ethylene oxide, a highly reactive alkylating agent used in chemical synthesis, and to a lesser extent for sterilization and fumigation, was tested by inhalation exposure in F344 rats at 10, 33, and 100 ppm (Snellings et al., 1984). At the end of the 2-year study there was an increased incidence of tumors in both sexes with increases in brain tumors in both sexes, mononuclear cell leukemia and mammary gland adenomas and adenocarcinomas in females, and peritoneal mesotheliomas in males (Table 6). There was a high incidence of Leydig cell tumors in all groups of male rats and a variety of endocrine neoplasms in both male and female rats. Snellings et al. (1984) used two equally sized but separate control groups. A different inhalation study at 50 and 100 ppm in male F344 rats also resulted in an increased incidence of peritoneal mesotheliomas (Table 6) (Lynch et al., 1984). This latter study also documented an increase in mixed

cell gliomas in the brain and mononuclear cell leukemia in the ethylene oxide exposed males.

The overall frequency of mesotheliomas in the Snellings et al., (1984) study was not statistically significant by a 2-tailed Fischer's exact test. However, there was a statistically significant trend test and the cumulative percent of rats developing mesothelioma was significantly increased in the 100 ppm group versus the controls, from the 21<sup>st</sup> month to study termination. The late-developing mesotheliomas were probably influenced by the altered hormonal milieu associated with age-associated Leydig cell tumors in F344 rats. In the Lynch et al., (1984) study there was a dose-related increase in mesotheliomas with a statistically significant increase in the 100 ppm exposed rats.

In both studies, treatment-associated mesotheliomas arose in the tunica vaginalis and some spread into the abdominal cavity. They were morphologically similar to spontaneously occurring mesotheliomas. While the mechanism for induction of mesotheliomas by ethylene oxide remains unclear, the spectrum of other lesions in endocrine tissues and testes potentially implicates a hormonal factor in their development. Ethylene oxide is positive in the Ames test (Table 29) and most in vitro and in vivo genetic toxicity tests.

#### 1,2-Dibromoethane.

1,2-Dibromoethane is a multisite, trans-species carcinogen following inhalation exposure, and produces nasal, pulmonary, and mammary tumors, as well as hemangiosarcomas (NTP TR 210). Inhalation of dibromoethane for 2 years produced a strong mesothelioma response in male F344 rats (Table 7). There was an increase in mammary fibroadenomas in female rats. Primary lung tumors, hemangiosarcomas, fibrosarcomas, nasal carcinomas, and mammary adenocarcinomas were induced in B6C3F1 exposed mice (NTP TR 210).

There was a high Leydig cell tumor frequency in the control and exposed groups. 1,2-Dibromoethane caused testicular degeneration that might explain the reduced number of Leydig cell tumors in the high exposure rats. In an older NTP gavage study in Osborne-Mendel rats, increased forestomach and liver tumors, as well as hemangiosarcomas were reported, but no mesotheliomas were present (NTP TR 86).

The mechanism by which 1,2-dibromoethane induced mesotheliomas is unknown. Glutathione conjugation of 1,2-dibromoethane leads to formation of an episulfonium ion that is DNA reactive, suggesting a genotoxic effect. The typically high incidence of Leydig cell tumors in the low exposure group and the known testicular toxicity even at low doses ([www.epa.gov/iris](http://www.epa.gov/iris)) suggest a profound perturbation of hormonal balance that might have contributed to the robust mesothelioma response. 1,2-Dibromoethane is mutagenic in the Ames and mouse lymphoma tests, and produces chromosome damage in mammalian cells in culture and in mouse bone marrow cells (Table 29).

### 1,2-Dichloroethane (DCE).

A low incidence of malignant mesotheliomas in the peritoneal cavity, especially in the scrotal sac, was reported at 160 ppm DCE in an inhalation study using F344 rats (Table 8.) (Nagano et al., 2006). The mesotheliomas at this highest concentration exceeded the historical control, but the incidence was not statistically significantly increased compared to the concurrent control.

Other tumor responses in the Nagano study included subcutaneous fibromas and mammary fibroadenomas in male and female rats, as well as mammary adenomas and adenocarcinomas in the female rats. In an older NCI gavage bioassay in Osborne-Mendel rats, mesotheliomas were not observed (NTP TR 55), and there was no mention of testicular Leydig cell tumors in the study report. DCE was carcinogenic in B6C3F1 mice causing mammary and endometrial tumors in females and lung tumors in both sexes (NTP TR 55). In an older inhalation study in F344 rats, exposure to 50 ppm DCE did not result in a tumor response (Cheever et al., 1990). DCE is mutagenic in the Ames and in vitro cytogenetics tests, but did not induce micronuclei in bone marrow of dosed male or female mice (Table 29).

### Specific Chemicals - Dosed Feed Route of Administration

Ethyl tellurac. A dose feed study of ethyl tellurac in F344 rats produced an equivocal tunica vaginalis mesothelioma response that showed a statistically significant trend, but was not significant by pairwise comparison (Table 9) (NTP TR 152). This was the only tumor response seen in rats in this study, and the chemical was judged to exhibit equivocal evidence of carcinogenicity. There was no mention in the report of Leydig cell tumors.

The judgment to consider the ethyl tellurac bioassay as not positive was based on a non-significant pairwise statistical comparison to the concurrent control, and the historical control incidence (12/416; 2.9%) for the testing laboratory. An increased frequency of Harderian gland adenomas in treated male and female mice was considered equivocal evidence of carcinogenicity. Ethyl tellurac is not mutagenic in the Ames test, mutagenic in the mouse lymphoma test, and produced an equivocal increase in chromosome aberrations in cultured mammalian cells (Table 29).

### *o*-Nitrotoluene.

Two prechronic and one carcinogenicity study on *o*-nitrotoluene have been conducted by the NTP (NTP Tox 23, NTP Tox 44, NTP TR 504). Mesothelial hyperplasia and mesotheliomas involving the tunica vaginalis surface of the epididymis were seen in rats receiving 5000 and 10000 ppm *o*-nitrotoluene in their diet for 13 weeks (Table 10). A follow-up 26-week prechronic study was conducted to compare the tumor responses of *o*-nitrotoluene and *o*-toluidine HCl given at equimolar concentrations in the diet, and to investigate the role of intestinal flora in metabolism of *o*-nitrotoluene (NTP

Tox 44). This 26-week study included a 13-week *o*-nitrotoluene exposure, followed by an additional 13 weeks on control diet (i.e., stop study). Mesothelial hyperplasia and mesotheliomas were seen at the 13-week interim sacrifice, in the stop-exposure group at study conclusion, and in the rats continuously exposed to *o*-nitrotoluene for 26 weeks (Table 11). The 2-year cancer bioassay of *o*-nitrotoluene included dietary doses of 625, 1250, and 2000 ppm, and incorporated a 3-month stop study in which rats were fed diets containing 2000 or 5000 ppm *o*-nitrotoluene followed by undosed feed for the remainder of the two years. All stop-study rats, and all but three of the rats given 1250 ppm, died before the end of the two years. The incidences of mesotheliomas in this study are summarized in Table 12.

In the 2-year study, the mesotheliomas were located in the tunica vaginalis of the testis or epididymis with some cases extending into the abdominal cavity. The majority of the mesotheliomas in treated rats were large and locally invasive. *o*-Nitrotoluene is not mutagenic in the standard Ames test. However its nitro group can be reduced by anaerobic gut flora to ultimately yield a DNA reactive metabolite. The formation of *o*-benzyl glucuronide is a critical step in leading to formation of the DNA-reactive metabolite. Basically, intestinal microflora hydrolyze the glucuronide and reduce the nitro group to form *o*-aminobenzyl alcohol. Upon reabsorption of the *o*-aminobenzyl alcohol, it is sulfated and binds to DNA.

Because reduction of the nitro group of *o*-nitrotoluene by anaerobic gut flora yields *o*-toluidine, which is mutagenic in the Ames test, a 26-week study comparing equimolar doses of *o*-nitrotoluene and *o*-toluidine was conducted. The incidence of mesothelioma was greater, and the latency less, for rats administered *o*-nitrotoluene (NTP Tox 44). Similarly, the liver effects, including cholangiocarcinomas, were greater for *o*-nitrotoluene than for *o*-toluidine. The lower potency of *o*-toluidine compared to *o*-nitrotoluene with respect to liver lesions and mesothelioma induction suggests that the effects of *o*-nitrotoluene involve more than the simple intestinal reduction of the nitro group. *o*-Nitrotoluene produced testicular degeneration in the 26-week toxicity study as well as in the two-year cancer study. This would lead to hormonal perturbations which, in the two-year study, were the likely cause of the reduced Leydig cell tumors in the high-dose males. An associated Leydig cell tumor reduction associated with testicular toxicity has been noted for other chemicals (Boorman et al., 1985). There was clear evidence of carcinogenicity in treated mice based on increased frequencies of hemangiosarcomas, large intestinal carcinomas and hepatocellular neoplasms.

*o*-Nitrotoluene was not mutagenic in the Ames test and did not induce chromosome aberrations in cultured mammalian cells, or micronuclei in mouse bone marrow cells when given in the feed to males and females, or when given i.p. to male mice or male and female rats.

#### *o*-Toluidine HCl.

*o*-Toluidine is a trans-species carcinogen that produced tumors in both sexes of F344 rats and B6C3F1 mice. Tumor types included a variety of splenic and other tissue mesenchymal tumors, urinary bladder transitional cell neoplasms, subcutaneous fibromas, hepatocellular neoplasms, hemangiosarcomas, and mammary gland fibroadenomas. *o*-Toluidine HCl induced a low incidence of epididymis mesotheliomas in F344 rats in a 26-week *o*-nitrotoluene/*o*-toluidine comparative study (NTP Tox 44) (Table 13). An older cancer bioassay had documented a high overall incidence of mesotheliomas involving multiple tissues in the abdominal cavity and the scrotal tunica vaginalis (NTP TR 153) (Table 14). An increase in mammary fibroadenomas was present in female rats.

The mesotheliomas in the 2-year study (Table 14) were morphologically similar to spontaneous and treatment-related mesotheliomas in other studies. A few of the more fibrous mesotheliomas contained foci of osseous metaplasia. In light of the known genotoxicity of *o*-toluidine (Table 29), it is likely that the mode of action for mesothelioma induction involves DNA damage to the tunica vaginalis mesothelium in addition to the contribution of hormonal imbalance associated with aging male F344 rats bearing Leydig cell tumors. Hemangiosarcomas and hepatocellular neoplasms were increased in *o*-toluidine-treated mice. *o*-Toluidine was mutagenic in the Ames and mouse lymphoma cell tests, produced chromosome aberrations in mammalian cells in culture, and contradictory results in two mouse bone marrow micronucleus tests.

#### 2,2-Bis(bromomethyl)-1,3-propanediol.

2,2-Bis(bromomethyl)-1,3-propanediol is a widely used flame retardant. It is genotoxic in a number of test systems. A dosed feed 2-year bioassay in F344 rats, which included a 3-month exposure stop study, produced a multi-site tumor response, including an increased incidence of mammary fibroadenomas in male and female rats (NTP TR 452). There was a strong peritoneal mesothelioma response in the male rats (Table 15). Other tumor responses in rats were seen in the skin, Zymbal's gland, oral cavity, esophagus, forestomach, small and large intestines, urinary bladder, lung, thyroid gland, hematopoietic system, and seminal vesicle. Neoplastic responses were also present in both sexes of B6C3F1 mice.

2,2-Bis(bromomethyl)-1,3-propanediol is one of 14 brominated chemicals studied by the NTP in 2-year rodent carcinogenicity studies. Thirteen of those 14 brominated chemicals were found to be carcinogenic, but only three (1,2-dibromoethane, 2,2-bis(bromomethyl)-1,3-propanediol, and potassium bromate) produced TVM. There are two hypotheses for the carcinogenic activity of brominated chemicals: (1) oxidative damage to DNA and other cellular constituents resulting from the induction of ROS, and (2) formation of DNA adducts when the C-Br bond is broken leaving a carbon-containing electrophilic group. In oral administration studies with potassium bromate [see below], which also produces mesotheliomas in male F344 rats, there is a significant increase in 8-hydroxydeoxyguanosine, which is a biomarker of oxidative damage (Kurokawa et al., 1983; Kasai et al., 1987; Sai et al., 1992). 2,2-Bis(bromomethyl)-1,3-propanediol is mutagenic in the Ames test and produces chromosome aberrations in cultured

mammalian cells, but yielded equivocal results in a mouse bone marrow micronucleus test.

#### Nitrofurazone.

Mesotheliomas were induced in male F344 rats in the dosed feed study of nitrofurazone (NTP TR 337) (Table 16). The mesothelioma response, which was not dose-related, was considered equivocal evidence of carcinogenicity by the peer review panel, arose in the tunica vaginalis, with some mesotheliomas spreading to the peritoneal cavity and invading the underlying soft tissue. There was a treatment-related increase in preputial adenomas and carcinomas, and a significant increase in mammary fibroadenomas in the female rats. Previous studies suggested that nitrofurazone was a mammary gland carcinogen. There was an increase of ovarian cancer in mice. Taken together, the tumor responses indicate the nitrofurazone may act through hormonal effects.

Poor survival of the high dose group is the likely reason for the decrease in mesotheliomas at the 620 ppm dose as compared to the lower dose. There was a dose-related decrease in Leydig cell tumors, also partly a reflection of poor survival in the high dose group. The obligatory role for nitro reduction in nitrofurazone-induced mutagenicity may be related to the widespread tumorigenicity in rats and mice (Kari et al., 1989). Nitrofurazone was mutagenic in the Ames and mouse lymphoma mutation tests and produced chromosome aberrations in cultured mammalian cells, but did not induce micronuclei in bone marrow cells of mice (Table 29).

#### Pentachlorophenol.

Pentachlorophenol is a wood preservative, as well as an herbicide, fungicide, and germicide. In a dosed feed study with pentachlorophenol, an increase in peritoneal mesotheliomas was seen in the stop-study F344 rats but not in the continuously exposed rats (NTP TR 483) (Table 17). A marginal increase in nasal carcinomas (1/50 versus 5/50) was also present in the stop-study males. No other treatment-related neoplasms were present in the males, and no treatment-related neoplasms were present in the female rats. Increases in liver and adrenal tumors and hemangiosarcomas were seen in pentachlorophenol-treated mice (NTP TR 349).

The mesotheliomas arose in the tunica vaginalis and had the histomorphological characteristics of the spontaneous and chemically-induced mesotheliomas seen in other studies. Extension into the peritoneal cavity was evident in 5 of the mesotheliomas in the stop-study group and the 1 mesothelioma in the control. Pentachlorophenol was non-mutagenic in the Ames test, and only weakly positive in an *in vitro* chromosome aberration test in cultured mammalian cells, and did not induce micronuclei in mouse or rat bone marrow cells.

Although pentachlorophenol is not mutagenic in bacterial test systems, one of its major metabolites, tetrachloro-*p*-hydroquinone, is genotoxic, covalently binds to DNA, and can induce oxidative damage to DNA. Oxidative damage, as assessed by 8-

hydroxydeoxyguanosine adducts, has been found in livers of mice exposed to pentachlorophenol, as well as elevated hemoglobin adducts in males and females (NTP TR 483). Thus, it is probable that the mesotheliomas seen in rats exposed to the high dose of pentachlorophenol in the NTP study are a consequence of the oxidative damage to mesothelium of the tunica vaginalis. Given that there was also a high incidence of Leydig cell tumors in the treated rats the altered hormonal milieu associated with the proliferating Leydig cells may also have contributed to the development of tunica vaginalis mesotheliomas.

#### Specific Chemicals - Dosed Water Route of Administration

Tartrazine (FD&C Yellow No. 5). Tartrazine is a food, drug, and cosmetic coloring agent. In a 2-year dosed water study using F344 rats, mesotheliomas were present only at the lower dose (Table 18) of tartrazine (Maekawa et al., 1987). There was a persistent decreased body weight gain in the 2% group starting at experimental week 40. Based on a lower than expected incidence in the control group (historical incidence was 4.1%), absence of a positive trend, and absence of hyperplastic or preneoplastic lesions in the peritoneal cavity, the authors concluded that the occurrence of peritoneal mesotheliomas was not related to treatment. It is mentioned in the publication that the mesotheliomas are similar to those seen spontaneously in the F344 male. The incidence of Leydig cell tumors in this study was greater than 94% in the control and low dose groups and was 100% in the high dose group. There was an increased incidence of endometrial stromal polyps in the low dose female rats that the authors concluded was not treatment-related.

Tartrazine was not mutagenic in the Ames test but produced chromosome aberrations in *cultured mammalian cells* (Table 29).

#### 3,3'-Dimethoxybenzidine hydrochloride

In a chronic dosed water study, terminated at 21 months due to early tumor-induced mortality, there was induction of tumors at multiple tissue sites including a marginal peritoneal mesothelioma response in male F344 rats (Table 19) and a robust mammary gland adenocarcinoma response in female rats (NTP TR 372). 3,3'-Dimethoxybenzidine hydrochloride was considered to have clear evidence of carcinogenicity based on statistically significant increases in tumors at multiple sites. It is a member of the aromatic amine class of chemicals which when metabolically activated induce a variety of tumor types. Activation of *ras* oncogenes was identified in some of the induced epithelial tumors.

The incidences of mesothelioma were not statistically significant by pairwise comparison, although there was a significant positive trend. There was significant early mortality in all treated males and females with greater than 50% mortality by week 86. At study termination (94 weeks) only 8 low dose males were alive among the treated rats. The authors of the technical report suggested that the mesothelioma incidences might have been higher had the rats lived longer. 3,3'-Dimethoxybenzidine is mutagenic in the

Ames and mouse lymphoma mutation tests, but did not induce chromosome aberrations in cultured mammalian cells (Table 29).

*3,3'-Dimethylbenzidine hydrochloride.*

In a 14-month dosed water study in F344 rats, 3,3'-dimethylbenzidine HCl produced a peritoneal mesothelioma response (Table 20) that showed a positive trend and was statistically significant at the highest dose. The authors of the technical report attributed the mesotheliomas to the test chemical and suggested that the incidence of mesothelioma might have been higher except for the reduced survival in the two highest dose groups. 3,3'-Dimethylbenzidine HCl was considered to have clear evidence of carcinogenicity based on robust responses at multiple other tissue sites (NTP TR 390).

3,3'-Dimethylbenzidine is a congener of 3,3-dimethoxybenzidine. Activation of the *H-ras* oncogene was detected in several epithelial neoplasms. 3,3'-Dimethylbenzidine was mutagenic in the Ames and mouse lymphoma mutagenicity tests, and induced chromosome aberrations in cultured mammalian cells (Table 29).

*Potassium bromate.*

Potassium bromate is a rodent carcinogen and is nephrotoxic and neurotoxic in humans. Because potassium bromate is a biproduct of water disinfection by ozonation, there has been interest in testing it for adverse effects by dosing in drinking water. Four drinking water cancer bioassay studies have been conducted in F344 rats and peritoneal mesotheliomas were induced in each study. The incidences of mesothelial responses in these studies are summarized in Tables 21, 22, and 23.

In the 1983 study, the earliest mesothelioma was observed after 72 weeks of treatment. The mesotheliomas were frequently seeded throughout the abdominal cavity and were associated with massive hemorrhagic ascites which, according to the authors, lead to severe anemia and early death.

Mesothelioma responses in the 1986 study were observed at doses of 30 ppm and higher with a statistically significant increase at 500 ppm, but the tumor incidences between 30 and 250 ppm were not dose-related. The occurrence of Leydig cell tumors was 95 to 100% in all groups, including the controls.

The origin of the mesotheliomas in this study was the tunica vaginalis mesothelium with involvement of the vaginal tunic, including the mesotheliomas that were present throughout the abdominal cavity. The TVM tended to be bilateral with some exceptions. Based on the book chapter by Hall (1990) and a pathology peer review of this study, the additional peritoneal sites of mesothelioma are considered neither additional primary tumors nor metastases. TVM in F344 rats typically spread by extension and seeding rather than via vascular or lymphatic routes of metastasis.

The design of this study with interim time points permitted the opportunity to examine the temporal sequences associated with development of treatment-induced

mesotheliomas. While all mesotheliomas were considered malignant by the authors, a single case of mesothelial hyperplasia, and 1 rat with a small mesothelioma confined to the parietal vaginal tunic, were seen at 52 weeks. Spreading to other peritoneal sites was not present until after 78 weeks of treatment. Spreading was by extension or implantation (i.e., seeding) and most commonly involved spleen, gastrointestinal tract, mesentery, and pancreas. This study showed the origin of the mesotheliomas to be in the tunica vaginalis.

An extensive re-examination of the study materials from the Wolf et al., 1998 study was reported by Crosby et al., 2000. Using cross sections of the rat testes to map the TVM, it was concluded that the mesorchium was the major tissue target site for potassium bromate-induced mesotheliomas. The authors discuss several factors that may contribute to TVM development. However, as with other brominated chemicals, oxidative damage (DeAngelo et al., 1998) and formation of oxidative DNA adducts (Kurokawa et al., 1983; Kasai et al., 1987; Sai et al., 1992) are the most likely mode of action for induction of the TVM response. Potassium bromate is mutagenic in the Ames test (Table 29).

#### Acrylamide.

Two separate bioassays in which acrylamide was administered to F344 rats in drinking water have been reported (Johnson et al., 1986; Friedman et al., 1995). Mesotheliomas of the tunica vaginalis were documented in both studies (Table 24). Only some of the mesotheliomas were present in the abdominal cavity, while all were present in the vaginal tunics of the scrotal sac. Neither publication tabulates the incidence of testicular Leydig cell tumors, however, the laboratory study report for the Johnson et al. (1986) study shows that 57 of the 60 males in each group, including the control group, had Leydig cell adenomas. A retrospective examination of study slides from the Friedman et al. (1995) study was conducted by Iatropoulos et al., 1998, who found that the degree of morphological progression of the tunica vaginalis mesotheliomas was correlated with the size of the Leydig cell tumors. The malignant mesotheliomas, as classified by Iatropoulos, were seen only in rats that had 75% or greater of their testicular parenchyma replaced by Leydig cell tumors. The mesothelial tumors that they classified as hyperplasias were present in rats in which the Leydig cell tumors occupied 24% or less of the testicular parenchyma.

Acrylamide is not mutagenic in the Ames test, but produces chromosome aberrations in cultured mammalian cells. It produces chromosome aberrations and micronuclei in mouse, but not rat bone marrow cells, and chromosome damage in male germ cells of rats and mice.

The probable mode(s) of action for induction of TVM associated with exposure to acrylamide has been extensively reviewed (Shipp et al., 2006). Administration of acrylamide to rats produces a dose-related reduction in prolactin and testosterone thought to be centrally mediated via the dopaminergic system (Friedman et al., 1999; Agrawal et al., 1981; Ali et al., 1983, Uphouse et al., 1982). The enhanced dopamine signal, with its

associated decreases in prolactin secretion, would trigger down-regulation of Leydig cell LH receptors (Prentice et al., 1992), reduced testosterone, and a compensatory increase in LH, which in turn stimulates proliferation of Leydig cells (Cook et al., 1999). The altered hormonal milieu is then reflected as a transudate in tunical vaginalis fluid, and the exposed tunica vaginalis mesothelium proliferates via an autocrine response to growth factor production. A physical stimulus affecting tunica vaginalis mesothelium from testes enlarged by Leydig cell tumors may also lead to elaboration of growth factors by the mesothelium and an autocrine-mediated cell proliferative response.

#### Specific Chemicals - Gavage Route of Administration

##### *Methyleugenol.*

The gavage administration of methyleugenol (in 0.5% methylcellulose) to F344 rats resulted in induction of multiple tumor target sites (NTP TR 491) with a strong mesothelioma dose response (Table 25). Fifty of 60 males and females received 300 mg/kg methyleugenol for 52 weeks and then were administered methylcellulose vehicle, alone, for the next 53 weeks.

There was a dose-related increase of Leydig cell tumors in core study rats. Of the five 300 mg/kg treated rats and the five controls examined at 12 months, all had Leydig cell tumors, nine of which were bilateral. Of the 50 remaining stop study rats, five had TVM. Mammary gland fibroadenomas were also increased in dosed male rats. Other induced neoplasms included benign and malignant liver tumors, benign and malignant gastric neuroendocrine tumors, benign kidney tumors, and benign and malignant connective tissue tumors of the skin. Liver and glandular stomach neoplasms were increased in treated mice. While methyleugenol is not mutagenic in the Ames test, or induce chromosome damage in cultured mammalian cells or mouse bone marrow, its metabolism is associated with adduct formation, and beta-catenin mutations have been reported in methyleugenol-induced mouse liver tumors (Devereux et al., 1999).

##### *Benzaldehyde.*

In a 2-year gavage study of benzaldehyde in F344 rats (NTP TR 378) using corn oil as the vehicle, a marginal TVM response (Table 26) was not considered related to treatment, and the chemical was judged not to be a carcinogen in rats. This judgment was influenced by lack of a dose response and the laboratory's mesothelioma historic control incidence of 8% in male F344 rats. The incidences of Leydig cell tumors in the control and low dose groups were greater than 90%, while only 63% of the high dose males had Leydig cell tumors. There was some evidence of treatment-related neoplasia in mice based on forestomach squamous cell papillomas. Benzaldehyde was not mutagenic in the Ames test, but did induce mutations in the mouse lymphoma test, and it did not induce chromosome aberrations in cultured mammalian cells. (Table 29).

##### *Glycidol.*

Exposure to glycidol produces a marked carcinogenic response with tumors at multiple sites in both sexes of F344 rats and B6C3F1 mice (NTP TR 374). Peritoneal mesotheliomas are among the tumor responses in male rats that showed a dramatic increase in a 2-year gavage study in which glycidol was administered in a water vehicle (Table 27).

All mesotheliomas were present in the tunica vaginalis, many with extension into the abdominal cavity. They were classified into benign and malignant neoplasms. Mesotheliomas confined to the vaginal tunics were considered benign and those that spread into the abdominal cavity and/or had cytological features of malignancy were considered malignant. The histomorphological features of the malignant mesotheliomas included pleomorphism, cytological atypia, local invasiveness, and implant metastasis throughout the abdominal cavity. Malignant mesotheliomas were considered rapidly lethal; the first death attributed to mesothelioma occurred in a high dose male at study week 49.

Despite early tumor-associated mortality in the treated males, the control and dosed male groups all had high incidences of Leydig cell tumors. Mammary gland neoplasms were dramatically increased in female rats. Epithelial tumors were increased at multiple sites in treated mice. Glycidol is a direct alkylating agent, forming promutagenic adducts in DNA, and is mutagenic in the Ames test and produces chromosome damage in cultured mammalian cells and mouse bone marrow. The relationship between adduct formation and tumorigenesis is in part attributed to the relative susceptibility of the exposed tissue. The robust mesothelioma response observed in the glycidol study is most probably a consequence of the combined effects of localized genotoxicity and the susceptibility of tunica vaginalis mesothelium to the hormonal imbalance in F344 rats associated with aging and the development of Leydig cell tumors.

#### Specific Chemicals - Topical Application Route of Administration

##### 2,3-Dibromo-1-propanol.

Topical application of 2,3-dibromo-1-propanol produced a marginal mesothelioma response in male rats (Table 28) but clear evidence of carcinogenicity at other sites (NTP TR 400). There was also clear evidence of carcinogenicity in mice based on increased incidences of epithelial neoplasms.

The study was terminated after 51 weeks, because of reduced survival in the high-dose groups resulting from chemically induced neoplasms. Early mortality began at week 45. Major induced tumors involved the nasal cavity, skin, oral cavity, and gastrointestinal tract. The incidence of Leydig cell tumors was low because of early study termination, with the highest incidence of 34% seen in the low-dose group. However, the incidence of Leydig cell hyperplasia was up to 56% in the low dose group suggesting that the paracrine hormonal secretion by the proliferating Leydig cells also contributed to the early appearance of tunica vaginalis mesotheliomas in treated rats. 2,3-Dibromo-1-propanol is mutagenic in the Ames and mouse lymphoma mutation tests and produces chromosome aberrations in cultured mammalian cells, but did not induce micronuclei in mouse bone marrow cells (Table 29).

## **GENOTOXICITY**

Analyses of carcinogenicity and genotoxicity databases (Ashby & Tennant, 1988; Gold et al., 1993, 2001) have shown that some tumor types/locations are associated with genotoxic chemicals and some are associated with non-genotoxic chemicals, although the association appeared to be less strong in the Gold et al. (1993, 2001) compilations, which examined the NTP and other data sources than in Ashby and Tennant (1988). In this latter study that examined only chemicals tested by the NTP (Ashby and Tennant, 1988), there was an association of some tumor sites with mutagenicity. That is, some tumors/tumor sites were responsive primarily to chemicals that were mutagenic in the Salmonella test, some were responsive primarily to chemicals that were not mutagenic in Salmonella, and other sites appeared to be responsive to both mutagenic and non-mutagenic carcinogens.

Genotoxicity in this context is defined as positive results in the Salmonella mutagenicity (Ames) test. A positive response in an *in vitro* mammalian cell chromosome aberration test, by itself, is not considered to be definitive evidence of genetic toxicity because of the predilection of this test to produce positive results as a secondary response to cell toxicity, or to high osmolarity or changes in growth medium pH (Brusick, 1986; Scott et al., 1991; Morita et al., 1992).

Ashby and Tennant identified two chemicals among the NTP database that induced tunica vaginalis mesotheliomas, glycidol (Ashby and Tennant, 1991a) and 1,2-dibromoethane (Ashby and Tennant, 1991b), both of which were mutagenic in Salmonella. The (Gold et al., 1993, 2001) compilations of cancer site and mutagenicity do not distinguish tunica vaginalis mesotheliomas from other testicular tumors, and do not list mesothelioma as a tumor type.

Chemicals reported here to induce tunica vaginalis mesotheliomas (see Table 29) were classified according to the potency of their tumor induction, e.g., robust or nonsignificant-to-marginal, and their genetic toxicity. The criterion for a robust tumor response was that the magnitude of the highest incidence, regardless of dose, was >18%. This criterion was determined by examining the incidence data, the likely mode of action, and/or the final interpretation of the specific cancer bioassays. For 1,2-dichloroethane the 10% (5/50) TVM response was not statistically significant versus the concurrent control (0/50) (Nagano et al., 2006). The 10% TVM response in the low dose animals in the benzaldehyde study was judged to be a non-carcinogenic response by the NTP peer review board (NTP TR 378) because, although it was greater than the concurrent control, it was equivalent to the 8% historical control incidence in the testing lab. The 12% TVM response induced by tartrazine did not exhibit a dose response, and was considered to be not treatment-related by the author (Maekawa et al., 1987). The nitrofurazone TVM response of 14% was seen at the lower dose without evidence of a dose response (NTP TR 337). A 16% TVM response in the ethyl tellurac study, although dose-related was considered equivocal by the NTP peer review board (NTP TR 152). The TVM found in the acrylamide studies are considered centrally mediated and secondary to Leydig cell tumors (Shipp et al., 2006). The 18% pentachlorophenol response was seen only in a stop study where the dose exceeded the maximum tolerated dose (NTP TR 483). The gap between the 18% incidence of TVM in the pentachlorophenol study and a 24%

incidence of TVM for methyl eugenol, prompted selection of 18% as a cut-off incidence for classifying the potency of the TVM response. Latency, as defined by the week to first observed TVM, of less than 60 weeks was a feature of robust responses (Table 29).

Regardless of the conclusions of Gold et al. (1993, 2001) who found that genotoxic and nongenotoxic chemicals produced similar tumor induction patterns, there was a clear distinction between the chemicals that produced robust, and those that produced weak, tunica vaginalis mesothelioma responses. Of the 10 chemicals producing robust responses that had genetic toxicity test results, 8 (80%) were mutagenic in Salmonella. One of the outliers, nitrotoluene, requires anaerobic activation as present *in vivo*, in contrast to the aerobic conditions present in the Ames test. Where *in vitro* cytogenetics results were available, they supported the Salmonella results. In contrast, only 2 of the 7 chemicals (29%) that produced non-significant-to-marginal responses, were mutagenic in Salmonella. There were an additional three chemicals in this group that were negative in the Salmonella test but positive in the chromosome aberration test, one of which, acrylamide, also produced chromosome damage in the *in vivo* bone marrow test.

Three NTP studies were terminated early, i.e., less than 2 years, because of mortality from other tumors. 2,3-Dibromo-1-propanol, 3,3'-dimethoxybenzidine 2HCl, and 3,3'-dimethylbenzidine 2HCl, which are all mutagenic in Salmonella, had overall TVM frequencies of 8, 10, and 7%, respectively. Because TVM tend to be late occurring neoplasms, especially in controls, early study termination because of other tumor responses would not allow for adequate exposure time to fully assess the magnitude of a potential mesothelioma response in these 3 studies.

The chemicals in Table 29, and their putative metabolites, present a wide range of structures and chemical characteristics. Some, e.g., methyl(acetoxymethyl)nitrosoamine, glycidol, ethylene oxide, can form DNA adducts. Others, e.g., nitrilotriacetic acid, methyl eugenol, potassium bromate, ethyl tellurac, do not appear to have any direct DNA-reactivity, but may induce their damage through the induction of reactive oxygen species. And others, e.g., acrylamide, are known to be both DNA-reactive (through its metabolite, glycidamide) and capable of inducing oxidative stress and hormonal changes.

The one conclusion that is obvious from this compilation is that the induction of tunica vaginalis mesotheliomas is not confined to genotoxic chemicals. A significant fraction of these tumors are induced by chemicals that are considered to be nongenotoxic, presumably acting through a mechanism(s) that do(es) not involve direct DNA interaction.

### **RELEVANCE OF TUNICA VAGINALIS MESOTHELIOMAS IN RATS TO HUMAN HEALTH**

Of the 21 xenobiotics associated with a mesothelioma response in rats that are addressed in this document, 7 are judged to have a non-significant to marginal response, 3 are relatively non-informative with respect to their potency due to early study

termination because of tumors other than mesotheliomas, and 11 exhibited a robust mesothelioma response. Highlights of the findings in these studies are summarized in Table 30, and the categorization of the responses are in Table 29. If one excludes the three robust chemicals that were identified via the intraperitoneal route of exposure, where the xenobiotic would have direct contact with mesothelium, the remaining 18 studies were done using F344 rats. For the 11 chemicals with a robust mesothelioma response, a genotoxic mode of action may be associated with that target tissue response. However, the presence of Leydig cell tumors in the F344 rats, and the evidence linking Leydig cell tumors to tunica vaginalis mesotheliomas, suggests a contributory effect of the Leydig cell tumor burden.

The occurrence of xenobiotic treatment-associated tunica vaginalis mesotheliomas by other than the intraperitoneal route is a feature unique to the male F344 rat. This tumor response is an exacerbation of a well-documented, low spontaneous background rate of tunica vaginalis mesothelioma in these rats. A key event associated with the xenobiotic induction of TVM in the F344 rat is the age-associated and high incidence of testicular Leydig cell tumors. The local hormonal milieu in the tissues adjacent to the Leydig cell tumors is altered and the hormonal imbalance is reflected as a transudate in the tunica vaginalis fluid. This, in turn, leads to an autocrine growth factor response in the tunica vaginalis mesothelium as a primary mode of action, resulting in mesothelial hyperplasia and ultimately mesothelioma. Since it has been shown that mesothelial cells respond to pressure or shearing forces by elaborating autocrine growth factors, the markedly enlarged testes from the Leydig cell tumor burden can also initiate a mitogenic stimulus. Thus, a specific primary mode of action for developing tunica vaginalis mesotheliomas in the F344 rat is dependent upon enhanced mitogenesis caused by autocrine growth factors in the stimulated tunica vaginalis mesothelium. Given the extremely low incidence of Leydig cell tumors in humans, a F344 rat tunica vaginalis mesothelioma response attributed to this primary mode of action is not considered relevant to human cancer induction.

To further understand the factors associated with Leydig cell biology, an expert panel of scientists identified 7 mechanisms that could lead to Leydig cell hyperplasia and adenoma formation (Clegg et al., 1997). Two hormonal modes of action, viz., GnRH agonism and dopamine agonism, were considered not relevant to humans. GnRH agonism is unique to the rat since human as well as monkey and mouse Leydig cells do not express the LHRH receptor (Prentice and Meikle, 1995). Dopamine agonism leads to decreased prolactin secretion by the pituitary which, in turn, leads to down-regulation of Leydig cell LH receptors, decreased testosterone, and a compensatory increased circulating LH to raise testosterone levels (Cook et al., 1999; Prentice et al., 1992). The increased LH leads to Leydig cell proliferation and ultimately to Leydig cell tumors (Cook et al., 1999; Prentice and Meikle 1995). This dopaminergic mode of action is unlikely in humans because the number of LH receptors per Leydig cell is 14 times less than in the rat, and Leydig cell tumors are extremely rare in humans (Prentice and Meikle 1995; Foster, 2007). Five additional hormonal modes of action for Leydig cell tumor induction that are potentially relevant to humans include androgen receptor antagonism, 5-alpha-reductase inhibition, inhibition of testosterone biosynthesis, aromatase inhibition, and estrogen agonism. Rodents have greater sensitivity than humans to these hormonal

effects. The expert panel recommended a margin of exposure (MOE) approach be used when a rodent Leydig cell tumor response is attributable to one of these 5 modes of action. If the compound under investigation was mutagenic, then a case-by-case judgment regarding human health risk was recommended.

There are species and strain differences that indicate a tunica vaginalis mesothelioma response by other than the peritoneal route of exposure is specific to the F344 rat. Examination of the literature indicates that a tunica vaginalis response to xenobiotic exposure is generally not seen in other strains and stocks of rats, even following sustained increased LH levels (Prentice et al., 1992). The aging F344 rat has a more advanced development of testicular changes, including Leydig cell tumors, than other rat stocks (Kanno et al., 1987) and a greater background incidence of testicular mesotheliomas. In several hazard identification cancer bioassays conducted, in parallel, in F344 rats and B6C3F1 mice, a tunica vaginalis mesothelioma response was never seen in mice, nor was a mesothelioma response seen in female rats. Consequently, the male F344 rat specificity of tunica vaginalis mesothelial tumorigenesis is not likely to be relevant to other species or pose a human cancer risk.

Among the xenobiotics reviewed in this report, some are direct alkylating agents with clear genotoxicity and a robust tunica vaginalis mesothelioma response (Tables 30 and 31). Robust TVM responses have been observed in rats exposed to alkylating agents such as glycidol (NTP TR 374) and nitrosamines (Berman and Rice 1979; Lijinsky et al., 1985; Greenblatt and Lijinsky 1972). The relationship between adduct formation and tumorigenesis is, in part, attributed to the relative susceptibility of the exposed tissue. It has been suggested that tunica vaginalis mesothelium, as opposed to mesothelium elsewhere in the body, has unique properties making it more responsive to chemical carcinogens (Berman and Rice, 1979). The robust mesothelioma response observed in the glycidol study is most probably a consequence of the combined effects of localized genotoxicity and the susceptibility of tunica vaginalis mesothelium to the hormonal imbalance in F344 rats associated with aging and the development of Leydig cell tumors.

Another example of a robust tunica vaginalis mesothelioma response occurred following exposure to *o*-nitrotoluene. For this chemical, the formation of *o*-benzyl glucuronide is a critical step in leading to formation of DNA-reactive intermediates. Intestinal microflora hydrolyze the glucuronide and reduce the nitro group to form *o*-aminobenzyl alcohol. Upon reabsorption of the *o*-aminobenzyl alcohol, it is sulfated and binds to DNA. Two brominated chemicals, 2,2-bis(bromomethyl)-1,3-propanediol and potassium bromate, produced a robust mesothelioma response (Table 29). Hypotheses for the carcinogenic activity of brominated chemicals include oxidative damage to DNA and formation of DNA adducts when the carbon-bromine bond is broken (Kurokawa et al., 1983; Kasai et al., 1987; Sai et al., 1992; De Angelo et al., 1998). It is noted, however, that even for genotoxic xenobiotics producing a robust tunica vaginalis mesothelioma responses in male F344 rats, there are no mesotheliomas in female rats or in mice, thereby underscoring the unique sensitivity of the tunica vaginalis mesothelium in male F344 rats.

Some tissue-specific responses are characteristic of epigenetic modes of action in the non-significant to marginal tunica vaginalis mesothelioma responses (Table 29) .

Using acrylamide as an example, the adrenal pheochromocytoma, tunica vaginalis mesothelioma, and thyroid follicular adenoma responses in the male F344 rat (Johnson et al., 1986; Friedman et al., 1995) are consistent with rodent-specific targeting of endocrine-sensitive tissues, and have little relevance to human cancer risk (Cohen 2004). Exposure of F344 and Sprague-Dawley rats to acrylamide has been shown to increase replicative DNA synthesis in these tumor target tissues, but not in non-target tissues (Lafferty et al., 2004). Furthermore, blocking cytochrome P450 activity, and thus the formation of the DNA-reactive metabolite of acrylamide, glycidamide, did not abolish replicative DNA synthesis in the tunica vaginalis mesothelium. From these findings, it is apparent that the tunica mesothelioma response occurred through a mode of action independent of oxidative metabolism of the chemical to a DNA reactive metabolite (Lafferty et al., 2004). Acrylamide also has dopaminergic activity in the F344 rats, which leads to decreased circulating prolactin followed by enhancement of spontaneous, age-associated Leydig cell tumorigenesis (Friedman et al., 1999). As a result, the tunica vaginalis mesothelioma response in acrylamide-treated F344 rats is most likely caused by a hormonally mediated and autocrine growth factor-driven mesothelial mitogenesis mode of action. A similar autocrine growth factor-driven mode of action, although not necessarily amplified by dopamine agonism, is believed to be a primary cause of the observed tunica vaginalis mesothelioma responses seen for other chemicals with a non-significant to marginal response (Table 29). Thus, these xenobiotics with a non-significant to marginal tunica vaginalis mesothelioma response that is unique to the F344 rat do not pose a significant risk for human carcinogenesis (see Table 31).

## CONCLUSIONS

The primary conclusions based upon this review of tunica vaginalis mesotheliomas in rat bioassays are as follows:

- Tunica vaginalis mesotheliomas are low incidence spontaneous neoplasms in rats that can be exacerbated by treatment.
- Tunica vaginalis mesotheliomas in rats originate in the mesothelial lining of the scrotal sac, testes, epididymides, and mesorchium and can spread to the abdominal cavity by extension or seeding since the scrotal sac mesothelium is continuous with the peritoneal cavity mesothelium.
- A majority of chemicals that are associated with a non-significant to marginal tunica vaginalis mesothelioma induction are non-genotoxic based on the Ames test, whereas chemicals producing a robust response tend to be Ames test mutagens.
- The mesothelioma responses to xenobiotic exposure by other than the peritoneal route are male F344 rat-specific. They are never seen in female F344 rats or in either gender of mice in conventional cancer bioassays, and have not been reported in other rat strains used for carcinogenicity testing.
- Spontaneous, as well as several, xenobiotic-associated tunica vaginalis mesotheliomas are causally associated with Leydig cell tumors that lead to an autocrine growth factor-induced mesothelial mitogenesis.

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Table1. Some immunohistochemical stains used to distinguish human mesotheliomas from adenocarcinomas

Protein/peptide target	Mesothelioma	Adenocarcinoma
Cytokeratin 5/6	Positive	Negative
Thrombomodulin	Positive	Negative
Calretinin	Positive	Negative
Epithelial membrane antigen (EMA)	Positive	Most are Negative
Vimentin	Positive	Negative
WT1	Positive	Negative
HBME-1	Positive	Negative
Carcinoembryonic antigen	Negative	Positive
CK20	Negative	Positive
B72.3	Negative	Positive
BerEp[4]	Negative	Positive
BG8	Negative	Positive
TTF-1	Negative	Positive
Leu M1	Negative	Positive

Table 2. Xenobiotics associated with tunica vaginalis mesotheliomas in rat studies  
(arranged alphabetically)

<b>Xenobiotic Agent</b>	<b>CASRN</b>	<b>Reference</b>
Acrylamide	79-06-1	Johnson et al., 1986 Friedman et al., 1995
Benzaldehyde	100-52-7	NTP TR 378
2,2-Bis(bromomethyl)-1,3-propanediol	3296-90-0	NTP TR 452
Cytembena	21739-91-3	NTP TR 207
1,2-Dibromoethane	106-93-4	NTP TR 210
2,3-Dibromo-1-propanol	96-13-9	NTP TR 400
1,2-Dichloroethane	107-06-2	Nagano et al., 2006
3,3'-Dimethoxybenzidine	20325-40-0	NTP TR 372
3,3'-Dimethylbenzidine	612-82-8	NTP TR 390
Ethylene oxide	75-21-8	Snellings et al., 1984 Lynch et al., 1994
Ethyl tellurac	20941-65-5	NTP TR 152
Glycidol	556-52-5	NTP TR 374
Methyl(acetoxymethyl)nitrosoamine	56856-83-8	Berman & Rice 1979
Methyleugenol	93-15-2	NTP TR 491
Nitrilotriacetic acid ± ferric saccharate		Okada et al., 1989
Nitrofurazone	59-87-0	NTP TR 337
<i>o</i> -Nitrotoluene	88-72-2	NTP TR 504 NTP TOX 23 NTP TOX 44
Pentachlorophenol, purified	87-86-5	NTP TR 483
Potassium bromate	7758-01-2	Kurokawa et al., 1983 DeAngelo et al., 1998 Wolf et al., 1998
Tartrazine	1934-21-0	Maekawa et al., 1987
<i>o</i> -Toluidine HCl	636-21-5	NTP TR 153 NTP TOX 44

Table 3. Frequency of proliferative mesothelial lesions and Leydig cell tumors following a single intraperitoneal injection of methyl(acetoxymethyl)nitrosamine in three strains of male rats

Strain	Treatment	Mesothelioma	Mesothelial Hyperplasia	LCT
F344	Control	0/15 (0%)	0/15 (0%)	7/15 (47%)
	DMN-OAc	9/25 (36%)	5/25 (20%)	11/25 (44%)
Sprague-Dawley	Control	1/27 (3.7%)	2/27 (7.4)	4/27 (15%)
	DMN-OAc	4/27 (15%)	4/27 (15%)	1/27 (3.7%)
Buffalo	Control	1/25 (4%)	2/25 (8%)	0/25 (0%)
	DMN-OAc	12/26 (46%)	3/26 (11%)	0/26 (0%)

LCT = Leydig cell tumor

Table 4. Frequency of mesotheliomas following intraperitoneal injection of ferric saccharate in male Wistar rats

Treatment Groups	Mesothelioma
Physiological saline	0/20 (0%)
NTA (83.5 mg/kg/d)	0/20 (0%)
Ferric saccharate (5 mg Fe/kg/d)	9/19 (47%)
Ferric saccharate + NTA	13/19 (68%)

Table 5. Frequency of peritoneal and tunica vaginalis mesotheliomas in male F344 rats given 3 times weekly injections of cytembena for 2 years

Tumor	0 mg/kg (untreated)	0 mg/kg (vehicle)	7 mg/kg	14 mg/kg
Mesothelioma	1/50 (2%)	3/50(6%)	37/50 (74%)	36/50 (72%)

Table 6. Frequency of mesotheliomas in male F344 rats exposed to ethylene oxide vapor for 2 years

Study	0 ppm	10 ppm	33 ppm	50 ppm	100 ppm
Snellings et al., 1984	2/97*(2%)	2/51(4%)	4/39(10%)	-	4/30(13%)
Lynch et al., 1984	3/78(3.8%)	-	-	9/79(11%)	21/79(27%)

\* Combined controls (1/49 & 1/48)

Table 7. Frequency of mesotheliomas in male F344 rats exposed to 1,2-dibromoethane by inhalation for 2 years

Tumor	0 ppm	10 ppm	40 ppm
Mesothelioma	1/50 (2%)	13/50 (26%)	26/50 (52%)
Leydig cell	35/50 (66%)	45/50 90%)	26/50 (52%)

Table 8. Frequency of mesotheliomas in male F344 rats exposed by inhalation of DCE for 2 years (Nagano et al., 2006)

Tumor	0 ppm	10 ppm	40 ppm	160 ppm
Mesothelioma	1/50 (2%)	1/50 (2%)	1/50 (2%)	5/50 (10%)

Table 9. Frequency of mesotheliomas in F344 rats administered ethyl tellurac in the diet for 2 years

Group	0 ppm	300 ppm	600 ppm
Mesothelioma	0/20 (0%)	2/49 (4%)	8/50 (16%)

Table 10. Frequency of epididymal mesothelial lesions in rats receiving *o*-nitrotoluene in the diet for 13 weeks (NTP Tox 23)

Effect	0 ppm	625 ppm	1250 ppm	2500 ppm	5000 ppm	10000 ppm
Mesothelial hyperplasia	0/10	-	-	-	0/10	2/10
Mesothelioma	0/10	-	-	-	3/10	0/10

Table 11. Frequency of epididymal and testicular mesothelial lesions in rats in the 26-week dietary *o*-nitrotoluene study (NTP Tox 44)

<b>13-wk interim</b>	Normal GI flora		Altered GI flora*	
	0 ppm	5000 ppm	0 ppm	5000 ppm
Epididymis mesothelial hyperplasia	0/10	0/20	0/10	2/20
Epididymis mesothelioma	0/10	0/20	0/10	2/20
<b>Stop-exposure</b>	Normal GI flora		Altered GI flora	
	0 ppm	5000 ppm	0 ppm	5000 ppm
Testis mesothelioma	0/10	2/20	0/10	4/20
Epididymis mesothelial hyperplasia	0/10	2/20	0/10	1/20
Epididymis mesothelioma	0/10	4/20	0/10	8/20
<b>26-wk continuous exposure</b>	Normal GI flora			
Testis mesothelioma	0/10	2/20	nd	nd
Epididymis mesothelial hyperplasia	0/10	2/20	nd	nd
Epididymis mesothelioma	0/10	7/20	nd	nd

\* Rats treated with [antibiotic] to alter the intestinal flora

nd - not done

Table 12. Frequency of mesotheliomas in male F344 rats in the 2-year feed study of *o*-nitrotoluene (NTP TR-504)

Group	0 ppm	625 ppm	1250 ppm	2000 ppm	2000 ppm stop exposure	5000 ppm stop exposure
Overall rate	2/60(3.3%)	20/60(33%)	29/60(48%)	44/60(73%)	44/60(73%)	54/60(90%)
Terminal rate*	2/39(5.1%)	5/18(28%)	1/3(33%)	0/0	10/11(91%)	0/0

\* Rates in animals that were alive at 104 weeks.

Table 13. Frequency of mesotheliomas in F344 rats receiving dietary *o*-toluidine HCl for up to 26 weeks (NTP Tox 44)

Diet	0 ppm	5000 ppm
<b>13-Week Interim</b>		
Epididymis mesothelioma	0/10	0/20
<b>Stop-exposure</b>		
Epididymis mesothelioma	0/10	2/20
<b>26-Week continuous exposure</b>		
Epididymis mesothelioma	0/10	0/20*

\* One rat had mesothelial hyperplasia.

Table 14. Frequency of mesotheliomas in F344 rats  
receiving dietary *o*-toluidine for 2-years (NTP TR 153)

Group	0 ppm	3000 ppm	6000 ppm
Mesothelioma	0/20	17/50 (34%)	9/49 (18%)

Table 15. Frequency of mesotheliomas in male F344 rats administered

2,2-bis(bromomethyl)-1,3-propanediol in the diet

Group	0 ppm	2500 ppm	5000 ppm	10000 ppm	20000 ppm Stop study
Overall rate	0/51 (0%)	3/53(5.6%)	8/51(16%)	9/55(16%)	26/60(43%)
Terminal rate*	0/26(0%)	0/20(0%)	4/13(31%)	1/1(100%)	0/0

\* Rates in animals that were alive at 104 weeks.

Table 16. Frequency of mesotheliomas in male F344 rats administered nitrofurazone in the diet for 2 years

Group	0 ppm	310 ppm	620 ppm
Overall rate	0/50 (0%)	7/50 (14%)	2/50 (4%)
Terminal rate*	0/33 (0%)	2/30 (7%)	0/20 (0%)

\* Rates in animals that were alive at 104 weeks.

Table 17. Frequency of mesotheliomas in a 2-year study with continuous exposure and a stop study in which male F344 rats received pentachlorophenol in the diet for 1 year (NTP TR 483)

Group	0 ppm	200 ppm	400 ppm	600 ppm	1000 ppm for 1 year - stop study
Overall rate	1/50 (2%)	0/50 (0%)	2/50 (4%)	0/50 (0%)	9/50 (18%)
Terminal rate	0/12* (0%)	0/16 (0%)	0/21 (0%)	0/31 (0%)	4/27 (15%)

\* Rates in animals that were alive at 104 weeks.

Table 18. Frequency of mesotheliomas in male F344 rats in a 2-year dosed water study of tartrazine (Maekawa et al., 1987)

Group	0%	1%	2%
Mesothelioma	0/48 (0%)	6/49 (12%)	0/49 (0%)

Table 19. Frequency of mesotheliomas in male F344 rats administered

3,3'-dimethoxybenzidine hydrochloride in drinking water for up to 21 months (NTP TR 372)

Group	0 ppm	80 ppm	170 ppm	330 ppm
Overall rate	2/60 (3%)	1/45 (2%)	7/75 (9%)	6/60 (10%)
Terminal rate	1/44 (2%)	0/8	0/0	0/0

Table 20. Frequency of mesotheliomas in male F344 rats administered

3,3'-dimethylbenzidine HCl in drinking water for 14 months (NTP TR 390)

Group	0 ppm	30 ppm	70 ppm	150 ppm
Overall rate	0/60 (0%)	0/45 (0%)	3/75 (4%)	4/60 (7%)
Terminal rate*	0/60 (0%)	0/41 (0%)	3/50 (6%)	0/0

\* Rates in animals that were alive at 104 weeks.

Table 21. Frequency of mesotheliomas in a drinking water study of potassium bromate in F344 rats reported by Kurokawa et al.

Tumor (Study)	0 ppm	15 ppm	30 ppm	60 ppm	125 ppm	250 ppm	500 ppm
Mesothelioma (Kurokawa et al., 1983)	6/53 (11%)	--	--	--	--	17/52 (33%)	28/46 (61%)
Mesothelioma (Kurokawa et al., 1986)	0/19 (0%)	0/19 (0%)	3/20 (15%)	4/20 (20%)	2/24 (8%)	3/20 (15%)	15/20 (75%)

Table 22. Frequency of mesotheliomas in the drinking water study of potassium bromate in F344 rats (DeAngelo et al., 1998)

Group	0 g/L	0.02 g/L	0.1 g/L	0.2 g/L	0.4 g/L
Mesothelioma	0/47 (0%)	4/49 (8%)	5/49 (10%)	10/47 (21%)	27/43 (63%)

Table 23. Frequency of mesotheliomas in the drinking water study of potassium bromate in F344 rats (Wolf et al., 1998)

Group	0g/L	0.02 g/L	0.1 g/L	0.2 g/L	0.4 g/L
Week 12	0/6(0%)	0/6(0%)	0/6(0%)	0/6(0%)	0/6(0%)
Week 26	0/6(0%)	0/6(0%)	0/6(0%)	0/6(0%)	0/6(0%)
Week 52	0/6(0%)	0/6(0%)	0/6(0%)	1/6(17%)	0/6(0%)
Week 78	0/6(0%)	0/6(0%)	0/6(0%)	0/6(0%)	4/6(67%)
Week 100	0	4/49(8%)	5/50(10%)	10/47(21%)	27/43(63%)

Table 24. Frequency of mesotheliomas in F344 rats in two separate studies in which acrylamide was administered in drinking water

Study	Doses in mg/kg/day				
	0	0.01	0.1	0.5	2.0
Johnson et al., 1986	3/60 (5%)	0/60 (0%)	7/60 (12%)	11/60 (1%)	10/60 (17%)
Friedman et al., 1995	8/204* (4%)		9/204 (4%)	8/102 (8%)	13/75 (17%)

\* Pooled control groups (4/102 and 4/102)

Table 25. Frequency of mesotheliomas in male F344 rats gavaged with methyleugenol for two years (NTP TR 491)

Group	0 mg/kg	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg 52-week exposure stop study
Overall rate	1/50 (2%)	3/50 (6%)	5/50 (10%)	12/50 (24%)	5/50 (10%)
Terminal rate*	0/20 (0%)	1/16 (6%)	0/15 (0%)	0/0	0/0

\* Rates in animals that were alive at 104 weeks.

Table 26. Frequency of mesotheliomas in male F344 rats administered benzaldehyde by gavage in corn oil (NTP TR 378)

Group	0 mg/kg	200 mg/kg	400 mg/kg
Overall rate	0/50	5/50 (10%)	2/50 (4%)
Terminal rate	0/37	4/29 (14%)	1/21 (5%)

Table 27. Frequency of mesotheliomas in male F344 rats in a 2-year water gavage glycidol study (NTP TR 374)

Group	0 mg/kg	37.5 mg/kg	75 mg/kg
Overall rate	3/49 (6%)	34/50 (68%)	39/47 (83%)
Terminal rate*	0/16	0/0	0/0

\* Rates in animals that were alive at 104 weeks.

Table 28. Frequency of mesotheliomas in male F344 rats treated topically with 2,3-dibromo-1-propanol for 51 weeks (NTP TR 400).

Group	0 mg/kg	188 mg/kg	375 mg/kg
Mesothelioma	0/50 (0%)	1/50 (2%)	4/50 (8%)

**Table 29. Genetic toxicity of chemicals associated with tunica vaginalis mesothelioma (TVM) induction in rats, arranged in order of decreasing maximum tumor frequency**

Carcinogen	CASRN	TVM frequency <sup>a</sup>	Wk to first TVM <sup>b</sup>	Ames test	In vitro cyto	In vivo cyto
<b>Robust TVM induction</b>						
o-Nitrotoluene	88-72-2	90%	13	—*	—	—
Glycidol	556-52-5	78%	49	+	+	+
Cytembena (ip)	21739-91-3	74%	45	+	+	—
Nitrilotriacetic acid ± ferric saccharate (ip)		68%	NA	**		
Potassium bromate	7758-01-2	63%	52	+		
1,2-Dibromoethane	106-93-4	52%	50	+	+	—
Methyl (acetoxymethyl)nitrosoamine (ip)	56856-83-8	46%	NA	+		
2,2-Bis(bromomethyl)-1,3-propanediol	3296-90-0	43%	52	+	+	<b>E</b>
o-Toluidine HCl	636-21-5; 95-53-4 <sup>c</sup>	34%	26	+	+	+/-
Ethylene oxide	75-21-8	27%	NA	+	+	+
Methyleugenol	93-15-2	24%	58	—	—	—
<b>Non-significant-to-marginal TVM induction</b>						
Pentachlorophenol, purified	87-86-5	18%	72	—	<b>w+</b>	—
Acrylamide	79-06-1	17%	66	—	+	<b>w+</b>
Ethyl tellurac	20941-65-5	16%	NA	—	<b>E</b>	
Nitrofurazone	59-87-0	14%	67	+	+	—
Tartrazine	1934-21-0	12%	NA	—	+	
Benzaldehyde	100-52-7	10%	80	—	—	
1,2-Dichloroethane	107-06-2	10%	NA	+	+	—
<b>Not Classifiable</b>						
2,3-Dibromo-1-propanol	96-13-9	***	51	+	+	—
3,3'-Dimethoxybenzidine HCl	20325-40-0	***	48	+	—	
3,3'-Dimethylbenzidine HCl	612-82-8	***	44	+	+	

Ames test, Salmonella mutagenicity result; in vitro cyto, chromosome aberrations in Chinese hamster cells in culture; in vivo cyto, chromosome aberrations or micronuclei in bone marrow cells of treated mice.

\*Nitrotoluene is mutagenic in Salmonella when activated by enteric organisms.

\*\* Nitrilotriacetic acid, by itself, is negative in the Ames test and was not tested in the chromosome aberration test. Nitrilotriacetic acid + ferric saccharate has not been tested in the Ames test or in chromosome aberration tests.

\*\*\* Studies were terminated early due to other tumor formation; cannot be classified as to TVM potency.

a. % of treated animals with TVM; maximum response recorded

b. Study week at which earliest TVM was identified

c. combined results from testing different salts of the same parent chemical

ip, intraperitoneal administration; +, positive response; w+, weakly positive; -, negative; E, equivocal response; +/-, conflicting results; blank, not tested; NA, data not available

Table 30. Summary of chemicals associated with tunica vaginalis mesotheliomas in rats, arranged by route of administration

Agent	Route	Strain	TVM	LCT	Other treatment-associated tumors
Methyl(acetoxymethyl) nitrosamine	IP	F344	36%	++	NR
Methyl(acetoxymethyl) nitrosamine	IP	SD	15%	+	NR
Methyl(acetoxymethyl) nitrosamine	IP	Buf	46%	0	NR
Ferric saccharate	IP	Wist	47%	NR	NR
Ferric saccharate + NTA	IP	Wist	68%	NR	NR
Cytembena NTP TR 207	IP	F344	74%	++	FR – Mammary fibroadenomas
2,3-Dibromo-1-propanol NTP TR 400	Topic	F344	8%	+	MR&FR – Tumors of the nasal cavity, skin, oral cavity, esophagus, forestomach, intestines, liver, kidneys, Zymbal gland MR – Splenic hemangioma/hemangiosarcoma FR – Clitoral gland tumors MM&FM – Skin & forestomach tumors MM – Liver and lung tumors
Ethylene oxide	Inh	F344	26%	++	MR&FR – Brain tumors FR – Mammary adenomas & adenocarcinomas
1,2-Dibromoethane NTP TR 210	Inh	F344	52%	++	MR&FR – Nasal carcinomas FR – A/B tumors; Mammary fibroadenomas MM – A/B tumors FM – Hemangiosarcomas; S/C Fibrosarcomas & Nasal carcinomas
1,2-Dichloroethane	Inh	F344	10%	NR	MR&FR – S/C fibromas and mammary fibroadenomas FR- Mammary adenomas and adenocarcinomas

<b>Agent</b>	<b>Route</b>	<b>Strain</b>	<b>TVM</b>	<b>LCT</b>	<b>Other treatment-associated tumors</b>
Ethyl telluric NTP Tr 152	Diet	F344	16%	++	MM&FM-Harderian gland tumors
o-Nitrotoluene NTP Tox 23; NTP Tox 44 NTP TR 504	Diet	F344	90%	++	MR&FR – S/C tumors & Mammary fibroadenomas MR – Liver tumors (including cholangiocarcinomas) & A/B tumors FR - Liver adenomas MM&FM – Hemangiosarcomas & Cecal carcinomas FM – Liver tumors
o-Toluidine Hydrochloride NTP Tox 44 NTP TR 153	Diet	F344	34%	++	MR&FR-Splenic sarcomas MR – S/C Fibromas FR – U. Bladder carcinoma & Mammary adenomas & adenocarcinomas MM – Hemangiosarcomas FM – Liver adenomas or carcinomas
2,2-bis(bromomethyl)-1,3-propanediol NTP TR 452	Diet	F344	43%	++	MR&FR – Mammary fibroadenomas; oral cavity and esophagus carcinomas; thyroid follicular cell tumors MR – Skin tumors; U. bladder carcinomas; A/B tumors; S/C fibromas; forestomach papillomas; intestinal tumors MM&FM – A/B tumors; Harderian gland tumors MM – Renal adenomas FM – S/C sarcomas
Nitrofurazone NTP TR 337	Diet	F344	14%	+	FR – Mammary fibroadenomas FM – Ovarian tumors
Pentachlorophenol NTP TR 483	Diet	F344	18%	++	MR – Nasal carcinomas MM & FM – Liver & adrenal tumors FM – Hemangiosarcomas
Tartrazine (FD&C Yellow No. 5)	Water	F344	12%	++	FR – Endometrial stromal polyps

Agent	Route	Strain	TVM	LCT	Other treatment-associated tumors
3,3'-Dimethoxybenzidine HCl NTP TR 372	Water	F344	10%	++	MR&FR – Tumors in the oral cavity, large intestine, liver, Zymbal gland and skin MR – Tumors in small intestine and brain FR – Mammary adenocarcinoma; tumors in clitoral gland and uterus
3,3'-Dimethylbenzidine HCl NTP TR 390	Water	F344	7%	+	MR&FR – Tumors of the skin, Zymbal gland, liver, oral cavity, intestines and lung MR – Preputial gland tumors FR – Clitoral gland tumors
Potassium bromate (Multiple published studies)	Water	F344	75%	NR	MR – Kidney and thyroid tumors
Acrylamide (2 published reports)	Water	F344	17%	++	MR&FR – Thyroid follicular tumors FR – Mammary fibroadenomas
Methyleugenol NTP TR 491	Gav	F344	24%	++	MR&FR – Liver tumors; Neuroendocrine stomach tumors MR – Kidney tumors; mammary tumors; S/C tumors MM & FM – Liver tumors MM – Glandular stomach tumors
Benzaldehyde NTP TR 378	Gav	F344	10%	++	MM&FM – Forestomach papillomas
Glycidol NTP TR 374	Gav	F344	83%	++	MR&FR – Brain, forestomach and thyroid tumors MR – Mammary fibroadenomas, Intestinal tumors, skin tumors; Zymbal gland tumors FR – Oral cavity tumors, clitoral gland tumors, leukemia MM&FM – Harderian gland and skin tumors MM – Forestomach, liver and lung tumors FM – Mammary tumors, Uterine tumors, S/C tumors

TVM = highest % incidence tunica vaginalis mesothelioma LCT = Leydig cell tumor response [+ = < 79%; ++ = ≥80%]

MR = Male Rat FR = Female Rat MM = Male Mouse FM = Female Mouse NR = Not reported

SD = Sprague-Dawley Buf = Buffalo Wist = Wistar NTA = Nitrlotriacetate acid

IP = intraperitoneal Tpoic = topical Inh = inhalation Diet = dietary Water = drinking water Gav = gavage

Table 31. Human relevance framework for tunica vaginalis mesothelioma induction in F344 rats secondary to enhanced mesothelial mitogenesis

Alternative Key Events	Degree of Certainty in F344 Rat	Human Relevance
Presence of Leydig cell tumors causally related to tunica vaginalis mesotheliomas	Reasonably certain. Size of Leydig cell tumors correlated with tunica vaginalis mesotheliomas and localized growth factors. Localized peritesticular hormonal imbalance stimulates mitogenic autocrine growth factors from mesothelial cells. (Turek & Desjardins 1979; Gerwin et al., 1987; Karpe et al., 1982; Bartke et al., 1985; Versnel et al., 1988)	Not relevant. Leydig cell tumors are extremely rare in humans. There are significant differences in production and responsiveness between rat and human mesothelial cells. (Clegg et al., 1997; Walker et al., 1995; Walker et al., 1992)
Physical pressure or shearing forces due to enlarged Leydig cell tumor-bearing testes	Good evidence. Evidence for altered growth factor expression in transformed mesothelial cells in vitro. (Tanigawa et al., 1987; Gabrielson et al., 1988; Gerwin et al., 1987; Waters et al., 1997)	Not relevant. Leydig cell tumors are extremely rare in humans. (Clegg et al., 1997; Walker et al., 1995; Walker et al., 1992)
Age-associated increased prolactin leading to decreased circulating testosterone	Certain. Increased prolactin causes decreased LHRH and LH and inhibition of testosterone production. (Mahoney & Hodgen 1995; Capen et al., 2002)	Not relevant. Human Leydig cells do not have LHRH receptors. LH receptors not responsive to prolactin. (Prentice & Meikle 1995)
Decreased prolactin secretion from pituitary via dopamine agonists	Certain for specific chemicals. Serum prolactin levels decrease in rats. Decrease in LH receptors. (Prentice et al., 1992; Prentice & Meikle 1995; Friedman et al., 1999; Uphouse et al., 1982)	Not relevant. Human LH receptors not responsive to prolactin. (Wahlstrom et al., 1983)
Spontaneous age-associated decrease in testosterone and LH receptors and compensatory increase in LH	Certain. Responsible for the high spontaneous incidence of Leydig cell tumors in older F344 rats. (Amador et al., 1985; Maekawa & Hayashi 1992; Takaki et al., 1989; Solleveld et al., 1984; Foster 2007; Tanigawa et al., 1987; Turek & Desjardins 1979; Prentice & Meikle 1995; Capen 1996)	Uncertain. The number of LH receptors is 14 times greater in rats compared to humans. (Prentice and Meikle, 1995)
LHRH receptor agonist induced Leydig cell tumors	Reasonably certain for specific chemicals. Binding to rat LHRH receptors on Leydig cells produces Leydig cell tumors. (Prentice & Meikle 1995)	Not relevant. Human Leydig cells do not have LHRH receptors. (Prentice & Meikle 1995)

# ATTACHMENT K

## Comments on

### **Acrylamide Review Panel Draft Report on “USEPA Toxicological Review of Acrylamide” In Support of Summary Information on the Integrated Risk Information System (IRIS) December 2007”**

*Submitted by*

*The Sapphire Group, Inc.*  
Bethesda, Maryland<sup>1</sup>  
Cleveland, Ohio<sup>2</sup>

Submitted to the Science Advisory Board  
US Environmental Protection Agency

*On Behalf of*

Grocery Manufacturers Association  
Washington, DC

20 October 2008

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<sup>1</sup>Robert G. Tardiff, Ph.D., ATS; M. Leigh Carson, M.S.

<sup>2</sup>Christopher R. Kirman, M.S.

**Comments on**  
**Acrylamide Review Panel Report on**  
*“USEPA Toxicological Review of Acrylamide*  
*in Support of Summary Information on the*  
*Integrated Risk Information System (IRIS)*  
*December 2007”*

**INTRODUCTION AND SUMMARY —**

On behalf of the Grocery Manufacturers Association (GMA), *The Sapphire Group, Inc.*, offers to USEPA’s SAB comments on the draft report prepared by the SAB’s Acrylamide Review Panel (ARP). This ARP report addresses USEPA’s draft Toxicological Review of Acrylamide. We applaud the efforts put forth by the Agency’s ARP in its review. That notwithstanding, we elaborate herein on some advanced information that we recommend be taken into account by the ARP before final issuance of its report and ultimately by the group within the Agency responsible for advancing the draft Toxicological Review of Acrylamide to its next level.

On 2 April of this year, we provided assorted, detailed comments to both the Agency and the ARP about the Agency’s draft Toxicological Review of Acrylamide. While some of the concerns we raised have been addressed, others appear to remain outstanding. Since that time, we have had occasion to study in considerable detail the scientific underpinnings of a central element of the Agency’s draft, most notably the physiologically-based toxicokinetic (PBTK) modeling employed in the Agency draft. We hope to demonstrate clearly that the ARP has not addressed some important limitations of the Agency draft with particular reference to the PBTK modeling and factors influencing the cancer potency for ingested acrylamide. We request the SAB to take particular note of the fact of acrylamide’s inadvertent formation in cooked foods, resulting in it being of potential public health importance not only in the United States but also around the world. We recognize that USEPA’s regulatory purview extends only to environmental exposures such as drinking water and not to exposures in the workplace or to foods and some consumer products. Although USEPA has no responsibility to accurately assess acrylamide in food, the Agency must recognize that its toxicological review of acrylamide may become a reference for food regulators and risk managers. It is therefore incumbent on USEPA to make this review as robust as possible, including a clear discussion of alternatives and uncertainties in the assessment.

**We ask, therefore, that the SAB commission the ARP to reconsider elements of its report before making its report final.** To do so, we realize, may require some modification to the ARP's original charge.

The bases of our recommendation are as follows.

**1.** The PBTK model used by the Agency is flawed. ARP correctly acknowledged the central importance of the Agency's PBTK model ("recalibrated Kirman model") for acrylamide to its estimate of the noncancer RfD (based on neurotoxicity) and of the cancer potency. The recalibrated model summarized in the Agency's draft Toxicological Review of Acrylamide is based on (a) the model code that seeks to integrate assorted data into a dynamic representation of the metabolism and biological interactions of acrylamide and its primary metabolite (glycidamide) in the body of the rat; and (b) the documentation describing the information used and the functionality of the model with sufficient detail so as to impart transparency to third-party reviewers and users of the recalibrated acrylamide model. While the ARP identified several shortcomings of the recalibrated Kirman model, it was unable to ascertain the serious flaws in the model.

According to its draft report, the ARP either did not have access to the model code and its documentation or had insufficient resources to examine them in detail. Thus, ARP could not have examined or critiqued in detail the model structure to determine its integrity, completeness, or validity. We obtained these documents, and our kineticists have analyzed them closely. Our findings about the acrylamide PBTK model include:

- The model has not been subjected to the validation steps considered necessary by the community of PBTK model developers as well as Agency guidelines. Without the use of these rigorous approaches, it is unclear if the model's results for acrylamide are indeed valid.
- The model is out of date since it was completed in 2005 and much relevant data have been published since that time.
- Some parameters used in the model are physiologically impossible, leading to misstatements of internal doses.
- The model documentation lacks sufficient transparency, making it difficult to understand the model and replicate it.
- The model code *The Sapphire Group* received from the Agency's contractor was not operational due to an original programming error. That information has been conveyed to its author, who has volunteered to address the matter

with the Agency. It is unclear whether the Agency had a different version of the model code when it prepared its draft assessment.

The details of our findings are found herein in **Appendix A. We ask that the SAB consider these observations before completing its report.**

2. Although several limitations of the recalibrated Kirman model, some of which have been identified by the ARP, were substantial, the charge to the ARP did not encompass reconstruction of the reconstructed model to overcome those deficiencies. Due to our experience with the original Kirman et al. PBTK model for acrylamide and our understanding of the kinetic data on acrylamide, we have undertaken to produce an update of the Kirman model employing (a) all the relevant and most up-to-date scientific data about the compound's kinetic behavior and (b) a validation step. The updated model is scheduled to be completed by the end of 2008, at which time it will be submitted for publication in a peer-reviewed journal. It can then be used by the scientific community to develop internal doses of acrylamide that would serve as inputs into estimation of RfD and cancer potency factors. A summary of our progress is described in **Appendix B. SAB should ask ARP to await that updated model and consider it in the Agency's update of its draft Toxicological Review of Acrylamide.**

3. The ARP current draft refers to only a small fraction of the irreversible binding or sequestration detoxification mechanisms (both capacities and rates) present in humans into its estimate of cancer potency and risk-specific doses. Detoxification is a means for the body protect itself from what might otherwise be potentially dangerous exposures to chemicals foreign to the body (details were provided in our earlier comments to ARP and the Agency). Particularly noteworthy is the fact that humans have a greater abundance of these defense mechanisms than do the rats used to test experimentally for the carcinogenicity of acrylamide. The existence of effective detoxification mechanisms is to be expected on biological grounds because hundreds of naturally-occurring toxicants are intrinsic to the diet. Compounds that form naturally with heating, like acrylamide, have been in the diet since the advent of cooking.

This one factor alone may reduce human cancer risk estimates from ingested acrylamide; however, the ARP report does not address with any emphasis the consequences of these binding/sequestration processes for estimating cancer potency at doses experienced by humans in tap water or the diet (see our earlier submission for details). We have completed and submitted for peer-reviewed publication a manuscript that details the role of detoxification of acrylamide and its primary metabolite glycidamide. Comments from reviewers were received recently with an indication that once the suggested changes are incorporated, the manuscript will be accepted for publication, with publication in two to four

months. **We ask the SAB to defer finalizing its ARP report until the ARP can consider this publication and conduct a more detailed analysis of detoxification of acrylamide in the diet.**

**4.** The ARP draft report deals only peripherally with alternative shapes of the dose-response curve for acrylamide carcinogenicity. **The ARP should direct USEPA to include a non-linear approach for extrapolating to low doses in the range experienced by humans in addition to the linear default approach used by the USEPA for any pertinent cancer endpoints. This situation is particularly important when the lower bound cancer risk may be zero, as is the case with acrylamide.** Unless these steps are taken, decision makers and others will not be fully informed of the range of possible cancer potency factors for — and potential cancer risks of — acrylamide exposures for humans.

The ARP review should promote balance and perspective in the Agency’s characterization of acrylamide’s cancer risk. First, the ARP should not dismiss out of hand the evidence that has been submitted to the ARP and the Agency that supports non-genotoxic MoA, or has been submitted to the ARP and the Agency to support at least a mixed MoA. Even if acrylamide were assumed to be causing cancer through a genotoxic mode-of-action (as yet an unproven hypothesis), it is important to present quantitatively and transparently the impact of both possibilities on the estimated potency. In other words, the ARP report should say that, depending on whether acrylamide is assumed to be acting through a genotoxic or non-genotoxic mode-of-action, the cancer potency factor would be either “X” or “Y.” Furthermore, when using the linear assumption, ARP should direct the Agency to report the lower-bound on cancer potency and risk as well as the upper-bound. Such an approach would be appropriate even if genotoxicity were the sole MoA. Precedents exist for genotoxic carcinogens to have non-linear dose-response relationships. [Relevant documentation has been provided to ARP and the Agency in our earlier submission.]

Based on the above, it seems prudent that the USEPA conduct an RfD-type cancer assessment (*i.e.*, NOAEL or BMDL and Uncertainty Factors), in addition to the default linear approach presently described, in the next version of the Agency’s document. Another appropriate methodology would be that called the Margin of Exposure (MoE), by which one compares a human equivalent NOAEL to known or anticipated human doses. Another approach worthy of serious consideration by ARP is the formulation of a biologically-based model that incorporates mixed MoA for low-dose extrapolation of cancer incidence in rats and subsequent application to humans.

**5.** The ARP draft is incomplete with regard to judging the relevance to humans of the tumors observed in acrylamide-exposed rodents. A **Mode-of-Action/Human Relevance Framework** exists to do so in considerable detail and with much reliability and transparency. USEPA has accepted this Framework as part of its assessment process (as mentioned in our

previously submitted comments). In this instance, the Agency has applied this Framework to an insufficient extent to acrylamide's Toxicological Review. Were it to apply the Framework more completely, it would find that all rat tumors are not relevant to humans. Missing from USEPA's draft are (a) taking into proper account kinetic and dynamic factors, (b) plausibility of MoA for humans, and (c) concordance analysis of animal and human responses (notwithstanding USEPA's default view that no such concordance need exist). Considering that acrylamide is intrinsic to the diet and cannot be completely eliminated, such an analysis would prove to be essential understanding whether there may be any public health implications and informing risk management decisions. **The ARP should address this matter in sufficient detail to demonstrate the value of this approach in the next iteration of the Agency's Toxicological Review of Acrylamide.**

We wish to provide the following additional comments related to the ARP Review.

- 1.** We agree with the ARP's conclusion that the Agency draft treatment of acrylamide's neurotoxic properties demonstrated a sound understanding of the underlying science, an appropriate application of its methodology in generating a draft RfD, and a clear and understandable rationale of how it developed the draft RfD. We agree that the proposed RfD based on neurotoxicity is amply protective of public health, and suggest that USEPA consider the prospect that protecting against neurotoxicity may also protect against cancer (when the cancer potency is properly classified as non-linear, as noted in our comments).
- 2.** The ARP draft report does not recognize the restricted value of USEPA's approach to the time-to-response model, and recommend that the Agency address early mortality by methods other than that employed in the Agency draft.
- 3.** Some dose-response data from the Johnson et al. (1986) study may be useful for estimating the cancer potency of acrylamide and should be considered for inclusion in the quantitative assessment. The draft ARP report is silent on this point but should include an indication that the data may have some utility.
- 4.** We agree with ARP that AUC for glycidamide is the best dose measure for a genotoxic MoA. We recommend, however, that ARP indicate that to the extent that a non-genotoxic MoA can be justified, use of AUC acrylamide could be supported for a non-linear as well as linear assessment.
- 5.** ARP should inform the Agency that its use of a time-to-response model for acrylamide carcinogenicity is unwarranted. The model for male rats does not appear to be supported for tunica vaginalis mesothelioma data. On a practical level, the multistage-Weibull time-to-response model used by the Agency in its dose-response assessment for the carcinogenic effects of acrylamide is no longer available or supported, thereby adversely affecting the transparency and reproducibility of USEPA's assessment.
- 6.** The ARP draft review should inform USEPA to present not only central tendency and upper-bound estimates of cancer potency for acrylamide but also a lower bound estimate, as indicated by the Agency's current carcinogen assessment.
- 7.** Because acrylamide has been recently reported as being present in widely consumed foods, USEPA's judgments about human safety and risks may well have impacts in our society and globally that greatly transcend its legislated mandates. The SAB and the Agency should recognize the existence of major ongoing toxicological studies (sponsored by the National Toxicology Program) whose findings, expected by the end of 2009, may change, perhaps greatly, the USEPA's estimates of cancer potency of ingested acrylamide.

Therefore, the SAB and the Agency should proceed with particular caution in finalizing its Toxicological Review of Acrylamide and in the formulation of its IRIS documentation for acrylamide. **We ask, therefore, that the SAB recommend that the Agency defer issuance of its final Toxicological Review of Acrylamide and its IRIS documentation until the peer-reviewed NTP data become publicly available.**

## APPENDIX A

### Findings from a Detailed Review of USEPA's PBTK Acrylamide (AA) Modeling Documentation

*Prepared for*  
Grocery Manufacturers Association  
Washington, DC

*Prepared by*  
*The Sapphire Group, Inc.*  
Dayton<sup>3</sup> and Cleveland<sup>4</sup>, Ohio; Bethesda, Maryland<sup>5</sup>

October 2008

## INTRODUCTION

USEPA released a draft Toxicological Review in December 2007 in which toxicity reference values (a chronic Reference Dose, chronic Reference Concentration, cancer oral slope factor, and cancer inhalation unit risk) were derived for acrylamide (AA) for the Integrated Risk Information System (IRIS) (USEPA, 2007). An important part of the toxicity reference value derivation process was the use of a physiologically based toxicokinetic (PBTK) model for interspecies and route-to-route extrapolation.

USEPA had identified the PBTK model of Kirman et al. (2003) as being of potential value in deriving the AA toxicity reference values and employed a contractor to modify ("recalibrate") and apply it for the IRIS AA draft to calculate Human Equivalent Doses for noncancer and cancer. The contractors' efforts were completed in 2006, and the results of that modeling were incorporated in the Agency's draft IRIS document for AA.

A Science Advisory Board (SAB) was convened by USEPA to provide a peer review of the AA Toxicological Review. Several charge questions pertaining to PBTK modeling were put before the SAB for their discussion.

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<sup>3</sup>Lisa M. Sweeney, Ph.D., DABT,

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Charge Question 8:

*Please comment on whether the documentation for the recalibrated Kirman et al. (2003) PBTK model development, evaluation, and use in the assessment is sufficient to determine if the model was adequately developed and adequate for its intended use in the assessment. Please comment on the use of the PBTK model in the assessment, e.g., are the model structure and parameter estimates scientifically supportable?*

Charge Question 9:

*Do you agree with the conclusion that the recalibrated Kirman model is the best for deriving toxicity values?*

Charge Question 11:

*Is the recalibrated PBTK model appropriate to use for route-route extrapolation?*

*The Sapphire Group, Inc.* reviewed the IRIS AA draft and evaluated the PBTK model used by USEPA and its application in the toxicity reference value derivations. Particular concerns were the transparency of the model development/calibration and validation processes and the failure to test whether or not the AA model used by USEPA was consistent with recently developed data (*The Sapphire Group, 2008*). *The Sapphire Group's* comments were provided to the SAB by GMA. Some of the concerns could not be fully evaluated because we did not have access to a copy of the model and the documentation provided by USEPA was limited. Subsequently we were provided access to electronic copies of the model and additional documentation. The assessment in this current report therefore reflects our understanding based on this additional information.

## **PROCESS AND APPROACH**

Following the SAB public meeting, *The Sapphire Group* has conducted a follow-up review for which the contractor to USEPA provided copies of the spreadsheets and reports which documented the analyses used in the USEPA assessment. Access to these documents allowed us to investigate some of our previously identified concerns.

We reviewed the spreadsheets and reports to identify which of them documented the versions of the model that USEPA ultimately used in the IRIS draft Toxicological Review. We reviewed the equations in these spreadsheets to confirm that AA and its metabolite glycidamide were appropriately accounted for throughout the computations. For example, if AA is eliminated from the liver by metabolism (subtracted in the liver AA mass balance equation), a molar equivalent amount of glycidamide must be created and added to a mass balance equation for glycidamide in the liver. We also compared the model parameter values

in the key spreadsheets to those in the contractor's reports to the USEPA and USEPA's IRIS document.

## **FINDINGS**

The findings below reflect those findings related to PBTK modeling that were identified in our previous effort (The Sapphire Group, 2008), updated to reflect additional insights in light of the additional items which have subsequently been reviewed.

1. The recalibrated rat PBTK model lacked validation at the time it was completed (e.g., Doerge et al., 2005 a,b).

In the recalibration, the contractor emphasized fit to cumulative measures (end-of-exposure hemoglobin adduct levels and 24 hr urinary excretion). The Doerge et al. (2005a,b) data include measurements of AA and glycidamide in serum at several time points for rats exposed to AA or glycidamide by iv injection, gavage, or dietary exposure. Combinations of model parameter values that produced adequate simulations of metrics at the end of the simulation may not necessarily adequately describe how the concentrations of AA and glycidamide change during the exposure. That is, the model simulations may have given accurate predictions of the original data set for the wrong reasons, and this may not be apparent until the model is tested against a different type of data. Since PBTK models are frequently applied to dosing scenarios that are dissimilar to those used in the studies considered during model development (e.g., extrapolation to lower doses, repeated dosing), it is important to compare the model to an extensive, diverse data set, if such data are available. USEPA failed to fully validate their AA model in the draft Toxicological Review.

2. The USEPA PBTK documents lack transparency. While the absence of transparency may not change the results of USEPA's analysis or their toxicity reference values, it limits understanding and replication of USEPA's findings, thereby impairing the credibility of the IRIS documentation.

Physiologically impossible parameter values were listed in the limited model documentation provided by USEPA (USEPA, 2007). The human liver volume listed in Table E-8 is 0.183, which happens to be the same as the fractional liver blood flow in this table. Likewise, the fractional tissue blood flow and fractional tissue volumes in Table E-8 are also identical (0.8842). These values are problematic as the sum of tissue volumes and blood flow to tissues in humans are greater than 100%, a physical impossibility. It is surprising that no one identified this error during the preparation, internal review, and interagency review of the IRIS draft document. These same values are found in the report provided to USEPA by the

contractor, but these are not the values that were actually used in the spreadsheet calculations.

A further discrepancy between the model documentation is that most of the metabolism parameters were not correctly reported in the USEPA Toxicological Review (2007) or the contractor reports. The spreadsheet calculations typically included “adjustment factors” that were apparently manipulated by the user to achieve optimal fits to the experimental data. The reports neglected to include these adjustment factors in the calculation of the final best-fit model parameters, and as a result, the parameter values actually used in the model calculations differ by as much as 3.6-fold from the reported values. Without inspecting the spreadsheet, it is unlikely that these reporting errors could have been identified.

We have brought these findings to the contractor’s attention, who upon review of the spreadsheets and reports, concurred with *The Sapphire Group*’s conclusions.

The calculations in Appendix Tables E-9 (human model predictions) and Table E-10 and E-11 (AUCs for AA and glycidamide for various AA doses via a drinking water exposure or inhalation) values were calculated using the same parameter set. Thus there was no error introduced between human model development and application of those model parameters in the two human exposure scenarios.

3. *The Sapphire Group, Inc.* found that the spreadsheets do not accurately recreate the Kirman et al. (2003) rat model.

In Appendix A of Kirman et al. (2003), reaction rates are clearly specified as being expressed as function of liver venous blood concentrations. In the USEPA recalibrated model, these same rates are in terms of the concentration in liver tissue. Thus, the IRIS simulations of the Kirman et al. (2003) model as originally calibrated (e.g., Table E-3 in Appendix E of the IRIS document) are in error. The error may not be large, however, since the liver tissue: blood partition coefficient was 0.83. As a result, in the USEPA model calculations, the AA metabolism rate was underestimated by as much as 17 percent. However, since USEPA used an update of the rat model in their risk value derivations, any error in describing the Kirman model does not propagate into the quantitative aspects of the assessment itself.

4. The human PBTK model has limitations that could be explored using additional data and by conducting sensitivity analyses which USEPA has either not conducted or not reported.

The USEPA’s contractor used a very limited data set to parameterize the human model, and the data of Fuhr et al. (2006) and Kopp and Dekant (2008) (abstract only) were not used. The impact of the omission of these data cannot be determined until we know whether or not USEPA’s model output is consistent with the data of Fuhr et al. [Comparison to the data of

Kopp and Dekant would also be of value, though no firm conclusions should be drawn until a full, peer-reviewed report of the data is available.] When faced with highly uncertain parameter values with limited validation, USEPA should conduct sensitivity analyses of the dose metrics used in the assessment. Without this information, model confidence cannot be assigned, and thus the confidence in the quantitative aspect of the assessment cannot be assessed.

## **CONCLUSIONS AND RECOMMENDATIONS**

We conclude that the USEPA has used a model that was out-of-date by the time their assessment was completed. At a minimum, USEPA should have conducted additional simulations that would test whether their model was or was not consistent with the new data, and either revised their assessment or provided appropriate caveats. New or revised rat and human models should be developed and the assessment should be updated accordingly. Furthermore, the public and the reviewers of an updated assessment should be provided with sufficient, accurately-prepared documentation of the PBTK modeling so that a practitioner in the field could accurately reproduce the model and the simulations in the assessment.

## APPENDIX B

### Progress report for updating the acrylamide-glycidamide PBPK model

*Prepared for*  
Grocery Manufacturers Association  
Washington, DC

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#### INTRODUCTION

Based on the findings of our review of the USEPA's draft Toxicological Review for acrylamide (AA) and the available literature on the disposition of AA and its key metabolites, we concluded that the development of an updated PBPK model for AA was needed for a state-of-the science risk assessment of AA. The following report provides an overview of the work completed thus far toward the development of an updated PBPK model for AA and its metabolites in the rat. Information on planned subsequent activities is also provided. As model development is typically an iterative activity, there may be further changes to the model structure and changes in optimization/parameterization strategies prior to the completion of this effort. Human modeling efforts will be initiated once the rat model is deemed satisfactory. A more detailed description of the final model structure, the process for deriving the parameters for the model, and strengths and limitations of the model will be provided in the draft manuscript which is the intended outcome of this effort.

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## MODEL STRUCTURE

It was decided within our project team that alterations to the Kirman et al. (2003) model structure would be of value, in consideration of data that have come available since the publication. We plan to implement the following changes/additions to the model structure:

- The 2003 model consisted of blood (plasma plus erythrocytes), liver, and “tissue” compartments for AA and glycidamide (GA). The “tissue” compartment will be subdivided into compartments corresponding to the brain, kidneys, slowly perfused (muscle and skin), and other perfused tissues.
- Urinary elimination of GA from the kidney will be added to the model.
- Simple one-compartment model descriptions will be included for the major urinary metabolites (the mercapturic acids derived from conjugation of AA and GA with GSH, and glycidol) rather than direct excretion.
- GSH conjugation of AA and GA will be described as saturable with respect to GSH concentration, first order with respect to AA and GA concentration.
- Liver GSH synthesis and depletion will be incorporated into the model.

## IDENTIFICATION OF DATA SETS AND ORGANIZATION OF DATA

The data used in developing the Kirman et al. (2003) and Young et al. (2007) models, as well as additional data identified via literature review, have been reviewed, digitized (if necessary), and organized into Excel spreadsheets. The spreadsheets will facilitate the transfer of the data into the PBPK modeling program (ACSLx) as well as for export into software producing publication-quality graphics (GraphPad Prism).

## PRELIMINARY PARAMETER ESTIMATES

Physiological/anatomical parameter values (e.g., blood flows, tissue volumes) have been identified from the literature. Protein turnover rates for some tissues (liver and muscle) have been identified from the literature.

Tissue:blood partition coefficients for AA and GA have been estimated from the data of Doerge et al. (2005a). A single set of partition coefficients for male and female animals will be used.

Initial estimates for metabolism parameters will be derived from a variety of sources. The parameters determined by Young et al. (2007) for iv kinetics of AA and GA in male F344 rats will be converted into body weight-normalized first-order reaction rate estimates. Km estimates will be taken from other sources (e.g., Kirman model, Walker et al., 2007 model, *in vitro* studies). If reported *in vivo* serum and tissue levels are sufficiently high, metabolism will be described as saturable; otherwise, first order reaction rate equations will be used. Because Young et al. (2007) did not include hydrolysis of GA in their model, the Kirman et al. (2003) values for GA hydrolysis will be used in the initial estimates.

Oral absorption rate estimates will be taken from Young et al. (2007). Blood and tissue protein binding rates from the Kirman et al. (2003) model will be retained as initial estimates.

### OPTIMIZATION/PARAMETER ESTIMATION

In general, the approach to parameter estimation will be similar to that employed by Young et al. (2007). We will focus on the F344 rat data and start with the GA iv studies (GA serum time course). Because we will include a metabolic pathway not included by Young et al. (2007), other parameters will need to be adjusted from those determined by Young et al. (2007) to maintain consistency between the model predictions and the data. Next, we will consider the AA iv studies (AA and GA serum time courses and urinary metabolites). Unlike Young et al. (2007), we intend to identify metabolism parameter values that adequately describe all the data for male rats rather than optimizing parameter values for each data set separately. We will consider whether the male-female parameter differences in the Young et al. (2007) optimizations reflect a true gender difference or simply a body weight difference or experimental variability. The Edwards (1975) data were not used in any previous modeling efforts, but the GSH depletion data in this study may provide a useful “reality check” as to the total flux through the GSH-conjugation pathways, though this study was not conducted in F344 rats. The 8-24 hr data in Miller et al. (1982) study and Ramsey et al. (1982) study will provide a reality check for the protein binding rates, while the longer term data provide insights into the protein turnover in the blood and tissues. Once the iv data are reasonably well simulated, absorption rates for ip, gavage, and dietary administration of AA and GA will be estimated from the available blood, tissue, and urinary data. Metabolic parameters will be revised as necessary to provide adequate fit to all of the F344 rat data, or “outlier” data sets may potentially be identified. The parameters derived for F344 rats will be used to simulate kinetic studies conducted in other strains—Long-Evans (Crofton et al., 1996; Raymer et al., 1993), Sprague-Dawley (Kadry et al., 1999; Barber et al., 2001), and Wistar (Sanchez et al., 2008). Based on the findings in other strains, we may consider re-optimizing key parameters that can reasonably be anticipated to vary among strains.

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**UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
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The Honorable Stephen L. Johnson  
Administrator  
U.S. Environmental Protection Agency  
1200 Pennsylvania Avenue, N.W.  
Washington, D.C. 20460

Subject: SAB Advisory on Aquatic Life Water Quality Criteria for Contaminants of Emerging Concern

Dear Administrator Johnson:

The Science Advisory Board (SAB) Ecological Processes and Effects Committee, augmented with additional experts, reviewed the EPA White Paper titled *Aquatic Life Criteria for Contaminants of Emerging Concern* (“White Paper”). EPA’s 1985 *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (“Guidelines”) specify procedural and data requirements for deriving ambient water quality criteria for the protection of aquatic life (aquatic life criteria). The Agency is faced with a number of technical issues and challenges in deriving aquatic life criteria for contaminants of emerging concern (CECs). To address these technical issues, the Office of Water and Office of Research and Development have proposed recommendations for interpreting and/or adapting principles in the 1985 Guidelines. EPA’s White Paper describes the proposed recommendations, focusing in particular on CECs that disrupt endocrine function in animals. The White Paper also explores these recommendations in the context of a case example CEC, ethynylestradiol, a synthetic pharmaceutical estrogen.

EPA’s Office of Water (OW) requested that the SAB: 1) comment on the technical merit, practicality, and implementability of recommendations in the White Paper; 2) comment on whether the White Paper identifies the appropriate issues to be addressed in deriving aquatic life criteria for CECs; 3) suggest ways to improve the utility of the ethynylestradiol case example; and 4) offer other suggestions to assist the Agency in implementing recommendations in the White Paper. The enclosed advisory report provides the advice and recommendations of the Committee.

1 Overall, the SAB finds that, in the White Paper, EPA has identified appropriate  
2 technical issues to be considered in deriving aquatic life criteria for CECs. However,  
3 EPA was constrained by the 1985 Guidelines which, although excellent when developed,  
4 were never envisioned for use with the current CECs. The derivation of aquatic life  
5 criteria needs to be risk-based, using a transparent and consistent framework that  
6 provides necessary flexibility not presently possible within the algorithm approach of the  
7 1985 Guidelines. Hence, the SAB recommends that, to the extent practicable, the  
8 derivation of aquatic life criteria be risk-based using the principles defined in EPA's 1998  
9 *Guidelines for Ecological Risk Assessment*.

10  
11 Within the context of risk-based aquatic life criteria, we recommend that EPA  
12 consider issues in addition to those identified in the White Paper. In particular, we urge  
13 EPA to create a conceptual model to guide development of aquatic life criteria for CECs.  
14 Such a conceptual model should include consideration of probable direct and/or indirect  
15 impacts on food webs, ecological processes and services, and endangered or unique  
16 species of special value or concern. We also recommend that EPA develop multiple lines  
17 of evidence, consider uncertainty, and bolster consideration of mode of action in the  
18 criteria development process. We suggest that mammalian pharmacology data available  
19 from the drug discovery process, genomics/proteomics/metabolomics, and quantitative  
20 structure activity relationships (QSARs) be used to screen CECs for modes of action and  
21 assess potential multiple modes of action for individual CECs. To increase efficiency,  
22 parallel processes could then be considered when developing aquatic life criteria for  
23 compounds with similar modes of action.

24  
25 The SAB generally supports EPA's proposed approaches for interpreting and/or  
26 adapting principles in the Guidelines to address technical issues discussed in the White  
27 Paper. However, we have noted specific concerns about these approaches and provide  
28 recommendations to improve the White Paper. We emphasize that many CECs will  
29 require special consideration because they do not fit the effect model discussed in the  
30 White Paper (i.e., disruption of endocrine function), or may not be well enough  
31 understood to allow appropriate judgment of their mode of action. In addition, we note  
32 that specific issues such as the potential for joint interactions affecting toxicity exist for  
33 many CECs that may occur in mixtures in the environment and which may also interact  
34 with environmental variables such as temperature. Such possible interactions should be  
35 considered. As more information is developed on CECs, it is possible that water quality  
36 criteria may be revised up or down for individual CECs based upon data on joint  
37 interactions; use of such data would produce more risk-based criteria.

38  
39 The SAB finds that the ethynylestradiol illustrative example in the White Paper is a  
40 well written and thorough review of the existing literature. It illustrates the complexities  
41 inherent in generating aquatic life criteria for CECs. However, we do provide  
42 recommendations to clarify the example and make it more useful.

43 The SAB also provides other suggestions to assist EPA in implementing the proposed  
44 recommendations in the White Paper. These suggestions focus on: data collection and

This draft SAB Committee report has been prepared for quality review and approval of the chartered SAB.

This report does not represent EPA policy.

1 research activities; developing tissue residue-based criteria; developing exposure and  
2 effect indicators that could be used in future derivation of criteria; special considerations  
3 for sensitive or commercially/recreationally important species; and obtaining input from  
4 private industry and state governments.  
5

6 Thank you for the opportunity to provide advice on this important topic. The SAB  
7 looks forward to receiving your response to this advisory.  
8

9 Sincerely,  
10

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12 Dr. Deborah Swackhamer, Chair  
13 Science Advisory Board  
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Dr. Judith L. Meyer, Chair  
Ecological Processes and Effects  
Committee

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Science Advisory Board  
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**Augmented for the Advisory on the EPA's Aquatic Life Water  
Quality Criteria**

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**TABLE OF CONTENTS**

4

**1. EXECUTIVE SUMMARY ..... xii**

5

**2. INTRODUCTION..... 1**

6

**3. CHARGE TO THE COMMITTEE ..... 3**

7

**4. RESPONSE TO CHARGE QUESTIONS..... 6**

8

**4.1 Charge Question 1. Comments on Recommendations in the White Paper.....6**

9

**4.1.1 Relevance of Acute Toxicity Effect Concentrations .....6**

11

**4.1.2 Defining Minimum Data Requirements Regarding Taxonomic Coverage .....9**

13

**4.1.3 Use of Non-resident Species in Criteria Development.....13**

15

**4.1.4 Defining Appropriate Chronic Toxicity Data .....16**

17

**4.1.5 Selection of Effect Endpoints for Criteria Development.....19**

19

**4.1.6 Involvement of an Expert Panel.....23**

21

**4.2 Charge Question 2. Comments on Technical Issues Addressed in the White Paper.....24**

22

**4.3 Charge Question 3. Comments on Part II of the White Paper.....29**

23

**4.4. Charge Question 4. Suggestions to Assist EPA in Implementing the Recommendations .....33**

24

**6. REFERENCES..... 39**

25

## 1. EXECUTIVE SUMMARY

EPA's Office of Water (OW) requested that the Science Advisory Board (SAB) provide advice on the Agency's proposed recommendations pertaining to derivation of water quality criteria for the protection of aquatic life (aquatic life criteria) for contaminants of emerging concern (CECs). The Agency's proposed recommendations are provided in a white paper titled *Aquatic Life Criteria for Contaminants of Emerging Concern* (White Paper). The White Paper, prepared by the EPA Office of Water/Office of Research and Development Emerging Contaminants Workgroup, was reviewed by the SAB Ecological Processes and Effects Committee (Committee). To augment the expertise on the Committee for this advisory activity, several environmental toxicologists with specific knowledge of the effects of endocrine disrupting chemicals also participated in the review.

EPA's Office of Water develops ambient water quality criteria that provide guidance to states and tribes for adoption of water quality standards. The EPA document, *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (hereafter referred to as the "Guidelines") (Stephan et al., 1985), sets forth a methodology for deriving ambient water quality criteria for the protection of aquatic life. The Guidelines specify various data and procedural recommendations for criteria derivation and also define general risk management goals for the criteria. Most of EPA's aquatic life criteria have been derived using methods in the Guidelines, and EPA has stated that the Agency intends to continue using the Guidelines to derive aquatic life criteria. However, EPA has also indicated that it faces a number of technical challenges in deriving aquatic life criteria for CECs. In its White Paper, the Agency described these technical challenges and proposed recommendations to interpret and/or adapt Guidelines principles to address the challenges. One of the Committee's key recommendations is that EPA incorporate risk assessment principles, as defined by the 1998 *Guidelines for Ecological Risk Assessment*, within the framework of the 1985 Guidelines. Criteria derived within the risk assessment framework will provide additional consistency with other on-going work at EPA and will provide necessary flexibility not presently possible within the algorithm approach of the 1985 Guidelines.

The term "contaminant of emerging concern" or CEC has been used by EPA to identify a variety of chemical compounds that have no regulatory standard, have been recently discovered in the natural environment because of improved analytical chemistry detection levels, and potentially cause deleterious effects to aquatic life at environmentally relevant concentrations. The Agency is particularly concerned about pharmacologically active chemical compounds and personal care products because: 1) they are commonly discharged at wastewater treatment plants, and 2) some of these compounds are designed to stimulate a physiological response in humans, plants, and animals.

This draft SAB Committee report has been prepared for quality review and approval of the chartered SAB.  
This report does not represent EPA policy.

1 The first part of EPA’s White Paper (Part I), *General Challenges and*  
2 *Recommendations*, describes: 1) the technical challenges EPA faces in deriving  
3 aquatic life criteria for CECs; and 2) the proposed recommendations to address those  
4 challenges. The second part of the White Paper (Part II), *Illustration of*  
5 *Recommendations Using Data for 17 $\alpha$  – Ethynylestradiol (EE2)*, explores EPA’s  
6 recommendations in the context of an example CEC, ethynylestradiol (EE2), which is  
7 a synthetic pharmaceutical estrogen. In its charge to the SAB, EPA requested  
8 comments on the technical merit, practicality, and implementability of  
9 recommendations in the White Paper to address: a) relevance of acute toxicity effect  
10 concentrations in setting aquatic life criteria for CECs; b) defining minimum data  
11 requirements regarding taxonomic coverage in toxicity testing; c) use of non-resident  
12 species in criteria development; d) defining appropriate chronic toxicity data; e)  
13 selection of effect endpoints upon which to base criteria; and f) involvement of an  
14 expert panel in the criteria development process. In addition, EPA asked the SAB to:  
15 comment on whether the Agency has identified the appropriate issues to be addressed  
16 in deriving aquatic life criteria for CECs; offer suggestions that may improve the  
17 utility of Part II of the White Paper; and offer suggestions that would assist the  
18 Agency in implementing proposed recommendations in the White Paper. In response  
19 to the charge questions, the Committee has provided comments and recommendations  
20 to improve the White Paper and assist EPA in deriving aquatic life criteria for  
21 contaminants of emerging concern.

22  
23 *Relevance of acute toxicity effect concentrations in deriving aquatic life criteria for*  
24 *CECs*

25  
26 Many CECs are physiologically active at concentrations orders of magnitude  
27 lower than those causing acute lethality, and concentrations sufficient to cause  
28 lethality may never occur in the environment. Therefore, in the White Paper the  
29 Agency recommends that, when sufficient information demonstrates a negligible risk  
30 of acute lethality for a CEC, the “contaminant continuous concentration” (i.e., the  
31 concentration intended to protect against the longer term effects of exposure on  
32 survival, growth, and reproduction) be used to derive aquatic life criteria. In  
33 principle, the Committee supports EPA’s suggestion to derive aquatic life criteria  
34 solely from criteria continuous concentrations for CECs when available information  
35 indicates that this is appropriate. However, we have recommended the following  
36 amendments in the White Paper:

- 37  
38 • Not enough is known about some classes of CECs (e.g., nanoparticles) to  
39 determine whether acute toxicity needs to be taken into account in deriving  
40 aquatic life criteria. Therefore, all available data on any new class of CECs  
41 should be used in determining whether acute toxicity is likely to occur in  
42 environmentally relevant settings.  
43  
44 • Some CECs appear to have differing modes of action for acute toxicity vs.  
45 chronic toxicity. Lowest Observed Effect Concentrations (LOECs) and LC50s

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1 (test concentrations that result in mortality to 50% of the test population) are  
2 within one order of magnitude for some CECs, making acute toxicity relevant in  
3 deriving aquatic life criteria. Therefore, “criteria maximum concentrations” to  
4 protect against acute effects should be derived for compounds where LOECs are  
5 found to be within one order of magnitude of LC50s.  
6

- 7 • Pulsed discharges of CECs may occur during natural disasters and spills and  
8 result in atypically high concentrations in the environment. As further discussed  
9 in Section 4.1.1 of this report, aquatic life criteria derivations should consider  
10 whether concentrations capable of causing acute toxicity may occur during these  
11 pulsed discharges. Under this scenario, it may be important to use Criterion  
12 Maximum Concentrations (CMCs) in addition to Criterion Continuous  
13 Concentrations (CCCs) in the aquatic life criteria derivation process.  
14
- 15 • Mixtures of CECs with comparable modes of action may result in higher effective  
16 concentrations than would be expected based on the concentrations of any single  
17 compound. Therefore, research is needed to determine how aquatic life criteria for  
18 CECs can take into account the fact that aquatic organisms are exposed to  
19 mixtures of chemicals with similar modes of action.  
20
- 21 • To maintain transparency in cases when criteria maximum concentrations are not  
22 used in criteria development, a summary of all available data that provide  
23 information on the relevance of acute toxicity should be included in any aquatic  
24 life criteria document.  
25

26 *Defining minimum data requirements regarding taxonomic coverage in toxicity*  
27 *testing*  
28

29 In the White Paper, EPA has recommended that, for CECs without complete  
30 chronic toxicity data sets to fulfill minimum data requirements, there be an evaluation  
31 of whether sufficient information exists to conclude that certain taxa would not be  
32 sensitive to a particular chemical. Thus, EPA recommends that the minimum data  
33 requirements for taxonomic coverage (specified in the Guidelines) be viewed as  
34 information requirements instead of toxicity test requirements. The Committee  
35 understands and appreciates the desirability of avoiding the extra work required to  
36 develop chronic data on species that are unlikely to be sensitive to certain CECs.  
37 However, we emphasize that it is equally important to perform adequate testing to  
38 ensure protection of aquatic life. We generally support the broad taxonomic coverage  
39 requirements in the Guidelines but agree that these could be viewed as information  
40 requirements instead of test requirements. We find that, if sufficient information  
41 exists on the insensitivity of certain taxa to particular chemicals, expert judgment  
42 concerning data development should prevail. This would result in a more focused  
43 approach to data development, keeping in mind weight of evidence rather than a  
44 requirement for testing all taxa specified in the Guidelines. As indicated below, we

1 have provided specific recommendations to improve the process of determining  
2 appropriate taxonomic coverage to develop aquatic life criteria for CECs:

- 3
- 4 • EPA needs to define what constitutes a sufficiently robust set of chronic data for  
5 criteria development. Although the example used in the White Paper generally  
6 illustrates EPA's proposed process for making decisions concerning taxonomic  
7 coverage, it would be helpful if EPA were more explicit in identifying what  
8 constitutes a "sufficiently robust set of chronic data" and "a reasonable  
9 understanding of the mode of action for the chemical that may allow inferences."  
10
  - 11 • The White Paper should place greater emphasis on information useful for  
12 development of aquatic life criteria, rather than just toxicity test requirements.  
13 Incorporating effects on ecological processes (e.g., food webs, nutrient cycling,  
14 primary production) rather than only target species would be valuable in criteria  
15 development, and would follow more recent scientific thinking.  
16
  - 17 • As further discussed in Section 4.1.2 of this advisory report, EPA should consider  
18 shifting from an approach requiring a minimum level of taxonomic coverage to  
19 the approach of determining receptors of potential concern (ROPCs).  
20
  - 21 • Examples showing the unanticipated effects of CECs on non-target organisms  
22 (e.g., the impact of antibiotics on plants and effect of atrazine on the quality of  
23 algae available as food for other species) should be used in Part I of the White  
24 Paper to help describe how the aquatic life criteria development process needs to  
25 be more flexible depending on the compounds under evaluation.  
26

### 27 *Use of non-resident species in criteria development*

28

29 Historically, EPA has not included data from toxicity testing with non-resident  
30 species in the actual criteria derivation process. In the White Paper, EPA  
31 recommends that "non-resident" species data be used in the aquatic life criteria  
32 derivation process if such data would enable a better estimation of species sensitivity  
33 distributions. The Committee agrees; we find that the exclusion of non-resident  
34 species data from criteria derivation is biologically and practically inconsistent with  
35 the intent of the Guidelines (i.e., providing an objective, internally consistent,  
36 appropriate, and feasible way of deriving national criteria). We have provided a  
37 number of specific recommendations concerning the use of non-resident species data:  
38

- 39 • Because of the frequent use of non-resident species in toxicity testing, such  
40 species could potentially be over-represented in aquatic life criteria databases.  
41 Therefore, the proportion of the data set that should include resident species  
42 should be carefully evaluated by an expert advisory panel assembled to review  
43 each criterion.  
44

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- 1 • Although non-resident species can be used for criteria development, in no case  
2 should a criterion be developed on the basis of non-resident species data alone.  
3 Although the Guidelines have been designed to protect aquatic communities  
4 (including endangered species), EPA should support research that addresses the  
5 suitability of the use of surrogate species in assessing the responses of various  
6 resident aquatic species (e.g., endangered or long-lived species and species with  
7 varying life history strategies) to endocrine disrupting and other CECs.  
8
- 9 • Differences in strains, husbandry, health, and parasite and pathogen load (i.e.,  
10 other stressors) contribute to variations in toxicity test response and thus should  
11 be considered in the criteria development process.  
12
- 13 • Issues to be considered in prioritizing species responses should include their  
14 vulnerability, endangerment status, and recreational, commercial and ecological  
15 value.  
16
- 17 • Non-resident and resident species data must meet test guidelines for data and  
18 method validity.  
19

#### 20 *Defining appropriate chronic toxicity data*

21

22 In the White Paper, EPA recommends that the Guidelines requirements for chronic  
23 toxicity test data be tightened by requiring at least one full life-cycle test for a fish  
24 (life-cycle tests are already required for invertebrates) unless there is a compelling  
25 body of information indicating that life processes outside the early life stage or partial  
26 life-cycle exposure/observation window are not critical to capturing the biologically  
27 important effects of chronic exposure to the chemical. As further discussed in  
28 Section 4.1.4 of this report, the Committee strongly supports the use of fish full life-  
29 cycle test data in appropriate cases to develop aquatic life criteria. We find that it  
30 would be useful to develop a tiered testing approach to determine an appropriate  
31 rationale for use of data from fish full life-cycle, partial life-cycle, and possibly  
32 multigenerational testing to derive aquatic life criteria for CECs with parallel modes  
33 of action. We have provided additional recommendations concerning the requirement  
34 for chronic toxicity data.  
35

- 36 • EPA should critically review data dealing with transgenerational responses of  
37 aquatic species and evaluate whether this additional testing would provide  
38 significant new information to inform the criteria development process.  
39
- 40 • Test guidelines should include flexibility to include assessment of key  
41 developmental events, and professional judgment from an expert panel should be  
42 used to evaluate the relevance of non-traditional endpoints such as immune  
43 function and organism behavior. Behavioral endpoints (e.g., predator-prey  
44 interactions) may hold some promise for criteria development if the assays can be  
45 related to population-level responses and variability can be understood.

1

2 *Selection of effect endpoints upon which to base criteria*

3

4 In the White Paper, EPA has identified a number of endpoints that could be  
5 considered in developing aquatic life criteria for CECs. Moreover, the Agency has  
6 recommended more thorough exploration of the use of such endpoints in criteria  
7 development. Generally, the Committee agrees that EPA should continue to explore  
8 the possibility of using sublethal endpoints in helping to set aquatic life criteria.  
9 However, we caution EPA that such “non-traditional” endpoints must ultimately be  
10 linked to population endpoints (i.e., they must consider potential impacts to  
11 populations, not solely effects on individual organisms). We have provided a number  
12 of recommendations concerning use of these endpoints:

13

- 14 • EPA should use “non-traditional measures” to develop an understanding of and  
15 confirm mode of action of CECs.
- 16
- 17 • As further discussed in Section 4.1.5 of this advisory report, EPA should use  
18 human health information and toxicology tools (genomics/physiologically based  
19 pharmacokinetic models [PBPKs]) to reduce the uncertainty of aquatic life criteria  
20 for CECs.
- 21
- 22 • EPA should consider the following key points concerning use of the non-  
23 traditional endpoints discussed in the White Paper: 1) vitellogenin in males and  
24 juveniles is an indicator of exposure to feminizing stressor, but its linkage to  
25 population effects is limited; 2) strong correlations between vitellogenin and  
26 fecundity have been observed in females, but this is not necessarily tied to altered  
27 endocrine mode of action; 3) anomalous intersex can be indicative of exposure to  
28 a feminizing stressor(s) but may not, at present, be directly tied to population  
29 effects; and 4) gender ratio can be indicative of endocrine alteration, but baseline  
30 information on appropriate life stages is necessary for this evaluation.

31

32 *Involvement of an Expert Panel*

33

34 Because the development of aquatic life criteria for CECs may be dependent on  
35 technical interpretations of a wide range of toxicological information, EPA has  
36 proposed that expert panels be used to provide professional judgment during criteria  
37 development. The Committee strongly supports the use of panels comprised of  
38 experts with a balanced range of perspectives to provide professional judgment  
39 during the process of developing aquatic life criteria. However, we note that the use  
40 of expert panels could lead to less consistency in how aquatic life criteria are  
41 determined if the panels are not selected carefully. To help alleviate this potential  
42 problem, we recommend that EPA develop specific guidance on the role of expert  
43 panels in problem formulation, data evaluation, and generation of advice to support  
44 criteria development. Specifically, we recommend that:

45

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- 1 • The process for the use and selection of expert panels be described in detail and  
2 that it be transparent.  
3
- 4 • The panels be given clear charges and understanding of their roles in the process.  
5
- 6 • EPA take advantage of similar expert panel processes occurring in Europe and  
7 Asia to the extent possible.  
8

9 *Technical issues addressed in the White Paper*

10  
11 The Committee was asked to comment on whether EPA has identified the  
12 appropriate technical issues in the White Paper, and whether there are additional  
13 important issues that the Agency has not identified. We find that EPA has identified  
14 appropriate technical issues in the White Paper. However, as further discussed in  
15 Section 4.1.6 of this advisory report, we recommend that the Agency address  
16 additional issues to customize and update the 1985 Guidelines and thereby increase  
17 the flexibility and specificity of the aquatic life criteria derivation process. The  
18 following additional issues are of particular importance:

- 19  
20 • In the White Paper, EPA should articulate principles that can be applied when  
21 modifying the 1985 Guidelines to develop water quality criteria for CECs. In  
22 particular, these principles should address: 1) obtaining a wide range of inputs  
23 from diverse perspectives; 2) developing a robust conceptual model; 3)  
24 developing criteria for using multiple lines of evidence; and 4)  
25 identifying/including uncertainties (quantitative and qualitative) associated with  
26 criteria development.  
27
- 28 • It is particularly important that understanding and presenting uncertainty become  
29 an intrinsic part of the aquatic life criteria development process. For example, the  
30 uncertainties inherent in understanding modes of action, concentration-response  
31 relationships, extrapolation of sensitivities, and derivation of ecological effects  
32 should be quantified and/or described in a narrative sense.  
33
- 34 • EPA should bolster the consideration of mode of action in the aquatic life criteria  
35 derivation process. As stated previously, aquatic life criteria for CECs, should  
36 take into account the fact that aquatic organisms are exposed to mixtures of these  
37 chemicals. Understanding the mode of action of a compound is very important in  
38 estimating mixture interactions. In fact, pharmacological mode of action is the  
39 basis for evaluating multiple drug prescriptions in humans by pharmacists. EPA  
40 should use mammalian pharmacology data available from the drug discovery  
41 process, genomics/proteomics/metabolomics and quantitative structure activity  
42 relationships (QSARs) to screen CECs for modes of action, identify CECs that  
43 may act in an additive manner as mixtures, and assess potential multiple modes of  
44 action for individual CECs. The Committee strongly recommends enhancing the  
45 communication and data transfer capabilities between agencies such as the U.S.

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1 Food and Drug Administration (FDA) and EPA to provide mode of action  
2 information.

3

- 4 • In deriving aquatic life criteria for CECs, EPA should bolster consideration of  
5 ecology and indirect ecological effects and also give special consideration to the  
6 protection of threatened and endangered species.

7

### 8 *Part II of the White Paper*

9

10 Part II of the White Paper uses ethynylestradiol (EE2) as a model chemical to  
11 illustrate the technical issues presented and provide a basis for understanding the  
12 recommendations in Part I. The Committee was asked to offer suggestions to  
13 improve the utility of Part II. The Committee finds that Part II is a well-written and  
14 thorough review of the existing literature on EE2. We agree that EE2 is an  
15 appropriate initial focal CEC given the extensive data available relative to other CECs  
16 and the ease with which it illustrates the complexities inherent in generating CEC-  
17 specific water quality criteria. We have provided a number of specific  
18 recommendations to improve Part II:

19

- 20 • EPA should explicitly recognize that EE2 is unique in being a data-rich CEC.  
21 The White Paper should highlight the fact that the Agency's interest in CECs goes  
22 beyond endocrine-active substances, and discuss how the process outlined for  
23 EE2 might be applied to other substances, particularly those for which less data  
24 are available and which have different modes of action.
- 25
- 26 • The Committee suggests that some of the illustrative pieces of Part II could also  
27 be presented in Part I in the form of succinct text boxes illustrating key concepts  
28 derived from the various recommendations, and that the recommendations could  
29 be best illustrated if the text boxes were not restricted to EE2 but rather included  
30 other CECs.
- 31
- 32 • Part II should discuss how the individual effects of EE2 on biota might be  
33 changed by mixtures of compounds, especially those with similar modes of  
34 action.
- 35
- 36 • As stated previously, a criterion should not be developed on the basis of non-  
37 resident species data alone. Therefore, Part II should indicate that resident species  
38 data, especially data from life-cycle tests using resident species, remain extremely  
39 valuable and that results from non-resident species tests may not be generalized to  
40 resident species without comparative sensitivity studies.
- 41
- 42 • The possibility of transgenerational effects should be explicitly addressed in Part  
43 II.

44

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- 1 • A broader array of endpoints should be included in Part II. For example, although  
2 EE2 is a potent estrogen receptor agonist, it can also affect the central nervous  
3 system (through steroid biotransformation), and an endpoint should be considered  
4 to reflect this. Part II should also note that relevant and reproducible endpoints  
5 indicative of adverse population level effects need to be used.  
6
- 7 • As further discussed in Section 4.3 of this advisory report, the use of weight of  
8 evidence is implicit in the evaluation done in Part II, and should be explicitly  
9 discussed. Furthermore, when appropriate data are available, EC<sub>x</sub> values (i.e.,  
10 concentration causing an effect in x percent of the test organisms) should be used  
11 in Part II instead of NOECs/LOECs (i.e., no observed effects  
12 concentrations/lowest observed effects concentrations). The use of the EC<sub>x</sub>  
13 values takes advantage of more of the information from a toxicity test, and  
14 confidence intervals can be generated. The raw data from most toxicity tests can  
15 be used to calculate an EC<sub>x</sub> value. The selection of a specific EC<sub>x</sub> value for  
16 derivation of an aquatic life criterion depends upon the level of protection or  
17 effect that decision-makers are willing to accept or detect in the field. However,  
18 an EC<sub>20</sub> has been used for most species and an EC<sub>10</sub> has been used for threatened  
19 and endangered species. The Committee notes that if data are not available to  
20 calculate an EC value, EPA should recommend in Part II that such values be  
21 developed and used in future criteria derivation. Published data sets are available  
22 for much of the fathead minnow and other species toxicity tests conducted at  
23 EPA's Duluth Laboratory and other laboratories. If the data are available then the  
24 regression should be calculated. The Committee also notes that if the data are not  
25 available then the value of the NOEL/LOEL should be carefully evaluated.  
26 Without information on the variability of the test results, and consequently the  
27 statistical power, it is not clear what the values represent.  
28
- 29 • As further discussed in Section 4.3 of this report, the clarity and transparency of  
30 Part II could be improved in a number of areas.  
31

32 *Suggestions to assist EPA in implementing recommendations discussed in the White*  
33 *Paper*  
34

35 In Section 4.4 of this advisory report, the Committee has provided comments and  
36 recommendations to assist EPA in implementing the approaches discussed in the  
37 White Paper. The following key recommendations are provided:  
38

- 39 • As noted at the beginning of this Executive Summary, the principles for  
40 conducting Ecological Risk Assessment should be incorporated into the process  
41 of deriving aquatic life criteria for CECs. The Committee recommends that,  
42 pending revision of the 1985 Guidelines, EPA develop a separate process  
43 document that discusses the intended application of aquatic life criteria for CECs.  
44 This process document should establish linkages between the Guidelines, EPA's

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- 1 Ecological Risk Assessment Principles (U.S. EPA, 1993, 1998), and the White  
2 Paper.  
3
- 4 • EPA should prioritize the list of CECs for which aquatic life criteria will be  
5 developed. Data needs for these chemicals should be identified, and EPA should  
6 fund the research and data collection activities necessary to support aquatic life  
7 criteria development for CECs. In this regard, the Committee recommends that  
8 EPA's Office of Water and Office of Research and Development look for  
9 opportunities to leverage EPA research with ongoing research in other federal  
10 agencies, international agencies, and industry groups.  
11
  - 12 • EPA should incorporate use of conceptual models and ecosystem-based criteria  
13 into the process of deriving aquatic life criteria for CECs. The Committee notes  
14 that EPA programs are moving toward developing more comprehensive  
15 ecosystem-relevant criteria that take into consideration population-community  
16 structure, ecosystem functions and processes, and ecosystem services.  
17 Accordingly, the Committee notes that it is important to develop the link between  
18 the protected resource, the assessment endpoint, and the measurement endpoint.  
19
  - 20 • For bioaccumulative CECs where food chain transfer is a concern, EPA should  
21 consider developing tissue-based criteria (i.e., expressing the criterion as a  
22 concentration of the pollutant in fish tissue rather than a concentration in the  
23 water).  
24
  - 25 • EPA should also consider expanding the definition of contaminants of emerging  
26 concern to include chemicals and other substances of increasing environmental  
27 concern due to anthropogenic activities and inadequate regulatory approaches.  
28 The White Paper focuses on endocrine disrupting chemicals. However, the  
29 Committee notes that some CECs do not fit the effect model of endocrine  
30 disrupting chemicals, or are not well enough understood at this time to allow a  
31 judgment of their mode of action. Nanoparticles are an example of such a class of  
32 compounds. Additional work is needed to further develop recommendations for  
33 deriving aquatic life water quality criteria for these other kinds of chemicals.  
34
  - 35 • In Section 4.4 of this advisory report the Committee recommends additional  
36 research to address important issues such as: the effects of mixtures of CECs,  
37 interactions between CEC and other stressors, modes of action of CECs,  
38 comparative sensitivities of resident and non-resident species, and use of field  
39 study results to inform the derivation of aquatic life criteria. The Committee also  
40 recommends that the discussion of taxonomic coverage in the White Paper be  
41 expanded to include specific recommendations concerning derivation of criteria to  
42 protect marine organisms. EPA's 1985 Guidelines call for assessment of marine  
43 organisms in the same manner as freshwater organisms. However, due to specific  
44 issues unique to marine organisms, such as physiological requirements (e.g.,  
45 maintenance of salt balance) and life-history strategies (e.g., reproduction tied to

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1 tidal cycles), more specific guidance for CECs is likely needed. We suggest that  
2 such guidance may be best addressed by convening a “Pellston” type workshop  
3 (Society of Environmental Toxicology and Chemistry, 2008) that is comprised of  
4 experts from multiple disciplines and types of organizations.

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**2. INTRODUCTION**

EPA’s Office of Water (OW) requested that the Science Advisory Board (SAB) provide advice on the Agency’s proposed recommendations pertaining to derivation of water quality criteria for the protection of aquatic life (aquatic life criteria) for contaminants of emerging concern (CECs) such as pharmaceuticals and personal care products that are commonly discharged in municipal wastewaters. EPA’s proposed recommendations are provided in a white paper titled *Aquatic Life Criteria for Contaminants of Emerging Concern* (White Paper). The White Paper, prepared by the EPA Office of Water and Office of Research and Development Emerging Contaminants Workgroup, was reviewed by the SAB Ecological Processes and Effects Committee (Committee). To augment the expertise on the Committee for this advisory activity, several environmental toxicologists with specific knowledge of the effects of endocrine disrupting chemicals also participated in the review.

EPA’s Office of Water is charged with protecting aquatic life, wildlife, and human health from the adverse water-mediated effects of anthropogenic pollutants. In support of this mission, OW develops ambient water quality criteria that serve as guidance to states and tribes for adoption of water quality standards. The EPA guidance document, *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (Guidelines) (Stephan et al., 1985), sets forth a methodology for deriving ambient water quality criteria for the protection of aquatic life. The Guidelines specify various data and procedural recommendations for criteria derivation and also define general risk management goals for the criteria. Most of EPA’s aquatic life criteria have been derived using methods in the Guidelines. EPA has informed the Committee that the Agency intends to continue using the Guidelines to derive aquatic life criteria. However, EPA has also stated that it faces a number of technical challenges in deriving aquatic life criteria for CECs. The white paper describes these technical challenges and proposes recommendations to interpret and/or adapt Guidelines principles to address the challenges.

The term CEC has been used by EPA to identify a variety of chemical compounds that have no regulatory standard, have been recently discovered in the natural environment because of improved analytical chemistry detection levels, and potentially cause deleterious effects to aquatic life at environmentally relevant concentrations. The Agency has indicated that it is particularly concerned about pharmacologically active chemical compounds and personal care products that are commonly discharged at wastewater treatment plants and may stimulate physiological responses in humans, plants, and animals. Many of these compounds are known to disrupt endocrine function in animals, and are thus referred to as endocrine disrupting chemicals. These chemicals may demonstrate low acute toxicity but cause significant reproductive effects at very low levels of exposure. In addition, the effects of exposure of aquatic organisms to CECs during the early stages of life may not be

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1 observed until adulthood. These chemicals may also have very specific modes of  
2 action that affect only certain types of aquatic animals (e.g., vertebrates such as fish).  
3 Therefore, EPA has suggested that traditional chronic toxicity test endpoints specified  
4 in the Guidelines may not be sufficiently comprehensive, and Guidelines  
5 requirements for taxonomic coverage in toxicity testing may not be appropriate to  
6 derive aquatic life criteria for these chemicals. The White Paper focuses on  
7 recommendations to derive aquatic life criteria for endocrine disrupting chemicals.

8 The first part of EPA's White Paper (Part I), *General Challenges and*  
9 *Recommendations*, describes: 1) the technical challenges facing EPA in deriving  
10 aquatic life criteria for CECs; and 2) the recommendations to address those  
11 challenges. The second part of the White Paper (Part II), *Illustration of*  
12 *Recommendations Using Data for 17α – Ethynylestradiol (EE2)*, explores EPA's  
13 recommendations in the context of an example CEC, ethynylestradiol (EE2), which is  
14 a synthetic pharmaceutical estrogen. In its charge to the SAB, OW requested  
15 comments on the technical merit, practicality, and implementability of  
16 recommendations in the White Paper to address: a) relevance of acute toxicity effect  
17 concentrations in setting aquatic life criteria for CECs; b) defining minimum data  
18 requirements regarding taxonomic coverage in toxicity tests; c) use of non-resident  
19 species in criteria development; d) defining appropriate chronic toxicity data; e)  
20 selection of effect endpoints upon which to base criteria; and f) involvement of an  
21 expert panel in the criteria development process. In addition, OW asked the SAB for:  
22 comments on whether the Agency has identified the appropriate issues to be  
23 addressed in deriving aquatic life criteria for CECs; suggestions to improve the utility  
24 of Part II of the White Paper; and suggestions to assist the Agency in implementing  
25 proposed recommendations in the White Paper.

26 The Committee generally supports EPA's proposed approaches for interpreting  
27 and/or adapting Guidelines principles to address the technical challenges discussed in  
28 the White Paper. However in this advisory report we have recommended  
29 improvements to the approaches proposed in the White Paper. In addition, we have  
30 noted a number of specific technical and practical issues and caveats that should be  
31 considered by EPA when implementing the proposed approaches.

32 The Committee finds that, in the White Paper, EPA has identified appropriate  
33 technical issues and challenges to developing aquatic life criteria for CECs.  
34 However, we recommend that the Agency address additional issues to customize and  
35 update the Guidelines and thereby increase the flexibility and specificity of the  
36 aquatic life criteria derivation process. We find that EPA could clarify the process of  
37 developing aquatic life criteria for CECs by articulating a clear set of principles that  
38 could be applied when modifying the Guidelines. We also emphasize the importance  
39 of developing a conceptual model to guide the process of developing aquatic life  
40 criteria for CECs. The Committee finds that Part II of the White Paper is a well  
41 written and thorough review of the existing literature on EE2 that illustrates the  
42 complexities inherent in generating aquatic life criteria for CECs. However, we have  
43 provided recommendations to improve the usefulness of this case example. In

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1 particular we suggest that EPA more explicitly describe how the illustration in Part II  
2 was developed from the recommendations in Part I of the White Paper.

3 The Committee has also provided other suggestions to assist EPA in implementing  
4 the proposed recommendations in the White Paper. These suggestions focus on:  
5 improved data collection and research activities; development of tissue residue-based  
6 criteria (i.e., expressing the criterion as a concentration of the pollutant in fish tissue  
7 rather than a concentration in the water) for bioaccumulative CECs where food chain  
8 transfer is a concern; use of indicators for future development of criteria; special  
9 considerations for endangered or commercially/recreationally important species;  
10 obtaining input from private industry and state governments; and consideration of a  
11 mixture strategy for CECs.

### 12 13 **3. CHARGE TO THE COMMITTEE**

14  
15 EPA's Offices of Water (OW) and Research and Development (ORD) sought  
16 advice from the Science Advisory Board on the scientific and technical merit of a  
17 draft white paper on aquatic life water quality criteria (ALC) for contaminants of  
18 emerging concern (CEC). The white paper developed by the EPA Emerging  
19 Contaminants Workgroup describes how the Agency intends to address the  
20 challenges it faces in developing ALC for CECs. The specific charge questions  
21 below were provided to the Committee:

- 22  
23 1. The following recommendations have been developed to address important  
24 technical challenges and issues in deriving water quality criteria for CECs. Please  
25 comment on the technical merit, practicality, and implementability of the  
26 recommendations addressing the following issues as described in Part I of the  
27 white paper and the ethynylestradiol (EE2) case study in Part II.

28  
29 *a. Relevance of Acute Toxicity Effect Concentrations in Setting ALC for CECs:*

30  
31 Criteria consist of a Criterion Maximum Concentration (CMC), intended to  
32 address acute lethality and a Criterion Continuous Concentration (CCC), intended  
33 to address effects of chronic exposures on survival, growth, and reproduction.  
34 Many CECs are physiologically active at concentrations orders of magnitude  
35 lower than those causing acute lethality, and the high concentrations sufficient to  
36 cause lethality may never occur in the environment. Rather than rotely requiring  
37 a robust acute toxicity data set for such chemicals, the workgroup recommends  
38 that aquatic life criteria consist of only a CCC and that no CMC be derived, when  
39 sufficient information demonstrates risks of acute lethality are negligible.

40  
41 *b. Defining Minimum Data Requirements Regarding Taxonomic Coverage:*

42  
43 If an acute criterion is not calculated, then the CCC cannot be calculated using the  
44 acute to chronic ratio (ACR) approach and must be instead calculated directly  
45 from chronic toxicity data. Procedures for this are included in the Guidelines

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1 (pages 40-42), but they require that acceptable chronic toxicity tests be conducted  
2 for a broad range of taxonomic groups. In the case of many CECs, toxicological  
3 research tends to focus on organisms for which the mode of action is most  
4 relevant (e.g., vertebrates for estrogen mimics) and may have limited data  
5 coverage for other taxonomic groups that will likely be less sensitive. To avoid  
6 generation of resource-intensive chronic toxicity data for insensitive species that  
7 will have little impact on the final criterion, the workgroup recommends  
8 interpreting the minimum data requirements for taxonomic coverage as  
9 information requirements instead of toxicity test requirements. By this we mean  
10 that, rather than requiring a specific chronic toxicity test, the data requirement for  
11 certain taxonomic group expected to be insensitive might be met by a body of  
12 information demonstrating insensitivity of the taxon to the CEC.

13  
14 *c. Use of Non-Resident Species in Criteria Development:*

15  
16 Historically, EPA has not used data derived from toxicity testing with non-  
17 resident species in the actual criteria derivation process. Excluding species  
18 simply because they are not resident may be unnecessarily restrictive for the  
19 purposes of deriving national criteria, and may actually increase rather than  
20 decrease uncertainty. The workgroup recommends that non-resident species be  
21 considered for use in criteria derivation calculations, focusing on those species  
22 with widely used and standardized test methods and for which there is reason to  
23 believe that they would represent the sensitivity of comparable resident species.  
24 Furthermore, the workgroup specifically suggest accepting data for zebrafish  
25 (*Danio rerio*) and Japanese medaka (*Oryzias latipes*), to reflect international  
26 efforts toward data equivalency.

27  
28 *d. Defining Appropriate Chronic Toxicity Data:*

29  
30 For fish, the Guidelines allow the use of early life stage (ELS; egg to juvenile)  
31 exposures in lieu of full life-cycle (F<sub>0</sub> egg to F<sub>1</sub> offspring) or partial life-cycle (F<sub>0</sub>  
32 adult to F<sub>1</sub> juvenile) exposures for determining chronic toxicity of chemicals,  
33 unless there is reason to believe this is inappropriate. Current understanding of  
34 many CECs, particularly endocrine disrupting compounds (EDCs), is that  
35 important effects of these chemicals may not occur, or at least not be expressed,  
36 until after the ELS exposure window; in fact, partial life-cycle exposures may also  
37 miss important effects, such as those on sexual development. For such chemicals,  
38 it is clear that the definition of an acceptable chronic test must include  
39 consideration of key windows of exposure and effect (e.g., to include sexual  
40 development and reproduction in assessments of steroid hormone  
41 agonists/antagonists). However, even more broadly, the workgroup recommends  
42 that the Office of Water consider amending the chronic data acceptability  
43 requirements in the Guidelines to require at least one full life-cycle test for a fish  
44 (for invertebrates, life-cycle tests are already required) unless there is a  
45 compelling body of information indicating that life processes outside the early life

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1 stage or partial life-cycle exposure/observation window are not critical to  
2 capturing the biologically important effects of chronic exposure to the chemical.  
3 This amended requirement would include all chemicals, not just EDCs/CECs.  
4

5 *e. Selection of Effect Endpoints upon Which to Base Criteria*  
6

7 Aquatic life criteria typically are based on direct measures of survival, growth,  
8 and reproduction; other measures of response are generally not included unless  
9 they can be shown to be closely linked to expected changes in population  
10 dynamics. The workgroup supports this existing guidance, but recognizes that  
11 many CECs, particularly those with very specific modes of action like steroid  
12 hormone agonists/antagonists, will have data for a wide variety of histological,  
13 biochemical, physiological, or behavioral endpoints that may warrant  
14 consideration as measures of biologically important effects. The degree to which  
15 such measures can be used to infer population level effects is likely endpoint-,  
16 chemical-, and/or organism-specific, and developing a universal list of  
17 recommended endpoints is therefore beyond the scope of the workgroup's  
18 activities. Rather, the recommendation here is simply that criteria development  
19 more thoroughly explores such possibilities.  
20

21 *f. Involvement of an Expert Panel:*  
22

23 While not addressed explicitly in the Guidelines, the complexities involved in the  
24 assessment of many CECs, and the reliance on professional judgment in making  
25 some of the determinations required under the workgroup's recommendations,  
26 make clear the need to bring the best scientific knowledge to bear in the  
27 development of criteria for CECs, as well as other chemicals. The workgroup  
28 supports the recommendation from a Society of Environmental Toxicology and  
29 Chemistry (SETAC) Pellston workshop held in 2003 (Mount et al., 2003)  
30 indicating that criteria development should involve recruitment of an expert panel  
31 early in the process to insure that all relevant issues are considered during initial  
32 development of the criterion and to provide scientific perspective on decisions  
33 that are made as part of the process. Such a panel would not undermine the  
34 authority of the Agency to make policy decisions regarding criteria, but would  
35 ensure that such policy decisions are made from the best possible technical  
36 foundation. It is envisioned that expert panels would be formed around specific  
37 chemicals, or perhaps groups of chemicals with chemical or toxicological  
38 similarities (e.g., same mode of action).  
39

- 40 2. Please comment on whether EPA has identified the appropriate issues to be  
41 addressed in deriving ALC for CECs. Are there additional important issues that  
42 EPA has not identified?  
43
- 44 3. Part II of this white paper was specifically developed as a companion to Part I and  
45 focuses on the use of ethynylestradiol as a model chemical to illustrate the

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1 technical issues presented by the workgroup, as well as providing a basis for  
2 understanding the recommendations. Does the Committee have suggestions that  
3 may improve the utility of Part II of this white paper for the purposes stated  
4 above?

5

6 4. Does the Committee have suggestions that would assist EPA in implementing the  
7 proposed recommendations discussed in the white paper, particularly with respect  
8 to developing the necessary scientific data and information and/or providing  
9 expert scientific input at the appropriate stages of the risk assessment process?

10

#### 11 **4. RESPONSE TO CHARGE QUESTIONS**

12

13 In the responses to each of the charge questions, the Committee has listed the key  
14 findings and comments as bullets. These comments are followed by numbered lists  
15 of the key recommendations.

16

17 **4.1 Charge Question 1. Please comment on the technical merit, practicality,  
18 and implementability of recommendations addressing the following issues  
19 as described in Parts I and II of EPA's white paper on Aquatic Life  
20 Criteria for Contaminants of Emerging Concern: a) relevance of acute  
21 toxicity effect concentrations in setting aquatic life criteria for  
22 contaminants of emerging concern; b) defining minimum data  
23 requirements regarding taxonomic coverage; c) use of non-resident  
24 species in criteria development; d) defining appropriate chronic toxicity  
25 data; e) selection of effect endpoints upon which to base criteria; and f)  
26 involvement of an expert panel.**

27

##### 28 **4.1.1 Relevance of Acute Toxicity Effect Concentrations**

29

30 As discussed in EPA's White Paper, aquatic life water quality criteria consist of a  
31 Criterion Maximum Concentration (CMC) intended to protect against severe acute  
32 effects of exposure to contaminants, and a Criterion Continuous Concentration (CCC)  
33 intended to protect against the longer term effects of exposure on survival, growth,  
34 and reproduction. EPA's Guidelines (Stephan et al., 1985) specify various data and  
35 procedural recommendations for criteria derivation. The CMC is determined based  
36 on available acute values (AVs). Acute values are median lethal concentrations or  
37 median effect concentrations from aquatic animal acute toxicity tests (48 to 96 hours  
38 long) meeting certain data quality requirements. The CCC is generally determined  
39 based on available chronic values (CVs), which are either: a) the geometric mean of  
40 the highest no-observed-effect concentration (NOEC) and the lowest observed effect  
41 concentration (LOEC) for effects on survival, growth, or reproduction in aquatic  
42 animal chronic tests; or b) in some recent criteria the EC<sub>20</sub> (the test concentration that  
43 would cause a reduction in survival, growth, or reproduction in 20% of the test  
44 population) based on concentration-effect regression analyses of the toxicity test data.  
45 If chronic toxicity test data are not available for at least eight genera of aquatic

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1 organisms with a specified taxonomic diversity, the CCC is derived on the basis of an  
2 acute to chronic ratio (ACR) (i.e., the ratio of the AV to CV from parallel acute and  
3 chronic tests for at least three species with a specified taxonomic diversity). EPA's  
4 White Paper states that many CECs are physiologically active at concentrations  
5 orders of magnitude lower than those causing acute lethality, and that concentrations  
6 high enough to cause acute lethality may never occur in the environment. Therefore,  
7 in the White Paper the Agency recommends that, when sufficient information  
8 demonstrates a negligible risk of acute lethality for a CEC, the ALC for that  
9 contaminant could consist of only a CCC.

10  
11 In principle, the Committee supports EPA's recommendation to derive aquatic life  
12 criteria directly from CCCs thus forgoing CMCs and ACRs. The Committee  
13 recognizes that, for many CECs, acute toxicity may only occur at concentrations  
14 several orders of magnitude greater than those likely to occur in the aquatic  
15 environment. The Committee also recognizes that the suggestion to forgo derivation  
16 of CMCs is not designed to truncate the aquatic life criteria development process, but  
17 rather is designed to allocate resources to areas most likely to affect the final aquatic  
18 life criteria and to avoid delaying implementation of aquatic life criteria due to a lack  
19 of data for species that are not likely to be sensitive.

20  
21 *Caveats concerning use of the Criterion Continuous Concentration for aquatic life*  
22 *water quality criteria*

23  
24 Although the Committee generally supports EPA's recommendation to derive  
25 aquatic life criteria for CECs directly from CCCs, we note that the following points  
26 should be considered by the Agency when implementing this recommendation:

- 27
- 28 • Some CECs do not fit the effect model of endocrine disrupting chemicals.  
29 Foremost on the Committee's list of concerns is that some CECs do not fit the  
30 effect model of endocrine disrupting chemicals (EDCs), or are not well enough  
31 understood at this time to allow a judgment of their mode of action.  
32 Nanoparticles are an example of such a class of compounds. Additional work is  
33 needed to further develop recommendations for deriving aquatic life water quality  
34 criteria for these other kinds of chemicals. EPA's White Paper focuses in  
35 particular on CECs that disrupt endocrine function in animals. Thus, many of the  
36 Committee's comments address deriving ALCs for CECs with modes of action  
37 similar to those of EDCs.  
38
  - 39 • For some CECs, acute toxicity may occur in environmental settings. The  
40 Committee notes that for some CECs, the LOECs and LC50s (test concentrations  
41 that result in mortality to 50% of the test population) are within one order of  
42 magnitude of each other, indicating that acute toxicity may occur in  
43 environmental settings. For these chemicals derivation of a CMC may be  
44 appropriate. Examples of such chemicals include fluoxetine (a selective serotonin  
45 reuptake inhibitor or SSRI) and gemfibrozil (a blood cholesterol regulator).

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- 2 • Some compounds have differing modes of action for acute and chronic toxicity.

3

4

5

6

7

- 8 • Pulsed discharge may result in high ambient concentrations of CECs. The  
9 Committee is concerned that the pulsed nature of some CEC releases (for  
10 example: pulsed industrial discharge; tidal action in the marine environment; and  
11 recurring natural events such as hurricanes that can cause flooding and release of  
12 untreated sewage) may result in short-term concentrations of CECs that could  
13 exceed what would generally be considered environmentally relevant  
14 concentrations. Although CCCs may be applicable in these situations, the  
15 Committee finds that acute toxicity should be considered to account for the effects  
16 of compounds where extreme pulses may occur more frequently than the three-  
17 year benchmark set by the Guidelines.

18

- 19 • Consideration of mixture effects is important. An additional concern of the  
20 Committee is the need for the consideration of mixture effects in determining  
21 whether acute toxicity could occur in natural settings. The White Paper explicitly  
22 references common modes of action for multiple compounds (as in the examples  
23 of EE2, estrone, and estradiol). The Committee feels strongly that mixture effects  
24 of compounds with similar modes of action should be taken into account in  
25 determining whether acute toxicity may occur in environmental situations. Thus a  
26 mixtures strategy is needed to guide development and interpretation of aquatic life  
27 criteria for CECs.

28

29 *Committee recommendations concerning the relevance of acute toxicity effect*  
30 *concentrations*

31

32 As a consequence of the Committee's discussion and concerns listed above, we  
33 provide the following recommendations to amend the White Paper text concerning  
34 derivation of aquatic life criteria on the basis of the Criterion Continuous  
35 Concentration:

36

- 37 1. Part 1 of EPA's White Paper contains a bulleted list (on page 28) identifying the  
38 kinds of information that should be reviewed in order to determine whether the  
39 differences between the CMCs and CCCs would be great enough to conclude that  
40 the CMC is not needed to develop ALC. The Committee finds that this list is very  
41 helpful. It addresses some of the concerns raised during the Committee's  
42 deliberation and it may be particularly useful in providing lines of evidence to  
43 determine whether acute toxicity data are needed. Therefore, we encourage  
44 expansion of this list in the final White Paper to include additional information  
45 addressing the points mentioned above.

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1

2 2. The Committee suggests that all available data on any new class of CECs should be  
3 used in determining whether acute toxicity is likely to occur in environmentally  
4 relevant settings. These data should be summarized to document when additional  
5 data are needed, or when it is justifiable to move aquatic life criteria development  
6 forward without the derivation of CMCs.

7

8 3. The Committee recommends that CMCs be derived for compounds where LOECs  
9 are found to be within one order of magnitude of LC50s.

10

11 4. The Committee recommends that the likelihood of pulses of exposure to  
12 contaminants be considered in determining the range of environmentally relevant  
13 concentrations for criteria development.

14

15 5. The Committee suggests that EPA consider the mixture effects of compounds with  
16 similar modes of action when determining the range of environmentally relevant  
17 concentrations for criteria development.

18

19 The Committee finds that, together with those in the White Paper, these  
20 considerations should allow a robust determination of whether CMCs are necessary  
21 for derivation of ALC for CECs.

22

#### 23 **4.1.2 Defining Minimum Data Requirements Regarding Taxonomic Coverage**

24

25 EPA's draft White Paper states that a consequence of dropping acute toxicity  
26 testing requirements for deriving aquatic life criteria for CECs is the inability to  
27 calculate a CCC using the ACR approach. The Committee notes that CCCs could,  
28 however, be developed directly from sufficiently robust sets of chronic data using  
29 procedures in the Agency's Guidelines (Stephan et al., 1985, pages 40-42). These  
30 procedures require that acceptable chronic toxicity tests be conducted for a broad  
31 range of taxonomic groups. EPA has suggested that, if insufficient data from actual  
32 toxicity tests are available to fulfill the minimum data requirements for CECs, a  
33 reasonable understanding of the toxicological mode of action for a chemical may  
34 allow inferences as to what taxa (and endpoints) are most likely to be insensitive, and  
35 measured chronic values for those taxa might not be needed. Thus, in the White  
36 Paper, EPA has recommended that, for CECs without complete chronic toxicity data  
37 sets to fulfill minimum data requirements, there be an evaluation of whether sufficient  
38 information exists to conclude that certain taxa would not be sensitive to the  
39 chemical. To accomplish this, EPA recommends interpreting the minimum data  
40 requirements for taxonomic coverage as "information requirements" instead of  
41 "toxicity test requirements." EPA notes that this would avoid generation of resource-  
42 intensive chronic toxicity data for insensitive species that would have little impact on  
43 the final criterion. The Committee agrees with EPA's recommendation. However, as  
44 further discussed below, the Agency needs to define: 1) what constitutes a sufficiently  
45 robust set of chronic data for criteria derivation, and 2) what constitutes a reasonable

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1 understanding of the mode of action for the chemical that may allow inferences  
2 concerning the insensitivity of particular taxa. In addition, the Committee has noted a  
3 number of concerns that should be addressed by EPA as it implements the proposed  
4 approach.

5  
6 The Committee finds that the White Paper contains a comprehensive discussion of  
7 the issue of taxonomic coverage for developing aquatic life criteria. EPA's 1985  
8 Guidelines require that data be available for the following organisms: a salmonid in  
9 the class Osteichthyes, a second family in the class Osteichthyes, a third family in the  
10 phylum Chordata, a planktonic crustacean, a benthic crustacean, an insect, a family in  
11 a phylum other than Arthropoda or Chordata, and a family in any order of insect or  
12 other phylum not already represented. This requirement is the same for freshwater as  
13 well as marine organisms. In the White Paper, EPA notes these taxonomic coverage  
14 requirements but recommends movement to a more "expert judgment" approach that  
15 is logical and should address some of the unique properties of CECs. The Committee  
16 understands and appreciates the desirability of avoiding the extra work required to  
17 develop chronic data for species that are unlikely to be sensitive to certain CECs. On  
18 the other hand, we emphasize that it is equally important to perform adequate testing  
19 to ensure protection of aquatic life. Therefore it is important to define what  
20 constitutes a sufficiently robust set of chronic data for criteria derivation and also to  
21 provide additional guidance concerning the data needed to infer that various taxa are  
22 insensitive to chemicals with specific modes of action.

#### 23 *Concerns regarding taxonomic coverage for testing CECs*

24  
25  
26 The Committee emphasizes that there are instances in which CECs have been  
27 shown to have unanticipated effects on non-target organisms. Examples include the  
28 impact of antibiotics on plants (Brain et al., 2008) and atrazine effects on the quality  
29 of algae (Pennington and Scott, 2001). These types of examples should be used in  
30 Part I of the White Paper to help describe how the aquatic life criteria development  
31 process might need to be more flexible depending on the compounds under  
32 evaluation. In addition, we note the following important points to be considered  
33 concerning appropriate taxonomic coverage for deriving aquatic life criteria for  
34 CECs:

- 35
- 36 • There is a need to maintain broad taxonomic coverage for development of aquatic  
37 life criteria. The White Paper suggests that knowing certain modes of action  
38 could potentially focus testing on a particular type of organisms (e.g., vertebrates  
39 for "estrogenic" compounds). This suggestion is not wholly supported by the  
40 Committee. As stated in the 1985 Guidelines, the procedure for estimating the 5<sup>th</sup>  
41 percentile final chronic value is to use the four lowest values in the data set. This  
42 approach considers primarily vertebrates, and it is appropriate for EE2. However,  
43 it is not always appropriate (e.g., in the case of the weak estrogenic compound  
44 bisphenol A) to give primary consideration to vertebrates. Staples et al. (2008)  
45 compared four species sensitivity distribution methods to develop a predicted no-

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1 effect concentration for the aquatic environment for bisphenol A. Their study  
2 indicated that when using the Guidelines approach, the four lowest predicted  
3 values belonged to three invertebrates and one vertebrate. Clearly, this finding  
4 suggests that there is a need to maintain a broad taxonomic coverage in the  
5 development of aquatic life criteria.  
6

- 7 • Little is known of chronic effects of CECs on “wild type” species. The  
8 Committee is concerned that much of the toxicity testing for CECs has been done  
9 on animals that are highly amenable to laboratory conditions and little is known  
10 of chronic effects of chemicals on "wild types." There is also some probability  
11 that criteria protecting "lab species" might not protect species of special concern  
12 like the endangered short-nosed sturgeon, several species of Pacific salmon, or the  
13 bull trout. Research is needed to evaluate the differences and similarities between  
14 life-histories and sensitivities of endangered/threatened and standard laboratory  
15 animals used for toxicity testing in order to have more confidence that surrogate  
16 species will provide sufficient information to develop protective criteria.  
17
- 18 • Modes of action are not known for some CECs. The Committee notes that it is  
19 not safe to assume that a known mode of action is the only mode of action for a  
20 CEC. Different organisms may be affected in different ways by the same  
21 compound both as adults and at earlier stages of development. There is also the  
22 potential for synergism among CECs in mixtures and in interactions with  
23 environmental variables. It is the exception rather than the rule that modes of  
24 action are known for CECs.  
25

26 *Committee recommendations to improve the process of determining appropriate*  
27 *taxonomic coverage*  
28

29 Although the example used in Part II of EPA’s White Paper to illustrate derivation  
30 of aquatic life criterion for an endocrine disrupting chemical is data rich, the  
31 Committee notes that the same cannot be said for all or even most CECs. As EPA  
32 correctly states in the White Paper, in many cases non-traditional endpoints (i.e.,  
33 endpoints not traditionally measured in toxicity testing) will almost certainly need to  
34 be considered for CECs. However, the use of non-traditional endpoints requires an  
35 understanding of their relevance to the health of the organism, and ultimately the  
36 population, and also an understanding of the variability inherent in the measure. The  
37 key to determining appropriate taxonomic coverage and endpoints is ecological  
38 relevance. These considerations call for keeping the taxonomic coverage as broad as  
39 possible, considering the trophic position of the test organisms, and establishing a  
40 clear process or set of guidelines to determine the "insensitivity" of taxa. The  
41 Committee provides the following recommendations to improve the process of  
42 determining appropriate taxonomic coverage for criteria development:  
43

- 44 1. EPA needs to define what constitutes a sufficiently robust set of chronic data.  
45 Although the example used in the White Paper generally illustrates EPA’s

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- 1 proposed process for making decisions concerning taxonomic coverage, it would  
2 be helpful to be more explicit in identifying what constitutes a "sufficiently robust  
3 set of chronic data" and "a reasonable understanding of the mode of action for the  
4 chemical that may allow inferences." The language in the White Paper introduces  
5 uncertainty in both the general approach and in setting up specific test conditions.  
6
- 7 2. EPA should consider emphasizing in the White Paper information necessary for  
8 development of aquatic life criteria rather than just toxicity test requirements. To  
9 that end, guidance on information needed to determine effects on ecological  
10 processes (e.g., food webs, nutrient cycling, and primary production) rather than  
11 only target species would be valuable in criteria development, and would follow  
12 more recent scientific thinking. In addition, there is a need for consideration of  
13 appropriate conceptual models that include fate pathways and exposure to the  
14 CECs. An understanding of exposure pathways could help direct testing toward  
15 more relevant species.  
16
- 17 3. An approach that might be considered by EPA would be to shift from a minimum  
18 level of required taxonomic coverage toward determining receptors of potential  
19 concern (ROPCs). EPA acknowledges in the White Paper example illustrating  
20 development of an aquatic life criterion for EE2 that certain types of organisms  
21 might be differentially sensitive or impacted by a compound. The Committee  
22 notes that, if sufficient information exists on sensitivity, then expert judgment  
23 concerning data development should prevail. This would result in a more focused  
24 approach to data development keeping in mind a weight of evidence rather than a  
25 broad requirement for testing all eight taxa specified in the Guidelines. This  
26 would be a more flexible risk-based rather than set approach and would be  
27 consistent with the risk-assessment terminology used throughout Part I of the  
28 White Paper.  
29
- 30 4. Examples showing the unanticipated effects of CECs on non-target organisms  
31 (e.g., the impact of antibiotics on plants and atrazine effects on the quality of  
32 algae) should be used in Part I of the White Paper to help describe how the  
33 aquatic life criteria development process might need to be more flexible  
34 depending on the compounds under evaluation.  
35
- 36 5. The Committee recommends that the discussion of taxonomic coverage in the  
37 White Paper be expanded to include specific recommendations concerning the  
38 marine environment. EPA's 1985 Guidelines call for assessment of marine  
39 organisms in the same manner as freshwater organisms. However, a discussion of  
40 testing marine organisms was omitted from EPA's White Paper. We note that  
41 including consideration of testing marine organisms would be consistent with the  
42 approach taken by the European Union as it developed its Water Framework  
43 Directive (European Commission, 2008). Due to specific issues unique to marine  
44 organisms, such as physiological requirements (e.g., maintenance of salt balance)  
45 and life-history strategies (e.g., reproduction tied to tidal cycles), more specific

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1 guidance for CECs is likely needed. The Committee suggests that this guidance  
2 may be best addressed by convening a “Pellston” type workshop (Society of  
3 Environmental Toxicology and Chemistry, 2008) that is comprised of experts  
4 from multiple disciplines and types of organizations. Since testing requirements  
5 for marine organisms are already being considered by EPA, this should be stated  
6 in the White Paper.

7

#### 8 **4.1.3 Use of Non-resident Species in Criteria Development**

9 EPA’s Guidelines limit the data used for aquatic life criteria development to tests  
10 with native species, while allowing use of non-resident species data to provide  
11 additional, narrative evidence for criteria development. In its White Paper, EPA  
12 suggests that excluding species from testing simply because they are not resident may  
13 be unnecessarily restrictive for the purposes of deriving national criteria, and may  
14 actually increase rather than decrease uncertainty. The White Paper recommends that  
15 these “non-resident” species data be used in the aquatic life criteria derivation process  
16 if the non-resident species data would enable better estimation of species sensitivity  
17 distributions (SSDs). EPA recommends that criteria derivation calculations focus on  
18 test data from species for which widely used and standardized test methods are  
19 available, and for which there is reason to believe that data would represent the  
20 sensitivity of comparable resident species. EPA specifically recommends accepting  
21 data for zebrafish (*Danio rerio*) and Japanese medaka (*Oryzias latipes*), to reflect  
22 international efforts in harmonization of test methods. As further discussed below,  
23 the Committee agrees with this recommendation.

#### 24 *Benefit of using non-resident species data*

25 The Committee finds that the exclusion of non-resident species data from criteria  
26 derivation is biologically and practically inconsistent with the intent of the Guidelines  
27 (i.e., providing an objective, internally consistent, appropriate, and feasible way of  
28 deriving national criteria). Furthermore, we find that, as advocated by the White  
29 Paper authors, use of such data would greatly benefit the development of  
30 scientifically sound aquatic life criteria CECs. Although geographic differences in  
31 species tolerance to contaminants have been documented (Chapman et al. 2006), it is  
32 important to note that the U.S. covers a wide range of geographic areas (from tropical  
33 [Florida, Hawaii] to arctic [Alaska]). Previous criteria development has focused on  
34 temperate species. Thus, inclusion of non-resident species has the potential to cover  
35 not only data needs but also the geographic (e.g., temperature) range of biota in the  
36 U.S. and arguably could increase the protectiveness of the derived criteria.

37 The White Paper states that only “species with recognized international  
38 equivalency [will] be included in criteria derivation with the full weight given to data  
39 from resident species.” This approach supports international test harmonization  
40 efforts. Specifically, the White Paper recommends use of zebrafish and Japanese  
41 medaka. These two species have been largely used for EDC testing and have shown

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1 sensitivity similar to native fathead minnows and other species. Tests conducted with  
2 the zebrafish and Japanese medaka provide insight into the biochemical and  
3 physiological mechanisms involved in the toxicity of CECs. More important is  
4 matching the mode of action with the appropriate test species. The conservative  
5 nature of the endocrine system, a target for most endocrine disrupting chemicals and  
6 likely many CECs, renders the exclusion of non-resident species from aquatic life  
7 criteria development biologically indefensible. Certainly the use of any test species  
8 would be useful if it could aid in the interpretation of modes of action, relative taxa  
9 tolerance, and endpoint sensitivity comparisons. For example, studies with surrogate  
10 species have been conducted to demonstrate the toxicity of CECs to resident species,  
11 such as the Rio Grande silvery Minnow and the North American Sturgeon, that are  
12 too endangered for laboratory testing (Beyers, 1995; Dwyer et al., 2000). Additional  
13 studies of the sensitivity of marine and freshwater test species are cited in the  
14 recommendations below. In such cases test data from closely related non-resident  
15 species may provide laboratory evidence useful in the development of protective  
16 aquatic life criteria for the endangered resident species

17

#### 18 *Concerns regarding the use of non-resident species data*

19 Although the Committee supports the use of non-resident species data for deriving  
20 aquatic life criteria for CECs, we note the following concerns that should be  
21 considered by EPA:

22

23 • Non-resident species are defined in different ways. The Committee notes that  
24 EPA's Guidelines define "non-resident" species as those not native to the  
25 continental United States and Canada. However, non-resident species have been  
26 defined in other ways. At the federal level, they have been defined as species that  
27 are not native to North America. Many states use the term non-resident species to  
28 mean species not native to their specific region. Hence local criteria are  
29 sometimes derived substituting species found locally. The definition of "non-  
30 resident" (or non-native) and invasive species should be clearly stated in EPA's  
31 White Paper. The White Paper should indicate whether organisms that have  
32 migrated (or invaded or been stocked) are considered "resident."

33

34 • Non-resident species data may dominate the criteria derivation process. The  
35 Committee is concerned that non-resident species and their large respective  
36 databases could dominate the criteria derivation process. The recommendation to  
37 use non-resident species data, as presented in the White Paper, is reasonable when  
38 looking at criteria derivation from a continental perspective. However, including  
39 non-resident species data in the criteria derivation process could lead to  
40 inappropriately biased criteria development in certain sensitive geographic areas,  
41 such as cold water and oligotrophic systems. More detailed information is needed  
42 in the White Paper to address this concern.

43

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- 1 • Variation in test organism response is often unknown. The Committee notes that  
2 variation among the strains of test organisms used in laboratory studies is often  
3 unknown. Therefore, it is difficult to understand whether the variation observed  
4 between native and non-native species is within the uncertainty of the test data for  
5 either species. Differences in husbandry, health, parasite and pathogen load (i.e.,  
6 other stressors) may contribute to differences in test results between resident and  
7 non-resident species. Within Pacific herring of Puget Sound there are apparent  
8 stock differences in the frequency of malformations of new hatchlings that are not  
9 related to spawning site (Hershberger et al., 2005). Differences in sensitivity have  
10 also been observed for clones of *Daphnia magna* (Baird et al., 1990). While the  
11 issue of response variation should be considered, many studies have shown  
12 parallel responses when fairly close relatives are used.

13 *Committee recommendations regarding the use of non-resident species data*

14

15 Excluding the use of use non-resident species data from the process of developing  
16 aquatic life criteria for CECs may result in failure to meet the minimum data  
17 requirements. Therefore, the Committee finds that use of available data for non-  
18 resident species is warranted. Although the use of resident species information is  
19 preferable to non-resident species, data from tests using non-resident species, such as  
20 zebrafish and Japanese medaka, can provide extremely useful information on modes  
21 of action. The appropriate use of non-resident species data in criteria development  
22 will allow better estimation of species sensitivity distributions and also improve  
23 international harmonization and equivalency efforts. The Committee provides the  
24 following recommendations concerning the use of non-resident species data:

- 25 1. As noted above, non-resident species could potentially be over-represented in  
26 aquatic life criteria databases. The proportion of the data set that should include  
27 resident species is a matter that should be carefully evaluated by the expert  
28 advisory panel assembled to review each criterion.  
29
- 30 2. In no case should a criterion be developed on the basis of non-resident species  
31 data alone. Certainly if it is shown that non-resident species are ecologically  
32 relevant and appropriately sensitive then they should be used for criteria  
33 derivation as long as the studies meet appropriate quality criteria. Test species  
34 used in toxicity testing tend to be easy to rear and test, and have appropriate  
35 sensitivity levels. However, other factors should be considered when ample data  
36 are available for prioritizing species responses for criteria development. These  
37 factors include vulnerability, endangerment status, and recreational, commercial  
38 or ecological value. In order to protect endangered species, studies should be  
39 completed to compare toxicity test responses of common test species and  
40 endangered organisms and thereby determine the relevance of surrogates in the  
41 criteria development process. Previously EPA and the U.S. Fish and Wildlife  
42 Service (Besser et al., 2005; Dwyer et al., 1999, 2005; and Sappington et al.,  
43 2001) compared the sensitivity of common freshwater and marine testing species

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1 with protected/endangered fish species and found that these surrogate test species  
2 (e.g., rainbow trout) may equally protect endangered species. However, these  
3 surrogate fish species do not necessarily provide protection for other threatened  
4 and endangered non-fish species such as marine mammals, wildlife and birds that  
5 reside and feed in aquatic ecosystems and utilize ecosystem goods and services.  
6 Additional consideration of these other non-fish protected species is required in  
7 developing risk-based approaches for CECs that fully protect all threatened and  
8 endangered species.

- 9
- 10 3. The statement that criteria would be developed "...with full weight given to data  
11 from resident species" should include a qualifier concerning the validity of the  
12 data. An available resident species study with no obvious protocol, no  
13 measurement of test concentrations, or other protocol concerns should be assigned  
14 a lower priority than a fully valid Organization for Economic Cooperation and  
15 Development (OECD)/EPA guideline study with a "non-resident" species.  
16 However, the Committee qualifies this recommendation by emphasizing that all  
17 scientifically valid data should be used in setting criteria.  
18
- 19 4. Differences in strains, husbandry, health, and parasite and pathogen load  
20 contribute to response variation and should be considered in the aquatic life  
21 criteria development process.  
22
- 23 5. Non-resident as well as resident species test data must meet Guidelines  
24 requirements for data and method validity.  
25

#### 26 **4.1.4 Defining Appropriate Chronic Toxicity Data**

27

28 EPA's Guidelines state that acceptable chronic tests for derivation of aquatic life  
29 criteria are full life-cycle exposures ( $F_0$  egg to  $F_1$  offspring) for vertebrates and  
30 invertebrates, as well as partial life-cycle (adult to juvenile) and early life-stage (egg  
31 to juvenile) tests for fish. EPA's White Paper states that some CECs may have potent  
32 effects on life processes that lie outside the exposure period represented by early life  
33 stage tests or effects may not be manifested until later in development. Thus, early  
34 life stage tests might not be good predictors of chronic toxicity for these chemicals.  
35 In the White Paper, EPA recommends that the Guidelines requirements for chronic  
36 toxicity data be tightened by requiring at least one full life-cycle test for a fish (for  
37 invertebrates, life-cycle tests are already required) unless there is a compelling body  
38 of information indicating that life processes outside the early life stage or partial life-  
39 cycle exposure/observation window are not critical to capturing the biologically  
40 important effects of chronic exposure to the chemical.

41

42 The Committee strongly supports EPA's recommendation to amend the chronic  
43 data acceptability requirements in the Guidelines. However, we are divided in our  
44 assessment of the "guilty until proven innocent" approach in the White Paper (page  
45 17). Some Committee members view it as appropriate while others view it as

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1 extremely precautionary. The White Paper states that "...it is probably wiser to  
2 require that the chronic toxicity data for fish include exposure and observation over a  
3 full life-cycle unless there is an affirmative reason to believe that it is not necessary."  
4 The statement is used in the context of requiring a full life cycle study instead of  
5 relying on an early life stage test for fish. Some Committee members find that the  
6 statement does not appear to fit the process of setting aquatic life criteria, whereas  
7 others find it to provide an important perspective for establishing aquatic life criteria.  
8

9 The Committee also supports extending the recommendation to amend the chronic  
10 data acceptability requirement to all chemicals, not just endocrine disrupting  
11 chemicals and CECs. The Committee finds that EPA's recommendation is justified  
12 based on evidence showing that a number of chemicals may exert effects during the  
13 period of gonadal differentiation, and that these effects may not be manifested until  
14 much later in life. Including at least one full life cycle test in the test guidelines for  
15 fish ensures that these types of effects are captured.  
16

17 *Issues to be considered in defining appropriate chronic toxicity data*  
18

19 Although the Committee supports EPA's recommendations concerning use of  
20 chronic toxicity data for development of aquatic life criteria, we note the following  
21 issues that should be addressed in defining appropriate chronic toxicity test data:  
22

- 23 • Transgenerational effects of CECs are potentially important and should be  
24 considered. There is evidence for some chemicals that exposure in one generation  
25 creates effects in a later generation that were not observed in prior generations  
26 even in the same life stage. Accordingly, the chronic toxicity data requirements  
27 include a full life-cycle test to be conducted for at least one species of fish. There  
28 is still some uncertainty as to whether a full life-cycle test might underestimate  
29 the chronic effects that would be seen in exposures extending over more than two  
30 generations (multigenerational testing). We do not recommend adding a  
31 requirement for multigenerational testing to the Guidelines, but suggest that EPA  
32 critically review data dealing with transgenerational responses of aquatic species  
33 and evaluate whether this additional testing provides significant new information  
34 that informs the evaluation process. This critical review should examine the  
35 utility of multigenerational tests relative to proposed fish full life-cycle (FFLC)  
36 tests as well as partial life-cycle (PLC) tests and early life-stage studies. The  
37 intent of this recommendation is to ensure that a full range of development (e.g.,  
38 early life stage to adult) is evaluated sufficiently to assure adequate aquatic life  
39 protection. The Committee generally supports the concept of fish full life-cycle  
40 testing because it spans the entire exposure window in the early life-cycle to  
41 adults. The Committee also supports further development of a tiered testing  
42 approach to derive an appropriate rationale for the use of FFLC, PLC, and  
43 possibly multigenerational testing for chemicals with parallel modes of action.  
44

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- 1 • Flexibility in test guidelines is needed to include key developmental events. Test  
2 guidelines must have the flexibility to include assessment of key developmental  
3 events (e.g., metamorphosis in amphibians, acquisition of saltwater tolerance),  
4 particularly if these processes are identified in a ROPC.  
5
- 6 • Test methods should include non-traditional measures that may be linked to  
7 ecologically relevant endpoints. There is a need to ensure that the test methods  
8 include provisions to consider non-traditional endpoints such as immune function  
9 and organism behavior. These endpoints may directly impinge on ecologically-  
10 relevant endpoints such as growth, reproduction and survival. In this case,  
11 professional judgment from an expert panel is needed to determine the relevance  
12 of these non-traditional endpoints.  
13

14 The Committee also notes the following practical issues that should be addressed  
15 if the chronic toxicity data recommendation in the White Paper is to be implemented:  
16

- 17 • Surrogate test species may be needed. A key issue to be addressed is the  
18 suitability of surrogate test species. Surrogates may be needed in the case of: 1)  
19 long-lived species with delayed sexual maturity; 2) organisms of large size (which  
20 precludes their suitability as a test species in the laboratory), 3) endangered  
21 species, and 4) species for which there is little knowledge of the husbandry  
22 conditions or background biology. There is also uncertainty in how differences in  
23 the physiology and life history strategies (i.e., long-lived versus short-lived  
24 species, differences in maternal-fetal transport of contaminants) may affect the  
25 response of aquatic species to CECs and endocrine disrupters. Many of these  
26 issues represent significant data gaps that need to be addressed. In these cases,  
27 expert opinion may be needed to assist EPA in determining the suitability of  
28 surrogate test species for use in criteria development.  
29

30 *Committee recommendations regarding defining appropriate chronic toxicity data*  
31

32 As discussed above, the Committee strongly supports EPA's recommendation  
33 concerning the use of at least one full life cycle test for a fish in appropriate cases for  
34 testing all kinds of chemicals when deriving water quality criteria for the protection  
35 of aquatic life in marine and freshwater environments. We provide the following  
36 recommendations to implement the requirement for chronic toxicity data:  
37

- 38 1. As discussed above, EPA should critically review data dealing with  
39 transgenerational responses of aquatic species and evaluate whether or not this  
40 additional testing provides significant new information that informs the evaluation  
41 process.  
42
- 43 2. EPA should support research that addresses the suitability of the use of surrogate  
44 species in assessing the response of aquatic species (e.g., endangered or long lived  
45 species; species with varying life history strategies) to CECs.

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3. Test guidelines should include flexibility to include assessment of key developmental events, and professional judgment from an expert panel should be used to evaluate the relevance of non-traditional endpoints such as immune function and organism behavior.

#### 4.1.5 Selection of Effect Endpoints for Criteria Development

In the White Paper, EPA has stated that the selection of endpoints appropriate to the derivation of aquatic life criteria must be tied to the goal of aquatic life criteria (i.e., to protect aquatic organisms and their uses). EPA further states that survival, growth, and reproduction are processes directly related to this goal. The Agency notes, however, that there are many more biological responses that have been observed in response to toxicant exposure. In the White Paper EPA has identified a number of sublethal endpoints that could be considered in developing aquatic life criteria for CECs. The Agency has recommended that the use of such endpoints be more thoroughly explored for development of aquatic life criteria.

##### *Points to be considered in selecting effect endpoints*

Generally, the Committee agrees that EPA should continue to explore the possibility of using sublethal endpoints to help set aquatic life criteria. However, we caution EPA that non-traditional endpoints must ultimately be linked to the population, and not solely to individual-level endpoints. The ultimate goal of any aquatic life criterion is to protect populations of aquatic organisms from the “harmful” effects of chemicals (or other stressors). Thus, reproduction, growth and survival are the predominant effect endpoints currently utilized in laboratory studies supporting criteria development. The Committee discussed: 1) the usefulness of information provided by the non-traditional endpoints identified in the White Paper; and 2) whether the endpoints might provide information to assess effects on populations, particularly when considering mixtures and indirect effects. We provide the following comments to be considered by EPA in selecting effect endpoints to develop criteria for CECs:

- Contaminants effects should be linked to different levels of biological organization. Definitions of “biologically important effect” and what constitutes a “good population” are needed. We also note that not all biological responses represent an “adverse” effect, consistent with a principle laid out in the White Paper (i.e., the White Paper states that chemicals such as endocrine disrupters have been shown to produce a wide variety of measurable changes at many different levels of biological organization, and the challenge is to select from among those endpoints that have sufficiently clear connection to expected effects on populations or communities of aquatic organisms).

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- 1 • Activational biological effects can provide useful information. CECs often  
2 induce changes in behaviors, secondary sexual characteristics, or levels of  
3 hormones or hormone-induced products. Many of these responses are transitory  
4 or may revert to their prior or normal condition with cessation of exposure.  
5 Accordingly, it is often difficult to interpret these activational responses in  
6 relation to higher level biological effects. Nevertheless, these endpoints do  
7 provide useful information, particularly regarding mode of action. Consideration  
8 of such effects would certainly help reduce uncertainty in a risk assessment  
9 paradigm. While it is clear that these endpoints alone could not be utilized to set  
10 criteria, the Committee notes that sublethal endpoints integrated with  
11 toxicodynamic and kinetic factors could provide useful data in a problem  
12 formulation step related to some CEC, and could also help identify data gaps that  
13 may help reduce uncertainty and aid in criteria development.  
14
- 15 • Use of non-traditional sublethal endpoints holds promise but further validation of  
16 such endpoints is needed. Behavioral endpoints related to population (e.g.,  
17 predator-prey interactions) and reproduction may hold some promise for criteria  
18 development if the assays can be validated and variability can be understood.  
19 Immune function and genetic variation are also endpoints that should be explored  
20 (Filby et al., 2007). In addition, models capable of extrapolating laboratory  
21 endpoints to the population level should be targeted for development (Ankley et  
22 al., 2008; Chandler et al., 2004). Exploration of endpoints related to ecological  
23 processes (e.g., primary productivity, decomposition rate) is also warranted.  
24
- 25 • Research is needed to determine how aquatic life criteria for CECs can take into  
26 account the fact that aquatic organisms are exposed to mixtures of these  
27 chemicals. As noted previously, in developing aquatic life criteria for CECs it  
28 will be particularly important to consider the effects of mixtures. The Committee  
29 provides a number of comments in this regard. We note that understanding the  
30 mode of action of a compound is extremely important in estimating mixture  
31 interactions. Mixtures of CECs with comparable modes of action may result in  
32 higher environmental concentrations than would be expected for any single  
33 compound. In fact, pharmacological mode of action is the basis for evaluating  
34 multiple drug prescriptions in humans by pharmacists. For example, if it is  
35 known that a vertebrate is exposed to aryl hydrocarbon receptor (AhR) agonists  
36 and estrogen receptor (ER) agonists, it is likely that antagonism of each effect  
37 could occur. Information regarding mode of action should be made available to  
38 EPA from manufacturers or other governmental agencies (e.g., available from the  
39 U.S. Food and Drug Administration [FDA] or from testing under the requirements  
40 of the Federal Insecticide, Fungicide, and Rodenticide Act [FIFRA]). It is  
41 through use of this information that non-traditional measures can confirm similar  
42 or different modes of action in targeted ROPCs. The Committee strongly  
43 recommends enhancing the communication and data transfer capabilities between  
44 agencies such as FDA and EPA to provide these data.  
45

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- 1 • Mode of action fingerprints developed by evaluating combined sublethal  
2 endpoints should be linked to *in vivo* species testing. The Committee notes that  
3 much of the toxicity testing for compounds such as pharmaceuticals and personal  
4 care products has been conducted using mammals and other vertebrates.  
5 Additional data are needed for other “keystone” species. We suggest that the  
6 choice of species, critical life stages and complicating stressors (i.e., salinity and  
7 temperature) could be potentially identified in a problem formulation/conceptual  
8 model stage of a risk assessment paradigm. If these data are not available,  
9 research and development could be undertaken to obtain mode of action  
10 “fingerprints” for a CEC or any other compound through combined sublethal  
11 endpoints (i.e., genomic-transcriptomic, proteomic, metabolomic) and  
12 toxicodynamic/kinetic feature evaluations within sentinel species (to cover  
13 taxonomic issues). It is likely that, through this process, additional “side-effects,”  
14 or species-specific modes of action, can be obtained. These data could be  
15 integrated with “fingerprints” of other compounds with different modes of action  
16 and utilized to help address mixture issues or potential indirect effects. The  
17 toxicity to a particular species at a particular trophic position could then be  
18 modeled to assess indirect impacts on other populations.  
19
- 20 • Additional research is needed to link biomarkers to effects. The Committee notes  
21 that the concept of using biological responses occurring prior to impacts on  
22 growth, reproduction and survival has been proposed for more than 20 years as a  
23 way to detect adverse effects in a population before the population is altered.  
24 While there are examples of such “biomarkers of effect,” we find that the linkages  
25 between biochemical, histological, and behavioral endpoints and reproduction,  
26 growth, and survival are likely life-stage dependent and are difficult to validate,  
27 particularly in the field. We note that “biomarkers of exposure” are available but  
28 research is needed to interpret their significance.  
29
- 30 • Vitellogenin production is an excellent biomarker of exposure to feminizing  
31 chemicals. One of the best examples of exposure biomarkers is the biological  
32 response of vitellogenin production in male or juvenile animals. Vitellogenin is  
33 an excellent *in vivo* biomarker for exposure to feminizing chemicals. If the  
34 response is measured in the whole animal, it incorporates estrogenic as well as  
35 anti-androgenic or other modes of action that can cause a feminized response  
36 (production of an egg-yolk precursor). It is important to point out that this assay  
37 is not identical to estrogen-receptor (ER) based *in vitro* bioassays. Some  
38 compounds such as EE2 are very potent ER agonists but also have other modes of  
39 action that may alter endocrine systems (Tabb and Blumberg, 2006) such as the  
40 inhibition of several isoforms of cytochrome P450 (e.g., CYP3A), which are  
41 important in the clearance of endogenous steroids (Parkinson, 2001).  
42 Nonylphenols also have multiple modes of action other than direct binding to the  
43 ER that lead to enhanced estradiol synthesis (Harris et. al, 2001; Kazeto et al.,  
44 2004; Martin-Skilton et al., 2006; Meucci et al., 2006; Thibaut and Porte, 2004).  
45 So the observation of vitellogenin induction within an oviparous male or juvenile

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1 organism does not indicate total specificity with regard to mode of action.  
2 Anything that increases endogenous estrogen biosynthesis or diminishes clearance  
3 would also provide this biological response. The Committee notes that the  
4 reduction of vitellogenin in females may not indicate anti-estrogenic effects or  
5 even alterations of endocrine activity, as basic hepatotoxicants in females can  
6 elicit a similar effect. However, we point out that the correlations between  
7 fecundity and vitellogenin in females have been observed to be strong even  
8 though this may not indicate mode of action (Miller et al, 2007) (see discussion  
9 below). Additional studies are needed to examine hepatotoxicants or compounds  
10 with modes of action exclusive of endocrine targets.  
11

12 • The linkage of vitellogenin production to biological effects is limited. While the  
13 linkage of vitellogenin to exposure is reasonably solid, linkages of vitellogenin in  
14 males/juveniles to higher biological effects such as altered reproduction, survival  
15 and growth are limited, even though the relationship may make intuitive sense.  
16 Several studies have shown relationships between vitellogenin and reproduction  
17 in the laboratory, often at concentrations beyond probable effect concentrations  
18 (Thorpe et al., 2007), but few examples of population alterations have been noted  
19 in the field. Even in the United Kingdom, where gender shifts to females were  
20 originally noted and correlated with vitellogenin induction within males, intersex  
21 individuals, and other histological anomalies, overall abundance declines within  
22 the species of interest have not been reported. In fact, only one study (Kidd et al.,  
23 2007) has linked population crash with vitellogenin or histopathological  
24 alterations in fish. A similar occurrence has been noted in laboratory studies  
25 where vitellogenin expression may or may not be linked to intersex (Grim et al.,  
26 2007), which in turn may or may not lead to gender shifts. Even the relatively  
27 clear signal of gender shift, while clearly impacting reproduction in laboratory  
28 animals optimized to a specific gender ratio, may not significantly impact field  
29 populations in an uncharacterized species (Munday et al., 2006). Clearly, a better  
30 understanding of the population dynamics of a ROPC is needed to determine the  
31 phenotypic plasticity of the gender ratio. Thus, gender shifts should be viewed  
32 with caution, particularly in species that have not been well studied.  
33

#### 34 *Committee recommendations regarding selection of endpoints*

35

36 The Committee agrees that EPA should continue to explore the possibility of using  
37 sublethal endpoints in helping to set aquatic life criteria. We provide the following  
38 recommendations in this regard:  
39

40 1. EPA should pursue the use of “non-traditional measures,” or endpoints for criteria  
41 development, as discussed in the White Paper. The Agency should ensure that  
42 such measures can be tied to impacts on populations or ecological processes, not  
43 just to effects to individual organisms.  
44

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- 1 2. EPA should use “non-traditional measures” when appropriate to develop an  
2 understanding of and confirm mode of action.  
3
- 4 3. EPA should use human health information and toxicology tools (genomics/  
5 PBPKs) when appropriate and available to reduce the uncertainty of aquatic life  
6 criteria.  
7
- 8 4. EPA should consider the following key points concerning use of the non-  
9 traditional endpoints discussed in the White Paper: 1) vitellogenin in males and  
10 juveniles is an indicator of exposure to a feminizing stressor(s), but its linkage to  
11 population effects is limited; 2) strong correlations between vitellogenin and  
12 fecundity have been observed in females, but this is not necessarily tied to altered  
13 endocrine mode of action; 3) Anomalous intersex is indicative of a gender  
14 stressor(s), but has not been strongly tied to population effects; and 4) gender ratio  
15 can be indicative of endocrine alteration, but baseline information on appropriate  
16 life history is necessary for this evaluation.  
17

#### 18 **4.1.6 Involvement of an Expert Panel**

19

20 Because development of aquatic life criteria for CECs may be dependent on  
21 technical interpretations of a wide range of toxicological information, EPA has  
22 proposed that expert panels be used to provide professional judgment during criteria  
23 development. The Committee concurs that strong, active participation by a panel of  
24 outside experts will be necessary to ensure that the approaches used (including the  
25 designs for toxicity testing, the selected endpoints, and the necessary species and tests  
26 to be used, i.e., the ROPCs) are the most appropriate for the compound under  
27 scrutiny. As the EPA moves away from firm requirements for species and tests, it  
28 will become increasingly important that expert panels comprising diverse expertise be  
29 utilized to ensure that the best data are selected for necessary decisions. The National  
30 Academy of Sciences and Society of Environmental Toxicology and Chemistry have  
31 suggested similar approaches. In a recent report dealing with ecological risk  
32 assessment in environmental decision making (U.S. EPA Science Advisory Board,  
33 2007), the SAB strongly recommended that expert panels be used to provide  
34 assistance to EPA during the problem formulation phase of ecological risk  
35 assessments. The same recommendations are appropriate for development of aquatic  
36 life criteria. Involving a suite of experts with a balanced range of perspectives during  
37 the very early stages of problem formulation, and continuing their involvement as  
38 external reviewers at strategic junctures throughout the process, will significantly  
39 improve quality, utility, and defensibility of the criteria.  
40

#### 41 *Committee recommendations concerning the use of expert panels*

42

43 As stated above, the Committee concurs with the use of expert panels to provide  
44 professional judgment during the process of developing aquatic life criteria. We offer  
45 the following recommendations concerning the formation and use of expert panels:

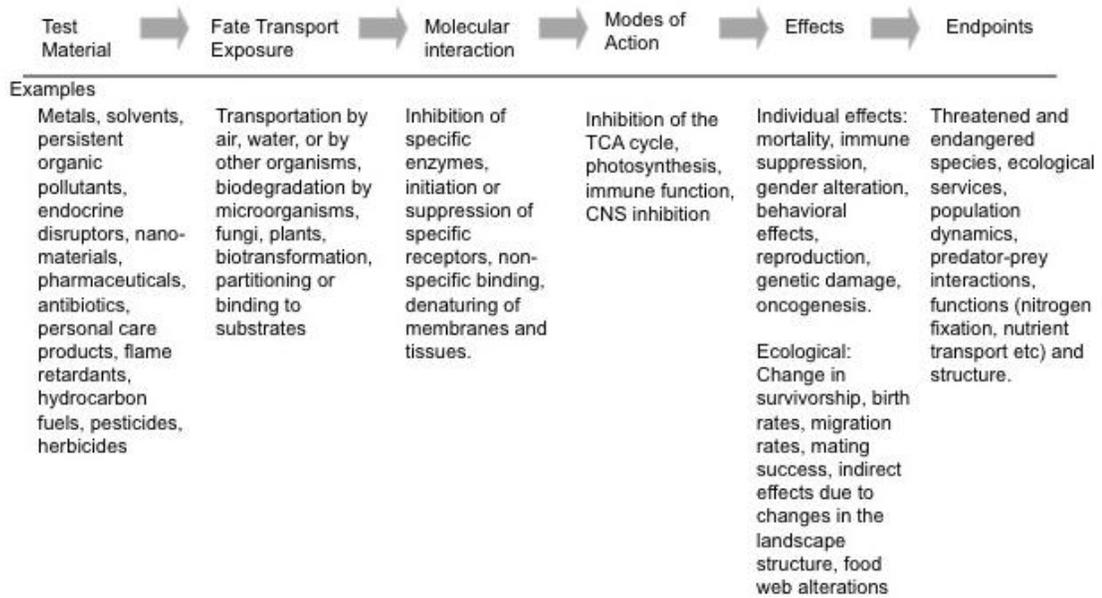
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2 1. The process for the use and selection of expert panels should be described in  
3 detail and should be transparent. The process used to select and convene the  
4 panels, the general attributes of panel composition, and methods used to address  
5 issues such as identification and elimination of conflicts of interest must be  
6 described (U.S. EPA, 2006). In this regard, one possible model to be considered  
7 is the process used to select SAB committees and panels, where national and  
8 international experts are identified from multiple sectors representing broad  
9 disciplinary expertise and professional affiliation (e.g., academic, appropriate  
10 governmental agencies [such as FDA], non governmental organizations, and  
11 private industry).  
12
- 13 2. The charge to the panel and the expected end result must be clearly defined.  
14
- 15 3. There are likely similar expert panel processes occurring elsewhere. The  
16 Committee recommends that EPA determine whether similar processes are  
17 underway in Europe and Asia, and if so, consider them as models to provide  
18 additional insight and/or expertise.  
19
- 20 4. The Committee is concerned that the use of expert panels could lead to less  
21 consistency in how aquatic life criteria are determined. To help alleviate this  
22 potential problem, we recommend that EPA develop specific guidance on the  
23 roles of expert panels in problem formulation, data evaluation, and the generation  
24 of recommendations leading to criteria derivation.  
25

26 **4.2 Charge Question 2. Please comment on whether EPA has identified the**  
27 **appropriate issues to be addressed in deriving ALC for CECs. Are there**  
28 **additional important issues that EPA has not identified?**  
29

30 As stated previously, EPA's White Paper identifies technical issues that need to  
31 be addressed in deriving aquatic life criteria for CECs. The Committee was asked to  
32 comment on whether the Agency has identified the appropriate issues in the White  
33 Paper and whether there are additional important issues that EPA has not identified.  
34 The Committee finds that appropriate technical issues have been identified in the  
35 White Paper. However, EPA could clarify the process of developing aquatic life  
36 criteria for CECs by articulating a set of principles that could be applied when  
37 modifying the 1985 Guidelines to develop water quality criteria for such  
38 contaminants. We also emphasize the importance of developing a conceptual model  
39 to guide the process of developing aquatic life criteria for CECs. The conceptual  
40 model should address more than the fate and direct effects of CECs. It should include  
41 consideration of probable direct and or indirect impacts on food webs, ecological  
42 processes and services, unique, endangered or keystone species or species of special  
43 societal value or concern. The example provided in Figure 1 illustrates components  
44 that could be included in such a conceptual model. Use of a conceptual model to  
45

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Figure 1. A Generalized Conceptual Model for Deriving Aquatic Life Criteria With Examples for Each Step

support criteria development would improve EPA’s ability to address emerging questions about unique mechanisms, fate processes, and effects endpoints. Use of the conceptual model is further discussed below.

*Committee recommendations concerning additional issues to be addressed*

Although the Committee finds that EPA has identified appropriate technical issues in the White Paper, we recommend that the Agency address the following additional issues in order to customize and update the 1985 Guidelines and thereby increase the flexibility and specificity of the aquatic life criteria derivation process:

1. In the White Paper, EPA should articulate principles that can be applied when modifying the 1985 Guidelines to develop water quality criteria for CECs. The Committee recommends that these principles be directly linked to EPA’s Guidelines for Ecological Risk Assessment (U. S. EPA, 1992, 1998). The committee in fact recommends that the 1985 Guidelines be updated to incorporate risk assessment principles and guidelines that did not exist when the Guidelines were developed over 20 years ago. In other words, the derivation of aquatic life criteria needs to be fully risk-based, using a transparent and consistent framework that provides necessary flexibility not presently possible within the algorithm approach of the 1985 Guidelines.
2. In line with using a risk-based approach, principles for developing aquatic life criteria for CECs should include the following: seek a wide range of inputs from diverse perspectives; determine appropriate ROPCs; develop a robust conceptual

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1 model; develop multiple lines of evidence; and identify uncertainties (quantitative  
2 and qualitative) associated with criteria development. Each of these risk  
3 assessment-based principles is further discussed below:

- 4
- 5 - Seek a wide range of inputs. EPA should seek input from a diversity of  
6 experts representing: Agency scientists, academic scientists, scientists in  
7 business and industry, state and tribal scientists, and the environmental  
8 community on the problem formulation, conceptual model development,  
9 modifications to the Guidelines dictated by the properties of a CEC, and the  
10 resulting recommendation for the aquatic life criterion. Adherence to this  
11 principle will ensure that the process stimulates a robust discussion and is  
12 informed by and acceptable from a diversity of perspectives. This diversity  
13 should include input from chemists, modelers, toxicologists, ecologists, and  
14 risk assessors.
  - 15
  - 16 - Determine appropriate ROPCs. The process needs to clearly identify the need  
17 to determine appropriate receptors of potential concern and not simply focus  
18 on “traditional” test organisms.
  - 19
  - 20 - Develop a robust conceptual model. At the start of the criterion development  
21 process, the available data on fate and effects should be examined and used to  
22 develop a conceptual model (e.g., Figure 1). Structure activity data and  
23 modes of action of similar compounds/materials should be consulted to inform  
24 model development. An expert panel should be convened to assist in the  
25 problem formulation and conceptual model development step. Uncertainty  
26 should be identified in the model and used to identify strategic efforts to  
27 reduce uncertainty. The conceptual model should include more than fate and  
28 effects data. It should include consideration of probable direct and or indirect  
29 impacts on food webs, ecological processes and services, and unique,  
30 endangered or keystone species or species of special societal value or concern  
31 (charismatic species).
  - 32
  - 33 - Develop multiple lines of evidence. The committee finds that a multiple line  
34 of evidence approach has the potential to inform decision making and the  
35 criterion recommendation. It also can serve to reduce uncertainty when the  
36 lines converge and reinforce each other.
  - 37
  - 38 - Identify uncertainties and conduct uncertainty analysis. As further discussed  
39 below, EPA should identify the uncertainties associated with the criteria  
40 developed for CECs. At all stages of criteria development, uncertainty should  
41 be quantified and/or qualitatively discussed. Uncertainty should be used to  
42 focus and prioritize data generation efforts.
  - 43
  - 44 3. EPA should develop a system or process to assist the development of criteria for  
45 CECs. The system would establish a set of rules to enable analysis of information

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1 supplied by the user and lead to recommendations concerning one or more  
2 courses of user action. The Committee finds that such a system would be an  
3 important tool for capturing and maintaining the state of the art in aquatic life  
4 criteria development. It would serve as a vehicle for connecting fate and effects  
5 assessment tools and capturing expert knowledge, and it could serve as a platform  
6 for deriving priorities for future research in assessing the risks of contaminants to  
7 aquatic life and ecosystems.

8

9 4. The Committee strongly recommends that understanding and presentation of  
10 uncertainty become an intrinsic part of the aquatic life criteria development  
11 process. The presentation of uncertainty needs to be an explicit and transparent  
12 part of the analysis. For example, the uncertainties inherent in understanding  
13 modes of action, determination of concentration-response relationships,  
14 development of species sensitivity distributions, and derivation of ecological  
15 effects should be quantified or described in a narrative sense. An important  
16 aspect of this is developing an a priori understanding of the amount and types of  
17 uncertainties that preclude the derivation of an aquatic life criterion. These  
18 uncertainties can be classified into the categories listed below:

19

- 20 - Uncertainties that preclude the derivation of an aquatic life criterion.
- 21
- 22 - Areas in which uncertainties may be important and can be resolved with  
23 additional modeling, research or a better understanding of the relationship of  
24 the uncertainty to the standard setting process.
- 25
- 26 - Uncertainties that do not preclude the setting of an aquatic life criterion but  
27 form the basis for future research programs.
- 28

29

30 Identification of uncertainties in these categories can be addressed in derivation of  
31 the conceptual model in consultation with the expert panel.

32

33 5. EPA should bolster the consideration of mode of action and ecology in the aquatic  
34 life criteria derivation process. A better understanding of the molecular  
35 interactions and modes of action will reduce uncertainty in that aspect of the  
36 conceptual model. A better understanding of the ecological effects and context  
37 will allow more specific and flexible predictions of risks to individuals,  
38 populations and ecological structure and function. This will reduce predictive  
39 uncertainty. The Committee encourages the developers of the aquatic life criteria  
40 to further integrate these advances into the criteria derivation process.

41

42 6. In the White Paper, EPA should discuss the importance of considering  
43 environmental context (i.e., site specific considerations) in deriving aquatic life  
44 criteria for CECs. These modifying factors should be mentioned in the CEC  
45 criteria themselves. For example, characteristics of the receiving environment  
affect bioavailability and toxicity to organisms (e.g., trophic status, dissolved

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1 organic carbon, pH, and substrate types) as well as longevity of their exposure  
2 due to impacts on the degradation and partitioning rates of these chemicals.  
3 Several CECs have the potential, based on their physical-chemical properties, to  
4 bioaccumulate and bioconcentrate, and this may result in diet-borne toxicity to a  
5 predator. Degradation/biotransformation products of CECs should be considered  
6 because there are instances where their toxicity is greater than the parent  
7 compound. In addition, the Committee recommends considering analytical  
8 chemistry because some aquatic life criteria have the potential to be set at  
9 concentrations that are at or below current (widely available) abilities to easily  
10 quantify CECs.

11

12 7. The Committee recommends that EPA keep abreast of the new science related to  
13 CECs in order to ensure that the latest approaches for assessing the effects of  
14 these chemicals are considered in criteria derivation. These types of effects may  
15 include impacts on natural selection and genetic diversity, indirect effects through  
16 changes in prey quality and quantity, and alteration of ecosystem function. We  
17 also point out that effects of CECs may be non-linear, which would pose  
18 challenges in derivation of aquatic life criteria. We note that consideration needs  
19 to be given to the diversity of phylogenies, functions, and habitats represented in  
20 the data used to establish an aquatic life criterion in order to ensure that the  
21 overall goals of the process (adequate, appropriate level of population-level  
22 protection) are met.

23

24 8. As mentioned previously, the Committee recommends that EPA use mammalian  
25 pharmacology data available from the drug discovery process,  
26 genomics/proteomics/metabolomics and QSARs to screen CECs for modes of  
27 action and assess potential multiple modes of action for individual CECs. This  
28 would facilitate exploration of the use of parallel processes to develop aquatic life  
29 criteria for CECs with similar modes of action. To increase efficiency when  
30 determining an aquatic life criterion for one compound (such as EE2), the process  
31 could be repeated (or developed in parallel) for compounds (such as estradiol or  
32 E2) with similar modes of action. In addition, some guidance should be provided  
33 for site-specific applications where mixtures of compounds occur that may have  
34 additive effects that exceed individual aquatic life criteria.

35

36 9. Natural history of a ROPC can determine the magnitude of effects of CECs and  
37 should therefore be considered in the derivation of aquatic life criteria. The  
38 timing of breeding seasons, immaturity periods, intrinsic rates of reproduction,  
39 survivorship, and life span all influence the magnitude and direction of possible  
40 changes in population size and age structure. Fisheries take should be considered  
41 for recreationally or commercially important species.

42

43 10. In developing aquatic life criteria for CECs, EPA should give special  
44 consideration to the protection of threatened and endangered species. Unlike  
45 other species, threatened and endangered species are managed so that effects on

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1 individuals, not populations, are avoided. Specific mortality of threatened and  
2 endangered individuals, along with the contribution of each to the survival of the  
3 population, are parameters requiring accuracy with a minimum of uncertainty. In  
4 certain cases specific populations or evolutionarily significant units are the  
5 assessment endpoints to be considered.  
6

7 **4.3 Charge Question 3. Part II of this white paper was specifically developed**  
8 **as a companion to Part I and focuses on the use of ethynylestradiol as a**  
9 **model chemical to illustrate the technical issues presented by the**  
10 **workgroup, as well as providing a basis for understanding the**  
11 **recommendations. Does the *Committee* have suggestions that may**  
12 **improve the utility of Part II of this white paper for the purposes stated**  
13 **above?**  
14

15 The Committee finds that Part II of EPA's white paper, which is intended to  
16 illustrate application of EPA's recommendations concerning aquatic life criteria for  
17 CECs (rather than serve as a comprehensive case-study) is a generally well-written  
18 and thorough review of the existing literature on EE2; however, some improvements  
19 are recommended to enhance clarity. The Committee agrees that EE2 is an  
20 appropriate initial focal CEC given: 1) the extensive data available relative to other  
21 CECs; and 2) the ease with which it illustrates the complexities inherent in generating  
22 CEC-specific water quality criteria to protect aquatic life. Nevertheless, there may be  
23 limitations as to how readily the insights gained from the EE2 illustration can be  
24 applied to other CECs. The following recommendations are provided to improve the  
25 usefulness of the EE2 example.  
26

27 *Committee recommendations to improve the usefulness of the illustrative example*  
28

- 29 1. In the White Paper, EPA should explicitly recognize that EE2 is unique in being a  
30 data-rich CEC. The White Paper should highlight the fact that the Agency's  
31 interest in CECs goes beyond endocrine-active substances, and discuss how the  
32 example of EE2 might be extrapolated to other substances, particularly to data-  
33 poor substances. EPA should consider conducting a similar assessment for a  
34 compound with a minimal data set (in contrast to the maximal set of data  
35 available for EE2) and evaluate the new approach accordingly.  
36
- 37 2. The Committee suggests that some of the illustrative pieces of Part II could also  
38 be included in Part I in the form of succinct text boxes illustrating key concepts  
39 derived from the various recommendations (e.g., why certain steps in the  
40 Guidelines were included and others were not). Further, we suggest that the  
41 recommendations could be best illustrated if the text boxes were not restricted to  
42 EE2 but rather included other CECs (e.g., non-endocrine-active compounds, data-  
43 poor CECs). In making these revisions, we urge the authors to ensure that the  
44 high level of readability inherent in the present version of Part I is retained.  
45

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- 1 3. Regarding the scope of the material included in the EE2 example, we note that the  
2 White Paper fails to address how the influence of EE2 might be affected by  
3 mixtures of compounds, especially those with similar modes of action (e.g.,  
4 estradiol, estrone), as well as environmental (e.g., temperature) and biological  
5 (e.g., disease, starvation) modifying factors. Although the Committee recognizes  
6 that various offices/groups within EPA are investigating mixtures of compounds,  
7 and the White Paper cannot address all relevant issues in the development of  
8 guidelines, the document needs to be explicit regarding the importance of  
9 considering multiple stressors as well as synergies among CECs. For example,  
10 the White Paper should, at the very least, state the rationale for not considering all  
11 estrogens within a given body of water, and should provide examples of mixtures  
12 and synergies that could affect the toxicity of EE2.  
13
- 14 4. Regarding choice of taxa for criteria derivation, the Committee agrees that,  
15 although use of non-resident species to assess EE2 effects appears to fit this case  
16 example, such may not always be the case. As such, the document should  
17 indicate that: 1) resident species data, especially life-cycle tests from resident  
18 species, remain extremely valuable, and 2) results from non-residents, while  
19 providing useful information, may not be generalized to resident species unless  
20 data are available to compare the sensitivities of the non-resident and resident  
21 species. We are also concerned that certain sensitive taxa such as amphibians  
22 were not included in Table 3.2, and that the key issue of development time to  
23 sexual maturity for long-lived, charismatic species, such as sturgeon, is not  
24 addressed in the document. Research should be conducted to develop  
25 comparisons between species that are long-lived and surrogate test species.  
26
- 27 5. The Committee is concerned that transgenerational effects were not considered in  
28 Part II of the White Paper. On page 14 in Part II of the White Paper, EPA states  
29 that “it does not seem that the evidence for transgenerational effects is sufficient  
30 for requiring their inclusion in the definition of an acceptable chronic test.” Given  
31 EE2’s role as an endocrine disrupting chemical, it is surprising that  
32 transgenerational effects were not included in the treatment of EE2. Further,  
33 given the “guilty until proven innocent” rule mentioned previously, the  
34 Committee recommends that the possibility of transgenerational effects be  
35 explicitly addressed in this illustration. Although transgenerational effects may  
36 not be expected in the case of EE2, potential transgenerational consequences must  
37 be addressed in a clear and transparent manner to ensure the development of a  
38 process that can also be applied to substances for which transgenerational effects  
39 are expected.  
40
- 41 6. The Committee recommends that a broader array of endpoints be included in Part  
42 II. For example, although EE2 is a potent estrogen receptor agonist, it also can  
43 affect the central nervous system through indirect effects (steroid  
44 biotransformation). Non-traditional endpoints such as genomic or physiologically  
45 based pharmacokinetic modeling (PBPK) studies might be considered. As noted

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- 1 previously, use of non-traditional endpoints requires an understanding of their  
2 relevance to the health of the organism and ultimately the population. The  
3 illustration in Part II needs to answer the question as to whether or not it is  
4 possible to calculate population-scale impacts with EE2 and, if not, how a  
5 criterion can be developed that will truly protect populations within a reasonable  
6 level of uncertainty (consistent with the intent of the Guidelines).  
7
- 8 7. Two key recommendations regarding Part I of the White Paper are repeated here  
9 for the sake of consistency. First, the use of weight of evidence is implicit in the  
10 evaluation, but it needs to be explicit in the Part II of the document. Interactions  
11 between weight of evidence and the Precautionary Principle (i.e., appropriate  
12 levels of uncertainty) should be clarified. Second, when appropriate data are  
13 available, EC<sub>x</sub> values (i.e., the concentration causing an effect in x percent of the  
14 test organisms) should be used rather than NOECs/LOECs (i.e., no observed  
15 effects concentrations/lowest observed effects concentrations). The EC<sub>x</sub> value  
16 reflects the information in the entire concentration-response curve and confidence  
17 intervals can be calculated as part of the curve fitting process. In contrast, the use  
18 of NOECs or LOECs by hypothesis tests are dependent upon the test  
19 concentrations that are used, the variability of the experimental technique, and the  
20 power of the statistical test. It is also not possible to generate confidence intervals  
21 for the NOEC/LOEC determinations. When available, the data used in a  
22 NOEC/LOEC determination should be used to calculate the EC<sub>x</sub> value. Curve  
23 fitting, which uses more of the information contained in a data set and enables  
24 derivation of confidence intervals in the estimation of the EC<sub>x</sub>, is the preferred  
25 method for representing dose (concentration)-response information.  
26
- 27 8. The Committee finds that the clarity and transparency could be improved in  
28 several areas. In particular, the authors need to more explicitly describe how the  
29 illustration was developed from the recommendations in Part I. Part II also needs  
30 to be more explicit regarding how specific conclusions and assessments derived  
31 from the data. The following specific revisions are suggested:  
32
- 33 - Data used to arrive at the values shown in Table 3.1 need to be provided in an  
34 appendix.
  - 35 - Table 1 arguably includes chronic data (*Lytechinus* and *Strongylocentrotus*  
36 echinoderm embryo development tests and the *Acartia* embryo test) that, not  
37 surprisingly, provide the most sensitive responses. While the Committee  
38 concurs that there is “ample evidence that a CMC is not needed and that it is  
39 unnecessary to conduct further tests to meet the minimum data requirements,”  
40 the differentiation between acute and chronic data needs to be more clear and  
41 transparent along with the implications of including equivocal data.  
42 Confusion between acute and chronic data can result in unnecessary levels of  
43 uncertainty and variability in criteria development. We note that slide 11 of  
44 the presentation provided by Dr. Russell Erickson of EPA ORD at the  
45

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- 1 Committee meeting on June 30 provides the requisite level of clarity and  
2 transparency and could usefully be included in the document.  
3
- 4 - More explicit discussion of what constitutes “sufficient information” at  
5 various decision points would be helpful.  
6
  - 7 - The validity of using non-resident species is justified by text referring to  
8 complex tables, which do not provide the level of clarity and transparency  
9 necessary. Given the importance of validating the use of non-resident species,  
10 a graphic representation of the data is required (e.g., SSDs or linear, horizontal  
11 lines indicating ranges for survival, growth and reproduction showing where  
12 the non-resident species fit).  
13
  - 14 - The Committee suggests that the authors add a concluding section that  
15 summarizes the process used to assess how the process of developing an  
16 aquatic life criterion for EE2 was modified by use of the new/updated  
17 guidelines. Part II should also provide an overview of how the process is  
18 expected to ultimately influence the criteria derived (in other words, what is  
19 the bottom line in terms of how the new recommendations changed the final  
20 outcome?).  
21
  - 22 - The EE2 example in Part II relies on nominal concentrations in addition to  
23 measured concentrations. The Committee assumes that criteria will not be  
24 based on nominal concentrations. However, it is acknowledged that as long as  
25 measured concentrations are within 20% of the nominal concentrations  
26 employed in a study, the concentrations reported could be the nominal  
27 concentrations. This needs to be made clear in the document.  
28
  - 29 - The first two paragraphs on page 13 of Part II would benefit from additional  
30 information on the timing of exposures to clarify that a 16% reduction in  
31 growth occurred after 28 days (paragraph 1, line 4), and the timing for lower  
32 reproduction at 0.2 and 1 ng/L (paragraph 1, line 9). We have a similar  
33 suggestion for effects on fertilization success (paragraph 2, lines 7-8).  
34
  - 35 - EPA should include in the appendix the residency status of each species or  
36 genus. The authors refer to residency in interpretations, but this information is  
37 missing from the document.  
38
  - 39 - A list of acronyms such as that provided for Part I also would be useful for  
40 Part II.  
41
  - 42 - A few questions are raised regarding citations: (1) Wenzel et al. (2002) is  
43 cited in the text (p. 14, paragraph 3, line 3) but not in the References; should  
44 the date of the reference be 2001?; (2) Is the Kolpin et al. (2002) reference  
45 correct (both here and in Part I) - it does not seem to apply as it is a 2-page

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1 response to a comment, not a full paper?; (3) Lee and Choi (2006) is listed in  
2 the References as “in press” but surely this is not still the case 2 years later?;  
3 and (4) the reliance on McKim et al. (1978) is questioned regarding the  
4 assertion that a “factor of 2 difference is generally found for other chemicals”  
5 (page 13, incomplete paragraph beginning the page, last line). We note that  
6 the McKim et al. (1978) paper only referred to one chemical, copper, and was  
7 published thirty years ago in a journal that does not have a high level of peer  
8 review.

9  
10 **4.4. Charge Question 4. Does the Committee have suggestions that would**  
11 **assist EPA in implementing the proposed recommendations discussed in**  
12 **the white paper, particularly with respect to developing the necessary**  
13 **scientific data and information and/or providing expert scientific input at**  
14 **the appropriate stages of the risk assessment process?**  
15

16 The Committee has provided comments and recommendations to assist EPA in  
17 implementing the proposed recommendations discussed in the White Paper. Many of  
18 our comments focus on actions that would assist in implementation of the  
19 recommendations in the White Paper. However, we have also provided broader  
20 suggestions to facilitate future development of aquatic life criteria for CECs. Some of  
21 our comments and recommendations elaborate upon points discussed in previous  
22 sections of this advisory report.

23  
24 *Points to be considered in implementing the proposed recommendations in the White*  
25 *Paper*  
26

- 27 • Developing new criteria for CECs will require intensive data collection /  
28 generation activities. In an ideal world, it would be the Committee’s  
29 recommendation that the same level of effort required to register a new chemical  
30 or pesticide also be required to develop aquatic life criteria for CECs.  
31 Acknowledging that this may not be possible in a world of limited resources, it  
32 will be important that OW/ORD prioritize the list of CECs for which aquatic life  
33 criteria will be developed. EPA should also identify data needs for these  
34 chemicals and leverage research development activities to develop the necessary  
35 data. Prioritization of CECs and data needs is further discussed below. In  
36 addition, EPA should conduct research to evaluate the sensitivity of test  
37 organisms that could be used as surrogates for resident and endangered species.  
38 Research should also compare the sensitivity of traditional and non-traditional test  
39 endpoints.  
40
- 41 • Leveraging research efforts of other agencies is essential. In a time of decreasing  
42 research funds within the federal government, it is important that OW/ORD seek  
43 opportunities to leverage research efforts of other government agencies (e.g.,  
44 FDA, U.S. Department of Agriculture [USDA], National Oceanic and  
45 Atmospheric Administration [NOAA]). The Committee was informed that EPA

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1 and the FDA are coordinating data sharing. We recommend that this activity  
2 continue and further that it be broadened to include other government agencies.  
3 We further support international collaboration between EPA, the European Union,  
4 Environment Canada and other appropriate non-U.S. environmental agencies. In  
5 addition, it is apparent that the regulated community, industries, animal husbandry  
6 organizations (e.g., National Cattlemen’s Beef Association) and Publicly Owned  
7 Treatment Works, are actively engaged in independent evaluation of CECs.  
8 Establishing a government/industry consortium may be a way of leveraging  
9 limited funds for broader data development opportunities.

10

11 • Linkages between ecological risk assessment and development of aquatic life  
12 criteria need to be articulated. The Committee finds that, in many ways, the 1985  
13 Guidelines contain the same principles of evaluating ecological risk that were  
14 subsequently incorporated into the 1989 *Risk Management Guidance for*  
15 *Superfund, Volume 2: Environmental Evaluation Manual*, (U.S. EPA, 1989), and  
16 in the 1992 *Framework for Ecological Risk Assessment* (U.S. EPA, 1992).  
17 Furthermore, it was apparent from the presentations made by EPA to the  
18 Committee that the ecological risk assessment principles have been considered by  
19 OW and ORD in planning further development of aquatic life criteria for CECs.  
20 However, the link between the 1989 Risk Management Guidelines and the aquatic  
21 life criteria derivation process is not apparent. The white paper needs to explicitly  
22 consider and illustrate risk assessment principles (e.g., identification of ROPCs,  
23 development of a conceptual diagram as previously recommended by the  
24 Committee).

25

26 • Tissue-based criteria should be considered for bioaccumulative CECs where food  
27 chain transfer is a concern. As mentioned previously, EPA should consider  
28 developing tissue-based criteria (i.e., expressing the criterion as a concentration of  
29 the pollutant in fish tissue rather than a concentration in the water). Aquatic life  
30 may be impaired directly by eating contaminated food, or indirectly by loss of  
31 prey or other ecosystem alterations that could stem from CECs. EPA is  
32 developing residue-based criteria for selenium (2002 and 2004 draft criteria  
33 documents [U.S. EPA, 2007]). Arguably, selenium can be considered a  
34 contaminant of emerging concern, but it does not fit the definition provided in  
35 Section 1.1 of Part I of the White Paper. The Committee finds that it may be  
36 useful to consider using selenium as an example for development of tissue-based  
37 aquatic life criteria for CECs.

38

39 • Quantitative linkages are needed between mode of action indicators and  
40 population-level endpoints. The proposed recommendations in the White Paper  
41 are consistent with bettering the risk assessment process. However, it will be  
42 important to set priorities for technical research that addresses significant gaps in  
43 knowledge needed to develop: 1) new indicators; 2) modeling capabilities; and 3)  
44 tools that provide integration and linkage of data sources. As mentioned  
45 previously, one of the most important challenges facing EPA will be linking mode

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1 of action indicators of exposure/effects to known population-level effects  
2 measurement endpoints such as survival, growth, reproduction and development.  
3 Developing conceptual models will guide criteria development but quantitative  
4 linkages will be needed to discern how mode of action indicators connect with  
5 population-level end points. The White Paper (p. 20, lines 21- 21) states that it is  
6 important to have a clear linkage between mode of action indicators such as  
7 histopathology and growth, reproduction and development. The Committee notes  
8 that in some instances it may be possible to define scaled risk (e.g., level of  
9 biological response in cell, tissue, etc.) and relative risk. This will make it  
10 possible to develop mode of action fingerprints that may provide earlier warning  
11 and greater sensitivity in predicting population-level effects.

12

13 • Additional factors may need to be considered to protect certain species. As noted  
14 previously, development of aquatic life criteria to provide adequate levels of  
15 protection for endangered, highly managed, protected and “charismatic” species  
16 (e.g., marine mammals, eagles, polar bears, sturgeon) may require consideration  
17 of additional factors. For example, in marine mammals a dive reflex can force  
18 more contaminant into tissue due to pressure gradients. Endangered species may  
19 have very different lag times for sexual differentiation and uptake characteristics  
20 of CECs than the commonly used test species. For example, sturgeons are both  
21 endangered and charismatic fishes, and they are known to readily accumulate  
22 many CECs for an extended developmental period prior to reproduction. Given  
23 their long lifespan, a life cycle chronic test to determine uptake would be  
24 impossible, and an early life cycle test would be inappropriate.

25

26 • There is a need to compile a list of priority CECs. To facilitate development of  
27 aquatic life criteria, the Committee finds that it would be useful for federal  
28 agencies working on CECs (e.g., EPA, the U.S. Geological Survey, the U.S. Food  
29 and Drug Administration, the National Oceanic and Atmospheric Administration,  
30 and others) to compile a list of priority CECs that may pose the greatest risks to  
31 aquatic life – in other words, use a risk assessment approach in a problem  
32 formulation exercise to determine contaminants of potential concern. Analytical  
33 chemistry methods should be developed for CECs that are not already being  
34 measured in aquatic environments. The Committee suggests that calculation of  
35 the ratios of the Maximum Environmental Concentrations to meaningful measures  
36 of biological effects (e.g., CCCs, or LC<sub>x</sub>s from toxicity testing) could initially be  
37 used to develop a list of high priority CECs. This kind of exercise would likely,  
38 but not certainly, show that estrogens should be a top priority for aquatic life  
39 criteria, as indicated in the White Paper.

40

41 • There is a clear need for continued development of analytical capabilities to  
42 measure levels of CECs in the aquatic environment. The ability to detect many of  
43 the CECs at appropriate concentrations in a controlled laboratory setting may be  
44 entirely different from detecting those same low concentrations in the aquatic  
45 environment. Addressing such issues will help current long term monitoring

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1 programs (e.g., NOAA National Status and Trends and Mussel Watch programs,  
2 U.S. Geological Survey National Water Quality Assessment Program, EPA  
3 Environmental Monitoring and Assessment Program) implement a coordinated  
4 approach to better define CEC exposures in the environment. Efforts to develop  
5 methodological approaches for lowering limits of detection and standards for  
6 CECs should involve discussion among agencies as well as the regulated  
7 community. It may be important to include the National Institute of Standards  
8 and Technology in the development of environmental standards for new CECs.  
9

- 10 • Input into the aquatic life criteria development process is needed from private  
11 industry and state government. The perspective of these important stakeholders is  
12 needed before finalizing the White Paper. These groups should be asked to  
13 provide input on the science associated with the modifications of the Guidelines  
14 related to CECs because aquatic life criteria will be used to develop state water  
15 quality standards.  
16
- 17 • It would make sense to consider using parallel processes to develop aquatic life  
18 criteria for compounds with similar modes of action (e.g., the estrogens, SSRIs).  
19 Since estrone, estradiol and EE2 all act through the estrogen receptor in the most  
20 sensitive taxa, fish, and there is growing evidence in the literature that their  
21 effects are additive (Thorpe et al., 2003), it would make sense to develop aquatic  
22 life criteria for the natural and synthetic estrogens using parallel processes.  
23 Similar approaches may be possible for other CECs with highly specific modes of  
24 action such as different classes of antibiotics, statin drugs and other  
25 pharmaceuticals that are CECs.  
26
- 27 • Further questions to consider. As EPA develops a research plan to support  
28 derivation of aquatic life criteria for CECs, it may be useful to consider the  
29 following questions mentioned previously: How can aquatic life criteria be  
30 developed to take into account the fact that aquatic organisms are exposed to  
31 mixtures of CECs and mixtures of CECs, known contaminants, and other  
32 stressors? What are the likely modes of action of CECs that are known to be  
33 present in the environment? How can field study results be used to inform the  
34 derivation of an aquatic life criteria for a CEC?  
35

36 *Committee recommendations to assist EPA in implementing proposed approaches to*  
37 *developing aquatic life criteria for contaminants of emerging concern*  
38

39 The Committee provides the following specific recommendations to assist EPA in  
40 implementing the Agency's proposed approaches to developing aquatic life criteria  
41 for CECs. Some of these recommendations have been discussed in the context of  
42 responses to the other charge questions in this report.  
43

- 44 1. EPA should develop a list of high priority CECs that may pose the greatest risks  
45 to aquatic life. Additional work should then be completed to further assess the

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- 1 potential risks posed by these chemicals and fund the research and data collection  
2 activities needed to support future development of aquatic life criteria. In this  
3 regard, we recommend that EPA's Office of Water and Office of Research and  
4 Development look for opportunities to leverage existing research with those on-  
5 going in other federal programs, similar programs with international agencies, and  
6 industry groups, to gather the data needed to develop the aquatic life criteria.  
7 The Agency should also work with other federal agencies to develop analytical  
8 chemistry detection methods and standards for these chemicals.  
9
- 10 2. EPA should explicitly incorporate the principles for conducting Ecological Risk  
11 Assessment into the process of deriving aquatic life criteria for CECs. The  
12 Committee recommends that the EPA develop a separate process document that  
13 discusses the intended application of aquatic life criteria for CECs, and cross-links  
14 the 1985 Guidelines, the EPA's 1992 Ecological Risk Assessment Principles, and  
15 the 2008 aquatic life CEC criteria White Paper. This cross-link document should  
16 also incorporate relevant ecological risk principles from other similar documents  
17 developed for FDA, the Toxic Substances Control Act, or the Federal Insecticide,  
18 Fungicide, and Rodenticide Act. The document should not only outline the  
19 process of aquatic life criteria development, but address elements such as  
20 contaminant exposure through food uptake, Water Effects Ratios, Whole Effluent  
21 Testing, mixtures of compounds with similar modes of action, and application of  
22 aquatic life criteria for CECs in sediment management programs. The Committee  
23 is not recommending the development of a large, comprehensive document, rather  
24 something short and concise similar to the Eco Update Bulletins that have been  
25 published by EPA's Office of Solid Waste and Emergency Response (OSWER).  
26
- 27 3. As previously discussed, the Committee recommends that EPA incorporate the  
28 use of conceptual site models and ecosystem-based criteria into the process of  
29 deriving aquatic life criteria for CECs. We note that EPA programs are moving  
30 toward developing more comprehensive ecosystem-relevant criteria that take into  
31 consideration population-community structure, ecosystem functions-processes,  
32 and ecosystem services. The data available to develop CCCs are often  
33 "traditional" toxicity test data. It is important to develop the link between the  
34 protected resource, the assessment endpoint, and the measurement endpoint. An  
35 appropriate conceptual model for deriving aquatic life criteria for a CEC (see  
36 Figure 1) may be used to develop the fate and effects data and data quality  
37 objectives needed to support the aquatic life criterion.  
38
- 39 4. As previously discussed, EPA should consider (where appropriate) developing  
40 tissue residue-based aquatic life criteria for CECs. The Agency should consider  
41 developing tissue-based criteria using the selenium example and expanding the  
42 definition of contaminants of emerging concern to include "chemicals and other  
43 substances of increasing environmental concern due to anthropogenic activities  
44 and for which current regulatory approaches are inadequate." Tissue residue-  
45 based criteria should be considered for CECs that have potential to bioaccumulate

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- 1 (e.g., carbamazepine) and bioconcentrate (e.g., flame retardants). At a minimum,  
2 the conceptual model could be used to help determine how to evaluate the  
3 available environmental data and models to assess the main routes of exposure for  
4 aquatic organisms.  
5
- 6 5. EPA should use a “mode of action” approach to develop more effective aquatic  
7 life criteria not only for CECs, but also for legacy contaminants and mixtures.  
8 Additional studies in genomic and toxicodynamics processes would provide  
9 necessary data for the identification of “mode of action” fingerprints and aid in  
10 this process, particularly in the problem formulation stage of risk assessment.  
11 This should help guide regulators to carry out the most efficient bioassays which  
12 will be used in setting thresholds or criteria.  
13
- 14 6. The Committee recommends that EPA appropriately use novel environmental  
15 indicators (molecular, genomics, proteomics) developed at other agencies,  
16 industry, and by academia in future development of criteria. For example, NOAA  
17 has developed a robust health effects assessment for bottle nosed dolphins that  
18 addresses many CECs including flame retardants and antibiotic resistance (Fair et  
19 al., 2006; Goldstein et al., 2006; Houde et al., 2006; National Oceanic and  
20 Atmospheric Administration, 2008; Reif et al., 2006). The assessment involved  
21 analysis of the immune function data and other health information on the animals  
22 such as clinical evaluation, blood chemistries, contaminants and hormones. Since  
23 dolphins are apex predators that breathe the air, swim in the water and constantly  
24 eat seafood, they provide a most exposed individual model. This type of insight  
25 may be pivotal in enhancing what EPA can do using the approach outlined in Part  
26 I of the White Paper.  
27
- 28 7. EPA should take into consideration appropriate additional factors to ensure that  
29 aquatic life criteria are protective of sensitive and commercially/recreationally  
30 important species. These species are protected by additional laws (e.g.,  
31 Magnuson Stephens, Marine Mammal Protection Act) and this may invoke other  
32 special considerations when developing aquatic life criteria.  
33
- 34 8. EPA should obtain input from private industry and state government on the  
35 Agency’s proposed approaches for developing aquatic life criteria for CECs  
36 before finalizing the White Paper.  
37
- 38 9. EPA should consider developing a mixture strategy to develop aquatic life criteria  
39 for classes of compounds with similar modes of action. As previously mentioned  
40 parallel processes could be used to develop aquatic life criteria for broad classes  
41 of CECs with similar modes of action (e.g., the estrogens, SSRIs).  
42  
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34

**SAB Comments on  
Aquatic Life Water Quality Criteria  
for Contaminants of Emerging Concern**

**1. Dr. David Dzombak:**

*(a) Are the original charge questions to the SAB Panel adequately addressed in the draft report?*

The SAB Ecological Processes and Effects Committee (EPEC) review panel has addressed all of the charge questions. Each of the charge questions appears to be addressed in sufficient depth, and specific recommendations have been developed for each of the charge questions and sub-questions.

*(b) Is the draft report clear and logical?*

The organization of the draft report by the SAB EPEC review panel follows the charge questions directly and is easy to follow. The Executive Summary is rather long considering the short length of the main report, but I don't think it's a problem and would not recommend condensing the Executive Summary further. There are two issues in the draft report that I recommend be addressed to improve clarity and strengthen it.

(i) I have identified a statement made in the letter to the Administrator, in the Executive Summary, and in the body of the report that is somewhat misleading and that I recommend be clarified. This statement is made on page 2 of the letter, on page xii of the Executive Summary, and on pages 25 and 37 of the main report. The version that is in the letter to the Administrator serves to illustrate my concern.

“The derivation of aquatic life criteria needs to be risk-based, using a transparent and consistent framework that provides necessary flexibility not presently possible within the algorithm approach of the 1985 Guidelines. Hence, the SAB recommends that, to the extent practicable, the derivation of aquatic life criteria be risk-based using the principles defined in EPA's 1998 *Guidelines for Ecological Risk Assessment*.”

The recommendation that EPA risk assessment procedures for determination of aquatic life criteria make direct use of the 1998 Guidelines is fine, but the preamble to this recommendation as given here and at the other locations in the report cited above implies that the 1985 Guidelines do not involve risk assessment, and thus that risk assessment has not been previously employed in establishing aquatic life criteria. This is not the case, and I recommend that the recommendation about use of the 1998 Guidelines be reworded to clarify the nature of the 1985 Guidelines.

(ii) In the Executive Summary (page xiii, bullet 1; page xxi, bullets 1 and 4) and in the main report (page 33, bullet 2; pages 36-37, item 1; and

perhaps elsewhere), the review panel discusses the potential for contaminants of emerging concern (CECs) to be from classes of chemicals other than pharmaceuticals and personal care products, and recommends that the Agency “consider expanding the definition of CECs to include chemicals and other substances of increasing environmental concern due to anthropogenic activities and inadequate regulatory approaches.” The review panel recommends that the Agency “look for opportunities to leverage EPA research with ongoing research in other federal agencies, international agencies, and industry groups.” These are useful and important observations and recommendations, but not mentioned in any of the discussion is the TSCA new product review process in which data are supplied by chemical manufactures in relation to the pre-manufacture notification required under TSCA. The search for possible CECs should begin at this stage. The TSCA new product review and its relationship to aquatic life criteria determination is not discussed in the report at all. At a minimum, aquatic life toxicity data provided by manufacturers in this process could be used to help set aquatic life criteria. There are other possibilities that could be considered, such as integrating parts of the aquatic life criteria establishment process into the TSCA new product review to aid in the assessment of the new product notifications. Also, data and other information supplied for the new product review could help the Agency prioritize CECs. Whatever the level of integration the review panel believes is appropriate, the main point is that aquatic life criteria determination for CECs should be conducted with knowledge of the data for new chemical products coming into commercial use provided by the TSCA new product review process.

*(c) Are the conclusions drawn, and/or recommendations made, supported by the information in the body of the draft SAB report?*

The conclusions drawn and recommendations made are supported by the information in the body of the draft report.

## **2. Dr. Meryl Karol:**

*a) Are the original charge questions to the SAB Panel adequately addressed in the draft report?*

The draft report clearly addresses the charge questions.

*b) Is the draft report clear and logical?*

The report is superb; clear and logical. One of the best reports I have read

*c) Are the conclusions drawn, and/or the recommendations made supported by information in the body of the report?*

Yes.

### 3. Dr. Thomas Wallsten

I have read the three draft reviews. It appeared to me that all three adequately addressed the charge questions, were logically laid out, and provided supporting information for their conclusions and recommendations. I have three comments on the reports:

- a) The review of the White Paper on "Aquatic Life Criteria for Contaminants of Concern" mentioned the use of expert panels to provide professional judgment during criteria development (Section 4.1.6). I concur that such panels can be very useful. My question is whether EPA has, or has not considered, guidelines for how such panels should operate to assure careful, unbiased judgmental extrapolations from available data to end points of concern?
- b) The same white paper urges that attention be paid to the possible effects of mixtures of contaminants, not just contaminants acting alone. This point would seem to apply to the "SAB Advisory on EPA's Third Drinking Contaminant Candidate List," yet I did not see it mentioned there (although I may have missed it).
- c) Finally, only the review of "Toxicological Review of Acrylamide" included a list of abbreviations. While some acronyms are common (e.g., LOEL, NOEL, DNA), others may be unique to specific fields or topics (e.g., CEC, ROPC, WBDO). It would helpful for all reports to have a list acronyms.

### 4. Dr. Terry Daniel

*The original charge questions to the SAB Panel are adequately addressed in the draft report, the report is clear and logical, and the conclusions and recommendations are supported by the information in the body of the report.*

Some suggestions for extensions to some sections of the Committee review are presented below.

#### 4.1.1

The Committee recommends that the White Paper pay greater attention to the possible interactions within "mixtures" of similar contaminants (especially viz. mode of action) and to the potential for environmental "pulses" of higher than normal concentrations to occur frequently in some contexts. A similar issue not specifically noted is the potential for periodic environmental concentration of contaminants as may occur, for example, in ephemeral water bodies due to evaporation. Of course, mixtures of contaminants and pulse/concentration phenomena might also interact to further magnify toxic effects.

#### **4.1.2**

The issue of minimum data requirements with regard to taxonomic coverage is a classic “proof of the null hypothesis” problem (“proof of innocence” in the white paper), and the Committee has appropriately noted the need for a clear and explicit specification of criteria for determining when data and understanding are sufficient for determining no effect. In this context, the suggestion presented later (4.2) of revising/updating the Guidelines in the direction of the Guidelines for Ecological Risk Assessment is appropriate here as well. A major factor in determining “proof of innocence” should be an assessment of the potential consequences of incorrectly concluding that a contaminant would have no effect (i.e., the payoff matrix). The ecological data requirements for supporting a conclusion of no effect (i.e., the level of “power” deemed sufficient for detecting a specified consequential effect) depend at least in part on an assessment of the social and biological values at risk and the potential for consequential losses. Moreover, because current goals extend to the protection of ecosystems and their services, rather than individual targeted organisms or specific sub-systems, there is a greater need to assure that biological assessments adequately address a broad range of taxa and environmental contexts.

#### **4.1.3**

The use of non-resident species models is well addressed by the Committee. This section additionally provides an opportunity to extend the Committee’s discussion of how to define “resident species” to include how global climate change and other factors potentially make this distinction a moving target. It seems clear that resident species is a “social construct,” and so some explicit involvement of publics/stakeholders in identifying appropriate species targets in given environmental/social contexts, and in determining the relevance of data based on surrogate non-resident species would seem both useful and prudent.

#### **4.1.4**

The relevance of a risk assessment approach noted above for taxonomic coverage issues applies equally well to decisions about the sufficiency of partial versus full life-cycle tests (perhaps extending to trans-generational testing) for determining chronic toxicity effects. Such decisions would seem to require a consideration of tradeoffs between the costs of additional testing and the values at risk and potential losses from missing an important effect. That is, such decisions cannot be made on the basis of biological data considerations alone.

#### **4.1.5**

The use of sub-lethal/”non-traditional” endpoints for toxicity assessments raises a number of issues addressed by the White Paper and refined by suggestions of the Committee. The rough implicit model is that biological

changes in individual organisms (in response to toxins) may produce changes in individual characteristics and behavior which may have implications for populations (and on to ecosystems). In that context, and consistent with the points raised by the Committee in recommendation 4, it should be noted that the model may at times work backwards, with social factors affecting individual behavior which in turn affects individual neurological and other systems and functions.

#### **4.1.6**

The discussion of expert panels emphasizes their use as a means for overcoming gaps in bio-ecological data and information. Consistent with the recommended move toward a risk assessment model (4.2) and with the issues raised in 4.1.2-4 above, this discussion might be extended to include both a wider range of disciplines (especially social sciences and economics) and some involvement of relevant publics/“stakeholders.” This may have been intended, but is not fully communicated by the call for a “balanced range of perspectives” in expert panels used for the development of aquatic life criteria.

#### **4.2**

The recommended shift toward an ecological risk assessment model (recommendation 1), including seeking inputs from diverse perspectives (recommendation 2), and aspects of several other recommendations in this section imply the need for explicit and systematic assessment of the concerns of relevant publics/stakeholders. This in turn implies the need for greater involvement of social and economic sciences in the aquatic life criterion setting process, especially in the context of identifying and prioritizing contaminants of emerging concern.

#### **4.3**

The Committee presents numerous good suggestions for improving Part II of the White Paper. The overall theme of many of these suggestions might be more forcefully presented in the Committee review—that the EE2 case should be presented more clearly as an example of the aquatic life water quality criterion setting process, rather than as a case study that is important in its own right (although the latter is certainly true). In that regard, more frequent and elaborated discussions of how the EE2 is similar to and contrasts with analyses for other classes of CECs and how the example illustrates points raised in Part I would be very useful. That is, the EE2 case could be used more forcefully to illustrate important issues and principles applicable across the breadth of CECs.

#### **4.4**

Perhaps the most important suggestion for implementing the recommendations in the White Paper is the need for some effective means to prioritize CECs and the related need for data to support the

development of criteria that are relevant to an expanded set of ecological and social goals (e.g., protection of ecosystems and ecosystems services). Consistent with the recommended risk assessment model and with the comments noted above, such prioritization can be facilitated by greater involvement of publics/stakeholders and relevant social sciences. Related to effective prioritization, there is also a need for some consistent classification of CECs into categories relevant to aquatic life criteria. The white paper, and the comments of the Committee suggest that mode of action may be a very useful basis for such classifications, as well as for addressing issues of mixtures of multiple contaminants and of environmental pulses and concentrations.

#### **5. Dr. Rogene Henderson**

I found this advisory to be exceptionally well-written. The charge questions were addressed in a clear and logical fashion and the recommendations were well-supported in the text. The tone of the report was supportive of the work of the Agency but the report also gave strong recommendations that should help the Agency improve their approach.

#### **6. Dr. David Allen**

Aquatic Life Water Quality Criteria Advisory: no comments.

#### **7. Dr. John Balbus**

a) Are the original charge questions to the SAB Panel adequately addressed in the draft report?

*Yes; the report is clearly organized according to the charge questions.*

b) Is the draft report is clear and logical?

*Yes; it is clearly written and appears logical.*

c) Are the conclusions drawn, and/or recommendations made, supported by information in the body of the report?

*Yes, the conclusions appear to be well supported by the text.*

#### **8. Dr. Valerie Thomas**

Overall, this advisory is well written, addresses the charge questions, is clear and logical, and the conclusions are supported by the body of the report. There are a few points which could be clarified, as discussed below:

Letter to the Administrator, p. 2, second paragraph: This paragraph would be more clear if the phrase “create a conceptual model to guide development of aquatic life criteria for CECs. Such a conceptual model should” were cut. By making this cut, the second sentence of the paragraph would read “In particular we urge EPA to include consideration of probably direct and/or indirect...” I think this would improve the clarity of the paragraph message because it would

emphasize the issues (topic of the first sentence of the paragraph) the Committee recommends EPA consider. If it is important to mention conceptual models, a new sentence could be added: “These issues could be incorporated through development of a conceptual model.”

Executive Summary, p. xiv, lines 15-10. “Mixtures of CECs.... Therefore research is needed.” The overall discussion of the importance of mixtures throughout the document, and in particular the discussion of the availability of approaches from pharmacology to identify the potential impact of mixtures suggests that the Committee may not have meant simply to recommend more research, but to actually recommend that the potential effect of mixtures be incorporated into the aquatic life criteria. In particular, page xviii says “As stated previously, aquatic life criteria for CECs, should take into account the fact that aquatic organisms are exposed to mixtures of these chemicals.” But, as far as I can see, this was not state previously. On page 8, lines 19-27, the Committee does state that “Consideration of mixture effects is important.... The Committee feels strongly that mixture effects of compounds ... should be taken into account.” The strength of this recommendation is not reflected in the Executive Summary.

Executive Summary, p. xviii, lines 16-17. “we recommend that the Agency ... customize and update the 1985 Guidelines.” This is an excellent and key point; this should probably also be stated in the Letter to the Administrator.

Executive Summary, p. xviii, line 23: “(2) developing a robust conceptual model.” It is not clear what this really means. The implication is that the EPA currently does not have a “concept” on which the criteria are based; this five-word phrase does not make clear what will be the benefit of the model, and it is not clear what a robust versus non-robust model is. Perhaps this would be more clear if the Committee said what content would be included. For example, a phrase might go something like this (the Committee would need to develop its own content: “(2) going beyond the fate and direct effects of CECs by including, at least at the level of a conceptual model, consideration of probable direct and or indirect impacts on food webs, ecological processes and services, unique, endangered or keystone species or species of special societal value or concern.” (Text is taken from p. 24 lines 39-43.)

Executive Summary, p. xviii. “As stated previously, aquatic life criteria for CECs, should take into account the fact that aquatic organisms are exposed to mixtures of these chemicals.” As far as I can see, this was not state previously.

Page 8, lines 19-27, “Consideration of mixture effects is important....The Committee feels strongly that mixture effects of compounds ... should be taken into account.” This idea is again emphasized on p. 9 lines 15-17. The strength of this recommendation is not reflected in the Executive Summary.

p. 24 line 39-p. 25, line 8. This is the discussion of the conceptual model approach that is heavily recommended by the Committee. This discussion does not mention how such a model might or might not be “robust.” Especially because the committee emphasizes the criteria for determination of robustness in other parts of the document, the Committee could add discussion of the robustness issue here, or drop the word “robust” from discussion of the conceptual model approach in the Executive Summary (p. xviii, line 23; also page 26 line 20).

p. 37, lines 27-28: “As previously discussed, the Committee recommends that EPA incorporate the use of conceptual site models....” Where were “site models” previously mentioned? Is this the same as the recommendation for “robust conceptual models”? Should all of these models be site models? Is a site model a model for one type of location (site) only, and if so, how many site models would be needed for a single CEC?

## **9. Dr. Duncan Patten**

**General Comment.** In all three cases, the SAB review committees have offered excellent review and advice to EPA. The reviews are comprehensive and in sufficient detail to allow EPA staff to reconsider their positions on topics of concern and to rewrite or rework the materials presented in the white papers.

In order to fully assess the responses of the SAB review committee, one would have to be more expert in the particular field of science than I am. Thus my comments are more general, but specific in some cases.

Here is an aside comment on Cumulative Effects and Synergism relevant to two of the reviews.

One question that comes to my mind as I read the reviews, and thus responses to EPA questions, especially those for “Aquatic Life Water Quality” and “Drinking Water Contaminant Candidate List” deals with the concepts of “cumulative effects” and “synergism” in effects of contaminants. Why aren’t these concepts considered more critically in testing or selecting contaminants of concern? Only in the Aquatic Life Water Quality review is the concept of synergism (page 11) even considered, and apparently only in passing. Are not the synergistic interactions as well as cumulative effects among and within contaminants of importance in selection and testing of toxic effects?

Specifically on the SAB Advisory on Aquatic Life Water Quality Criteria for Contaminants of Emerging Concern.

Initial comments are tied to the Executive Summary which offers most of the points of the review. The comments tend to point out the importance of an SAB panel response rather than to point out omissions or weaknesses. This is because the panel has done an excellent job of responding to EPA’s questions.

The amendments to EPA's "contaminant continuous concentration" proposed by the SAB review committee (e.g., page xiii) include cautionary statements which are very appropriate here but may be appropriate where other suggested changes or procedures are offered. Perhaps precaution should be a guiding rule for both EPA in selection of "tools" and SAB in its suggestion of alternatives.

The suggestion that EPA should "place greater emphasis on information useful for development of aquatic life criteria, rather than just toxicity test requirements" (e.g. page xv) gets to the heart of the review. The white paper was to offer guidelines for "development of aquatic life criteria" without creating some form of sideboards such as toxicity tests.

Comments dealing with use of "non-resident" species again offer guidance of precaution in their use. Good guidance to those who must rewrite the white paper.

In its response to using endpoints (e.g., page xvii), the SAB panel recommends use of "non-traditional measures" (e.g., line 14, page xvii)... One assumes this means "non-traditional" sublethal endpoints... is that what was meant?

In "involvement of an Expert Panel," the SAB panel suggests developing "specific guidance of the role of expert panels" (line 42, page xvii). They probably should also suggest establishing criteria for selection of expert panels for specific CECs.

Response to Technical Issues, the SAB panel recommends "obtaining a wide range of inputs from diverse perspectives (line 23, page xviii). The panel should suggest what they mean, for example, literature, experts, practitioners?

Executive Summary dealing with Part II of white paper on Aquatic Life Water Quality.

The SAB panel makes an excellent recommendation which might be useful for other EPA efforts when they say "the process outlined for EE2 might be applied to other substances, particularly for those for which less data are available and which have different modes of action." (Lines 22-24, page xix).

Page xx, line 22. Is there some reason why only one species (fathead minnow) is cited here?

In the main body of the text, under section addressing "concerns regarding taxonomic coverage..." (pages 10-11), the SAB panel comments under "modes of action are not known for some CECs, that "different organisms may be affected in different ways by the same compound both as adults and at earlier stages..." and that "there is also the potential for synergism among CECs in mixtures and in interactions with environmental variables." This point should have a major

emphasis as tests of CECs are done individually and this does not represent most conditions found outside the laboratory.

**10. Dr. Bernd Kahn:**

I have read the three draft Reviews and consider them to be well written.

**11. Dr. LD McMullen:**

I have read the documents and have found them to be well organized and easy to follow. I believe they answer the charge questions that were provided to the committee. These documents are not in my area of expertise and as such I have little to add on there technical merit.

I realize that mixing zone for the discharge is not a part of the Water Quality Standard. However, it is important to realize that some aquatic life find wastewater discharges a nutrient rich environment and will spend a significant amount of time in the discharge plume.

**12. Dr. Timothy Buckley:**

The ALC report looks rock solid. It is well organized and clearly responsive to the charge questions. I have no suggested edits or revisions.

**13. Dr. Jerry Schnoor:**

I have read the 43 page report from the Ecological Processes and Effects Committee (EPEC) of the SAB reviewing the EPA Agency Draft White Paper on Aquatic Life Criteria for Contaminants of Emerging Concern (CEC), and I find it to be an excellent report. It is well written, well organized, and full of important recommendations regarding a process that is central to the Agency's mission of protecting aquatic life.

The EPEC Report clearly speaks to the charges from the EPA to the Science Advisory Board on: 1) Reviewing the technical merit, practicability, and implementability of the White Paper; 2) Identifying the appropriate issues for deriving aquatic life criteria; 3) Providing suggestions for improving the utility of the Part II ethynlestradiol (EE2) case example; and 4) Providing guidance on implementing the recommendations.

The Executive Summary is rather long (10 pages), but it reflects quite accurately the discussion and recommendations found in the body of the report. Regarding EPEC's review of Part II, the case example on EE2, I might add that the Agency could probably benefit from the exercise of Part II with several other CECs of differing modes of action such as polybrominated diphenyl ethers (PBDEs), bisphenol A, and perfluorinated octynyl sulfonate (PFOS). These are also problematic and controversial CECs that have raised questions in the mind of the public over the capability of EPA's risk assessment procedures and aquatic life criteria to protect health and the environment. Also, these chemicals which differ from the stated concern in the White Paper over pharmaceutical and personal care products entering the aquatic ecosystem from wastewater treatment plants. But

they are nonetheless important and instructive case studies that might shed new light on revising the outdated 1985 Guidelines document. The EPEC Report recognizes this possibility in several parts of the report (p. 29, lines 29-35 and lines 40-44). I applaud the call to rely more on risk-based considerations, weight-of-evidence, and increased use of judiciously chosen expert panels to improve the 1985 Guidelines document and procedures.

Some specific comments on the EPEC Committee Report follow.

- a) Page xvii, lines 29-30) on vitellogenin as a biomarker. The fourth point here is a little unclear and not quite the way that the point is expressed in the main text of the Committee's report. I believe the report is overly cautious and subtly varies in its recommendations regarding vitellogenin as a biomarker (see page 22, lines 31-32, and page 23 lines 14-16). Gender alteration is listed as an important biological effect in Figure 1 on page 25. Certainly, if the sex ratio in humans changed due to chemical exposure, it would be an endpoint of considerable concern (not simply a biomarker of exposure). "Evidence of absence" of population change is not the same as "absence of evidence", and our techniques for detecting population changes *in situ* may not be sufficiently sophisticated for endocrine disruptors. In this case, a more precautionary approach may be recommended.
- b) Page xviii, lines 34-45. I whole-heartedly agree with this Committee recommendation. Grouping chemicals by their modes of action is a good research strategy for EPA. We need creative methods of simplifying the process if possible. It may accelerate EPA's ability to make ALC determinations and improve their efficiency. However, the Committee contradicts itself a bit on page 11 (lines 16-24) when it states that modes of action are not well known, and it casts some doubt on the whole exercise. In balance, I am in favor of recommending the grouping of chemicals by dominant modes of action, at least as a matter of research during the development of ALCs.
- c) Page xx, lines 17-27. I may have missed it, but I did not see any discussion of EC<sub>10</sub> and EC<sub>20</sub> in the main text of the report, only here in the Executive Summary.
- d) Page 9, lines 8-9. One order of magnitude seems a little excessive to me in Recommendation #3. I would suggest 1-2 orders of magnitude allowing some judgment regarding the uncertainty of the data and the possibilities of unmeasured pulses of chemical discharge.
- e) Page 10. References should be Brain et al., 2007 (not 2008); and Pennington et al., 2001.

#### **14. Dr. Steve Roberts:**

The panel has done an impressive job responding to charge questions related to a review of the subject EPA White Paper. Each of the charge questions is addressed in full, and the responses are clearly articulated. The organization of

the report is excellent, making the discussion and recommendations on specific topics easy to find and follow. The recommendations are logical and should be valuable to the Agency, both in finalizing the White Paper and in creating a scientifically sound process for developing aquatic life criteria for contaminants of emerging concern. I have no criticisms of the report.



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October 19, 2008

ATTACHMENT N

Mr. Tom Miller  
Designated Federal Officer  
Science Advisory Board  
US Environmental Protection Agency  
1200 Pennsylvania Avenue, NW  
Washington, DC 20460

Re: SAB white paper on aquatic life criteria for emerging contaminants

Dear Mr. Miller,

Thank you for the opportunity to comment on the EPA Science Advisory Board white paper on aquatic life criteria for contaminants of emerging concern (CEC). I am well aware of the issues of endocrine disrupting contaminants and literature on the topic, as I recently finished my PhD studying endocrine disruption in fish. I currently work on a project at UC Davis deriving pesticide water quality criteria and am also familiar with the EPA's 1985 Guidelines for setting water quality criteria.

Overall I find the approach to criteria for CECs to be technically sound and a timely response to Dr. Kidd's 2007 study about the collapse of a minnow population in the whole lake study. I agree that alternatives are needed for contaminants that do not display typical acute toxicity and to allow for the calculation of chronic criteria. Additionally I agree with the methods used to derive such criteria including: the call for full life cycle testing, the move to a "guilty until proven innocent" approach, that taxa shown to not be affected should not be required to set criteria, the use of data from compounds with similar MOA (mechanism of action) to show that certain taxa are insensitive, and use of biomarker data (such as vitellogenin) to demonstrate insensitive taxa.

My first comment relates to the main concerns over estrogenic compounds, which is occurrence of intersex and reproduced abnormalities in larger, longer lived fish species, such as carp, flatfish, bass, and sturgeon. Almost none of the data presented in the example reflect these species. The one data point used that does reflect these type of fish was for rainbow trout and was inconclusive (<16 ug/L). With no NEOC reported, the proper data for the EPA requirement for species in the family salmonidae is lacking. This is discussed in section 3.3 (in Part 2) of the report, in rearguards to rainbow trout "Actual criteria development will require a decision whether to (a) require more information for this species, (b) use other information to help estimate rainbow trout sensitivity or (c) justify setting the MDR aside." While I agreed with the methodology, I did not agree with this statement. According to the methodology outlined in part one, knowing that trout are sensitive and a species of concern would seem to make options "c" certainly unacceptable. It would be better to have this section more clearly in agreement with part one, which

appeared straight forward in that setting the MDR aside should only be an option for insensitive species. The white paper also discusses the preference for full life cycle tests, especially it seems, for known sensitive species. Considering that 1) rainbow trout is a species that best represents those for which there is concern, 2) the data obtained so far show that it is sensitive, and 3) that the partial life cycle test is not unreasonable to perform for this species, I feel that it should clearly be required, if not a full life cycle test.

Though minnows are fairly closely related to carp and fulfill the warm water fish requirement, we do not know that fish with short reproductive cycles are good surrogates for longer lived species ( as is discussed in section 3.4). With hormone mimics (and likely other contaminants with chronic effects) differences in life history may well make a difference in the chronic toxic effects. For instance a fish species with a longer time of sexual development could be more susceptible to endocrine disruption, because it will be open to influence of contaminants for a much longer time. Although studies with larger, longer lived fish are more difficult, this data is key for endocrine disruptors as these are the animals in which effects are observed in the wild.

My second comment is related to the first. EE2 (ethynylestradiol) represents an ideal case in the large amount of data that has been generated, but still it is lacking important information, as discussed above. If there is insufficient data for the case of EE2 how are these methods to be implemented for other CECs? Some of this was touched on in the methodology, using data from compound with a similar MOA, but this is unlikely to provide adequate data for the variety of CEC with different mechanisms noted in the white paper. Realistically, how will there be enough data to set limits for more than a few compounds? It is worth considering where this data will come from, as well as the responsibility of industry in providing adequate data. Without the data, the methods in white paper will have limited practical use.

Overall, the approach seems well thought out, however in the EE2 example it became apparent that situations that warrant use of substitute data should be more clearly defined, including situations when MDR should certainly be met, ie: when the required species is thought to be one of the sensitive species and standard tests would adequately address toxicological concerns. It seems very dangerous to suggest in the EE2 example that the most desirable data may not be required. Such data should be obtained so that a criterion for EE2 may be derived soon. I urge EPA to consider where the burden of generating such data should lie, as limited data is likely to be a common problem in setting effective criteria for CECs.

Sincerely,

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## Attachment O

### SAB Comments on Aquatic Life Water Quality Criteria for Contaminants of Emerging Concern

#### 1. Dr. David Allen

Aquatic Life Water Quality Criteria Advisory: no comments.

*Response - No changes needed.*

#### 2. Dr. John Balbus

a) Are the original charge questions to the SAB Panel adequately addressed in the draft report?

*Yes; the report is clearly organized according to the charge questions.*

b) Is the draft report is clear and logical?

*Yes; it is clearly written and appears logical.*

c) Are the conclusions drawn, and/or recommendations made, supported by information in the body of the report?

*Yes, the conclusions appear to be well supported by the text.*

*Response - No changes needed.*

#### 3. Dr. Greg Biddinger

In general the SAB panel has adequately addressed the draft Agency report and provided a clear and logical advisory.

As well, the conclusions drawn and recommendations are supported in the body of the advisory.

With the exception of the comments and recommendations for further consideration the draft advisory is worth forwarding to the Administrator.

SAB EPEC should cite their other guidance on Ecological Risk Assessment.

The SAB panel advisory recommends that ‘to the extent practicable’ the aquatic life criteria guidance for CECs should follow risk-based principles. This appears as a major recommendation in the cover letter, executive summary, and body of the report. This advisory should be linked to EPEC recommendations for improving ecological Risk Assessment developed through a workshop (February 7-8, 2007). A summary report of this workshop was published (January 2007) by the SAB subsequently in open literature

in SETAC journal *Integrated Environmental Assessment and Management* (V. Dale et al., 2008).

There are a number of references throughout the advisory that suggest the Agency incorporate ecological processes or link effects to population level impacts. These are explored in detail in the SAB review of Ecological Risk Assessment and the Agency should be directed to those discussions. The panel should look for opportunities in the executive summary and body of the advisory to highlight previous SAB EPEC advice on ecological risk improvements.

*Ecological Risk Assessment in Environmental Decision-Making, an Evaluation of the State of the Practice: A Workshop of the EPA Science Advisory Board Ecological Processes and Effects Committee, February 7-8, 2006. 120 pp.*

***Response - The previous SAB EPEC advice on ecological risk assessments will be highlighted and referenced at appropriate places in the report.***

It is not clear why the SAB Panel feels it is inappropriate to not calculate Criterion Maximum Concentration (acute value) in all cases. I suggest that the advisory panel reconsider this recommendation.

Although the Agency expects to make all of the appropriate acute toxicity data for CECs available for reference, they have suggested there is no need to calculate a CMC value for all compounds. It is not clear why the SAB Advisory panel has supported this position. Suggest the panel reconsider this position. There are certain field applications in which a CMC might be useful. For example if setting standard for effluent from an industrial operation which is designed to be a short-term batch operation, then considerations of chronic exposures from CECs in effluent don't apply. Additionally, an evaluation of risks associated with instantaneous emergency releases over a short period of time would need to be considered. Having an agency position on the CMC in the ALC guidance seems like a relatively easy calculation and a consistency that would add value.

The committee seems to sense there are some exceptions such as exposures linked to pulsed discharges, mixtures or disparate classes of CECs such as nanoparticles and called for them to be included in acute analysis. Suggest that the SAB panel may not catch all the exceptions so it would be more complete to follow a consistent approach of calculating both a CMC and CCC even and especially if the modes of action are different.

***Response – The report will be revised to indicate that in cases of emergency release of CECs, the potential for acute toxicity would need to be considered. Therefore, criteria documents for CECs should identify the CMC as a data gap when it is not used to derive criteria.***

Believe the committee missed an opportunity to discuss “Representative Species” as well as “resident species.”

While the panel has done a good job of discussing issues related to non-resident species, they should have also discussed a preferable concept of representative species. An underlying assumption is that non-resident species do not represent the response expected from native species in a geographic area. It is more important to consider the ecophysiological make-up of a species and its alignment with the ecological conditions in which the exposure occurs than the geographic home range of the species. It would be easy to postulate a case where resident or native warm water species are not as representative of risks to resident cold water species as the response of a non-resident cold water species which occupies the same or similar niche in a different geography.

The panel may want to consider adding a paragraph addressing this point.

***Response: Additional text will be inserted into discussion of non-resident species to emphasize the importance of selecting “representative” species to support criteria development.***

#### **4. Dr. Timothy Buckley**

The ALC report looks rock solid. It is well organized and clearly responsive to the charge questions. I have no suggested edits or revisions.

***Response - No changes needed.***

#### **5. Dr. Terry Daniel**

*The original charge questions to the SAB Panel are adequately addressed in the draft report, the report is clear and logical, and the conclusions and recommendations are supported by the information in the body of the report.*

Some suggestions for extensions to some sections of the Committee review are presented below.

4.1.1 The Committee recommends that the White Paper pay greater attention to the possible interactions within “mixtures” of similar contaminants (especially viz. mode of action) and to the potential for environmental “pulses” of higher than normal concentrations to occur frequently in some contexts. A similar issue not specifically noted is the potential for periodic environmental concentration of contaminants as may occur, for example, in ephemeral water bodies due to evaporation. Of course, mixtures of contaminants and pulse/concentration phenomena might also interact to further magnify toxic effects.

***Response - The sentence on page 8 line 11 will be revised as follows: “...recurring natural events such as fluctuations in environmental concentrations of contaminants in ephemeral water bodies due to evaporation and...”***

4.1.2 The issue of minimum data requirements with regard to taxonomic coverage is a classic “proof of the null hypothesis” problem (“proof of innocence” in the white paper), and the Committee has appropriately noted the need for a clear and explicit specification of criteria for determining when data and understanding are sufficient for determining no effect. In this context, the suggestion presented later (4.2) of revising/updating the Guidelines in the direction of the Guidelines for Ecological Risk Assessment is appropriate here as well. A major factor in determining “proof of innocence” should be an assessment of the potential consequences of incorrectly concluding that a contaminant would have no effect (i.e., the payoff matrix). The ecological data requirements for supporting a conclusion of no effect (i.e., the level of “power” deemed sufficient for detecting a specified consequential effect) depend at least in part on an assessment of the social and biological values at risk and the potential for consequential losses. Moreover, because current goals extend to the protection of ecosystems and their services, rather than individual targeted organisms or specific sub-systems, there is a greater need to assure that biological assessments adequately address a broad range of taxa and environmental contexts.

***Response - The following new paragraph will be inserted on page 10, line 22: “As further discussed in section 4.2 of this report, the derivation of aquatic life criteria should be risk-based and include consideration of probable direct and/or indirect impacts on food webs; ecological processes and services; and unique, endangered, and sensitive species. Thus, a major factor in determining that toxicity test data are not needed for particular taxa should be an assessment of the potential consequences of incorrectly concluding that a contaminant would have no effect. The ecological data requirements for supporting a conclusion of no effect (i.e., the level of “power” deemed sufficient for detecting a specified consequential effect) depend at least in part on an assessment of the social and biological values at risk and the potential for consequential losses. Moreover, because goals for aquatic life criteria should extend to the protection of ecosystems and their services rather than individual targeted organisms or specific sub-systems, there is a need to assure that biological assessments adequately address a broad range of taxa and environmental contexts.”***

4.1.3 The use of non-resident species models is well addressed by the Committee. This section additionally provides an opportunity to extend the Committee’s discussion of how to define “resident species” to include how global climate change and other factors potentially make this distinction a moving target. It seems clear that resident species is a “social construct,” and so some explicit involvement of publics/stakeholders in identifying appropriate species targets in given environmental/social contexts, and in determining the relevance of data based on surrogate non-resident species would seem both useful and prudent.

***Response - The following text will be inserted on page 14, at the end of line 32: “In this regard, the Committee notes that global climate change and other factors associated with the migration of organisms potentially make the definition of resident or non-resident species a moving target ”***

4.1.4 The relevance of a risk assessment approach noted above for taxonomic coverage issues applies equally well to decisions about the sufficiency of partial versus full life-cycle tests (perhaps extending to trans-generational testing) for determining chronic toxicity effects. Such decisions would seem to require a consideration of tradeoffs between the costs of additional testing and the values at risk and potential losses from missing an important effect. That is, such decisions cannot be made on the basis of biological data considerations alone.

***Response - The following text will be inserted at the end of line 43 on page 17. "In this regard, it is noted that the decision to use data from partial versus full life cycle and or multigenerational tests appears to require a consideration of tradeoffs between the costs of additional testing and the social and biological values at risk and potential losses from missing an important effect."***

4.1.5 The use of sub-lethal/"non-traditional" endpoints for toxicity assessments raises a number of issues addressed by the White Paper and refined by suggestions of the Committee. The rough implicit model is that biological changes in individual organisms (in response to toxins) may produce changes in individual characteristics and behavior which may have implications for populations (and on to ecosystems). In that context, and consistent with the points raised by the Committee in recommendation 4, it should be noted that the model may at times work backwards, with social factors affecting individual behavior which in turn affects individual neurological and other systems and functions.

***Response - The following text will be added at the end of line 18 on page 20: "The implicit model for considering behavioral endpoints is that biological changes in individual organisms in response to contaminants may produce changes in individual characteristics and behavior which may have implications for populations and ecosystems. It is also noted, however, that social factors can affect the behavior of individuals, which in turn can affect neurological and other systems and functions."***

4.1.6 The discussion of expert panels emphasizes their use as a means for overcoming gaps in bio-ecological data and information. Consistent with the recommended move toward a risk assessment model (4.2) and with the issues raised in 4.1.2-4 above, this discussion might be extended to include both a wider range of disciplines (especially social sciences and economics) and some involvement of relevant publics/"stakeholders." This may have been intended, but is not fully communicated by the call for a "balanced range of perspectives" in expert panels used for the development of aquatic life criteria.

***Response - The following text will be inserted at the end of line 39 on page 23: "It is noted that implementing a risk-based approach to deriving aquatic life criteria that protect ecosystems as well as their valued services will necessitate including social scientists, economists and relevant publics/stakeholders on expert panels."***

4.2 The recommended shift toward an ecological risk assessment model (recommendation 1), including seeking inputs from diverse perspectives

(recommendation 2), and aspects of several other recommendations in this section imply the need for explicit and systematic assessment of the concerns of relevant publics/stakeholders. This in turn implies the need for greater involvement of social and economic sciences in the aquatic life criterion setting process, especially in the context of identifying and prioritizing contaminants of emerging concern.

***Response - The following text will be inserted at the end of line 15 on page 25: "It is important to note that several of the following recommendations (e.g., the recommended shift toward an ecological risk assessment model and the recommendation to seek inputs from diverse perspectives) will require explicit and systematic assessment of the concerns of relevant publics/stakeholders. This in turn will require greater involvement of social and economic sciences in the aquatic life criterion setting process, especially in the context of identifying and prioritizing contaminants of emerging concern."***

4.3 The Committee presents numerous good suggestions for improving Part II of the White Paper. The overall theme of many of these suggestions might be more forcefully presented in the Committee review—that the EE2 case should be presented more clearly as an example of the aquatic life water quality criterion setting process, rather than as a case study that is important in its own right (although the latter is certainly true). In that regard, more frequent and elaborated discussions of how the EE2 is similar to and contrasts with analyses for other classes of CECs and how the example illustrates points raised in Part I would be very useful. That is, the EE2 case could be used more forcefully to illustrate important issues and principles applicable across the breadth of CECs.

***Response - The following text will be inserted before the second sentence on page 29, line 24: "Therefore, the EE2 illustrative example should be presented more clearly as an illustration of the aquatic life water quality criterion setting process, rather than the derivation of a criterion for a specific CEC that is important in its own right (although the latter is certainly true). In this regard, more frequent and elaborated discussions of how the EE2 is similar to and contrasts with analyses for other classes of CECs, and how the example illustrates points raised in Part I, would be very useful. That is, the EE2 example could be used more forcefully to illustrate important issues and principles applicable across the breadth of CECs."***

Perhaps the most important suggestion for implementing the recommendations in the White Paper is the need for some effective means to prioritize CECs and the related need for data to support the development of criteria that are relevant to an expanded set of ecological and social goals (e.g., protection of ecosystems and ecosystems services). Consistent with the recommended risk assessment model and with the comments noted above, such prioritization can be facilitated by greater involvement of publics/stakeholders and relevant social sciences. Related to effective prioritization, there is also a need for some consistent classification of CECs into categories relevant to aquatic life criteria. The white paper, and the comments of the Committee suggest that mode of action may be a very useful basis for such classifications, as well as for

addressing issues of mixtures of multiple contaminants and of environmental pulses and concentrations.

***Response - The following sentence will be inserted after the first sentence on line 32, page 35. "It is noted that compilation of a list of priority CECs can be further facilitated by greater involvement of publics/stakeholders and relevant social sciences. Related to effective prioritization of CECs for criteria derivation is the need for consistent classification of CECs into categories relevant to aquatic life criteria. As suggested in other parts of this report, mode of action may be a very useful basis for such classifications, as well as for addressing the issues of mixtures of multiple contaminants and of environmental pulses and concentrations."***

## **6. Dr. David Dzombak**

*(a) Are the original charge questions to the SAB Panel adequately addressed in the draft report?*

The SAB Ecological Processes and Effects Committee (EPEC) review panel has addressed all of the charge questions. Each of the charge questions appears to be addressed in sufficient depth, and specific recommendations have been developed for each of the charge questions and sub-questions.

*(b) Is the draft report clear and logical?*

The organization of the draft report by the SAB EPEC review panel follows the charge questions directly and is easy to follow. The Executive Summary is rather long considering the short length of the main report, but I don't think it's a problem and would not recommend condensing the Executive Summary further. There are two issues in the draft report that I recommend be addressed to improve clarity and strengthen it.

(1) I have identified a statement made in the letter to the Administrator, in the Executive Summary, and in the body of the report that is somewhat misleading and that I recommend be clarified. This statement is made on page 2 of the letter, on page xii of the Executive Summary, and on pages 25 and 37 of the main report. The version that is in the letter to the Administrator serves to illustrate my concern.

“The derivation of aquatic life criteria needs to be risk-based, using a transparent and consistent framework that provides necessary flexibility not presently possible within the algorithm approach of the 1985 Guidelines. Hence, the SAB recommends that, to the extent practicable, the derivation of aquatic life criteria be risk-based using the principles defined in EPA's 1998 *Guidelines for Ecological Risk Assessment*.”

The recommendation that EPA risk assessment procedures for determination of aquatic life criteria make direct use of the 1998 Guidelines is fine, but the preamble to this recommendation as given here and at the other locations in the report cited above implies that the 1985 Guidelines do not involve risk assessment, and thus that risk assessment has

not been previously employed in establishing aquatic life criteria. This is not the case, and I recommend that the recommendation about use of the 1998 Guidelines be reworded to clarify the nature of the 1985 Guidelines.

***Response – The second sentence on page ii, line 4 will be revised as follows: “The 1985 Guidelines established a complex process to evaluate risk by using information from many areas of aquatic toxicology. The SAB finds that the derivation of aquatic life criteria needs to be based on a risk assessment model that provides a transparent and consistent framework and necessary flexibility not presently possible within the algorithm approach of the 1985 Guidelines.”***

***The second sentence on line 20, page xii will be revised as follows to indicate that the 1985 Guidelines do evaluate risk. “The Guidelines specify various data and procedural recommendations for evaluating risk and deriving criteria and also define general risk management goals for the criteria.”***

(2) In the Executive Summary (page xiii, bullet 1; page xxi, bullets 1 and 4) and in the main report (page 33, bullet 2; pages 36-37, item 1; and perhaps elsewhere), the review panel discusses the potential for contaminants of emerging concern (CECs) to be from classes of chemicals other than pharmaceuticals and personal care products, and recommends that the Agency “consider expanding the definition of CECs to include chemicals and other substances of increasing environmental concern due to anthropogenic activities and inadequate regulatory approaches.” The review panel recommends that the Agency “look for opportunities to leverage EPA research with ongoing research in other federal agencies, international agencies, and industry groups.” These are useful and important observations and recommendations, but not mentioned in any of the discussion is the TSCA new product review process in which data are supplied by chemical manufactures in relation to the pre-manufacture notification required under TSCA. The search for possible CECs should begin at this stage. The TSCA new product review and its relationship to aquatic life criteria determination is not discussed in the report at all. At a minimum, aquatic life toxicity data provided by manufacturers in this process could be used to help set aquatic life criteria. There are other possibilities that could be considered, such as integrating parts of the aquatic life criteria establishment process into the TSCA new product review to aid in the assessment of the new product notifications. Also, data and other information supplied for the new product review could help the Agency prioritize CECs. Whatever the level of integration the review panel believes is appropriate, the main point is that aquatic life criteria determination for CECs should be conducted with knowledge of the data for new chemical products coming into commercial use provided by the TSCA new product review process.

***Response - The following new bullet will be inserted on page 34, line 10 (this point will also be included in the executive summary): “Aquatic life criteria determination for CECs should be conducted with knowledge of data provided by the Toxic Substances Control Act (TSCA) new product review process. Chemical manufacturers provide data to EPA on new products in accordance with the TSCA pre-manufacture notification requirements. The search for possible CECs should begin at this stage. At***

*a minimum, aquatic life toxicity data provided by manufacturers in this process could be used to help derive aquatic life criteria. EPA could also consider integrating parts of the aquatic life criteria setting process into the TSCA new product review to aid in the assessment of the new product notifications. Data and other information supplied for the new product review under TSCA could also help the Agency prioritize CECs for aquatic life criteria derivation.”*

*Text beginning on page 37, line 2 will be rewritten as follows: “In this regard, we recommend that EPA’s Office of Water and Office of Research and Development look for opportunities to leverage existing research and data collection activities with those ongoing in other federal agency and EPA programs, similar programs with international agencies, and industry groups in order to gather the data needed to develop aquatic life criteria. In particular, aquatic life criteria determination for CECs should be conducted with knowledge of data provided by the Toxic Substances Control Act new product review process.”*

(c) *Are the conclusions drawn, and/or recommendations made, supported by the information in the body of the draft SAB report?*

The conclusions drawn and recommendations made are supported by the information in the body of the draft report.

***Response - No changes needed.***

**7. Dr. Rogene Henderson**

I found this advisory to be exceptionally well-written. The charge questions were addressed in a clear and logical fashion and the recommendations were well-supported in the text. The tone of the report was supportive of the work of the Agency but the report also gave strong recommendations that should help the Agency improve their approach.

***Response - No changes needed.***

**8. Dr. Bernd Kahn**

I have read the three draft Reviews and consider them to be well written.

***Response – No changes needed.***

**9. Dr. Meryl Karol**

a) *Are the original charge questions to the SAB Panel adequately addressed in the draft report?*

The draft report clearly addresses the charge questions.

b) *Is the draft report clear and logical?*

The report is superb; clear and logical. One of the best reports I have read

- c) *Are the conclusions drawn, and/or the recommendations made supported by information in the body of the report?*  
Yes.

***Response - No changes needed.***

**10. Dr. LD McMullen**

I have read the documents and have found them to be well organized and easy to follow. I believe they answer the charge questions that were provided to the committee. These documents are not in my area of expertise and as such I have little to add on there technical merit.

I realize that mixing zone for the discharge is not a part of the Water Quality Standard. However, it is important to realize that some aquatic life find wastewater discharges a nutrient rich environment and will spend a significant amount of time in the discharge plume.

***Response - No changes needed. The issue of mixing zones is important but somewhat beyond the scope of this review. EPA's water quality standards regulation allows states to adopt provisions authorizing mixing zones for NPDES permitting. Criteria continuous concentrations and criteria maximum concentrations provide guidance for the development of acute and chronic criteria in state water quality standards that may be exceeded in mixing zones.***

**11. Dr. Duncan Patten**

**General Comment.** In all three cases, the SAB review committees have offered excellent review and advice to EPA. The reviews are comprehensive and in sufficient detail to allow EPA staff to reconsider their positions on topics of concern and to rewrite or rework the materials presented in the white papers.

In order to fully assess the responses of the SAB review committee, one would have to be more expert in the particular field of science than I am. Thus my comments are more general, but specific in some cases.

Here is an aside comment on Cumulative Effects and Synergism relevant to two of the reviews.

One question that comes to my mind as I read the reviews, and thus responses to EPA questions, especially those for "Aquatic Life Water Quality" and "Drinking Water Contaminant Candidate List" deals with the concepts of "cumulative

effects” and “synergism” in effects of contaminants. Why aren’t these concepts considered more critically in testing or selecting contaminants of concern? Only in the Aquatic Life Water Quality review is the concept of synergism (page 11) even considered, and apparently only in passing. Are not the synergistic interactions as well as cumulative effects among and within contaminants of importance in selection and testing of toxic effects?

***Response - The Committee has stressed the importance of considering the interactive effects of criteria. However, in the absence of a specific methodology to derive criteria for mixtures, the Committee has recommended additional research in this area and noted that in the future criteria may be revised up or down to account for the interactive effects of contaminants with similar modes of action.***

Specifically on the SAB Advisory on Aquatic Life Water Quality Criteria for Contaminants of Emerging Concern.

Initial comments are tied to the Executive Summary which offers most of the points of the review. The comments tend to point out the importance of an SAB panel response rather than to point out omissions or weaknesses. This is because the panel has done an excellent job of responding to EPA’s questions.

The amendments to EPA’s “contaminant continuous concentration” proposed by the SAB review committee (e.g., page xiii) include cautionary statements which are very appropriate here but may be appropriate where other suggested changes or procedures are offered. Perhaps precaution should be a guiding rule for both EPA in selection of “tools” and SAB in its suggestion of alternatives.

***Response - No changes needed. Thank you for the comment, the Committee has included cautionary statements various parts of the report.***

The suggestion that EPA should “place greater emphasis on information useful for development of aquatic life criteria, rather than just toxicity test requirements” (e.g. page xv) gets to the heart of the review. The white paper was to offer guidelines for “development of aquatic life criteria” without creating some form of sideboards such as toxicity tests.

***Response - No changes needed. The Committee has recommended that EPA consider revising the 1985 Guidelines to incorporate risk assessment principles that did not exist when the Guidelines were developed over 20 years ago.***

Comments dealing with use of “non-resident” species again offer guidance of precaution in their use. Good guidance to those who must rewrite the white paper.

***Response - No changes needed.***

In its response to using endpoints (e.g., page xvii), the SAB panel recommends use of “non-traditional measures” (e.g., line 14, page xvii)... One assumes this means “non-traditional” sublethal endpoints... is that what was meant?

***Response - The sentence on page xvii line 4 will be revised as follows to clarify this point. “In the White Paper, EPA has identified a number of endpoints that could be considered (in addition to the “traditional” endpoints of survival, growth, and reproduction) in developing aquatic life criteria for CECs.”***

In “involvement of an Expert Panel,” the SAB panel suggests developing “specific guidance of the role of expert panels” (line 42, page xvii). They probably should also suggest establishing criteria for selection of expert panels for specific CECs.

***Response - Given the range of issues to be addressed by expert panels, we would prefer not to develop a prescribed set of criteria for selection of panel members beyond the guidance that has been developed by the Science Advisory Board staff office. The SAB process is mentioned in the report (on page 24, line 6) as a model to be considered.***

Response to Technical Issues, the SAB panel recommends “obtaining a wide range of inputs from diverse perspectives (line23, page xviii). The panel should suggest what they mean, for example, literature, experts, practitioners?”

***Response - This point is discussed in more detail on page 26, lines 5-14. To reflect this, the second sentence on page xviii will be revised as follows: “In particular, as further discussed in section 4.2 of this report, these principles should address...”***

Executive Summary dealing with Part II of white paper on Aquatic Life Water Quality.

The SAB panel makes an excellent recommendation which might be useful for other EPA efforts when they say “the process outlined for EE2 might be applied to other substances, particularly for those for which less data are available and which have different modes of action.” (Lines 22-24, page xix).

***Response - No changes needed.***

Page xx, line 22. Is there some reason why only one species (fathead minnow) is cited here?

***Response - The fathead minnow is specifically mentioned here because it is a very commonly used test species. We would prefer not to change this sentence.***

In the main body of the text, under section addressing “concerns regarding taxonomic coverage...” (pages 10-11), the SAB panel comments under “modes of action are not known for some CECs, that “different organisms may be affected in different ways by the same compound both as adults and at earlier stages...” and that “there is also the potential for synergism among CECs in mixtures and in interactions with environmental variables.” This point should have a major emphasis as tests of CECs are done individually and this does not represent most conditions found outside the laboratory.

***Response - In various parts of the report the Committee has pointed out the importance of considering interactive effects. This point has been mentioned in the letter to the Administrator, executive summary and in the main body of the report.***

## **12. Dr. Steve Roberts**

The panel has done an impressive job responding to charge questions related to a review of the subject EPA White Paper. Each of the charge questions is addressed in full, and the responses are clearly articulated. The organization of the report is excellent, making the discussion and recommendations on specific topics easy to find and follow. The recommendations are logical and should be valuable to the Agency, both in finalizing the White Paper and in creating a scientifically sound process for developing aquatic life criteria for contaminants of emerging concern. I have no criticisms of the report.

***Response – No changes needed.***

## **13. Dr. Jerald L. Schnoor**

I have read the 43 page report from the Ecological Processes and Effects Committee (EPEC) of the SAB reviewing the EPA Agency Draft White Paper on Aquatic Life Criteria for Contaminants of Emerging Concern (CEC), and I find it to be an excellent report. It is well written, well organized, and full of important recommendations regarding a process that is central to the Agency’s mission of protecting aquatic life.

The EPEC Report clearly speaks to the charges from the EPA to the Science Advisory Board on: 1) Reviewing the technical merit, practicability, and implementability of the White Paper; 2) Identifying the appropriate issues for deriving aquatic life criteria; 3) Providing suggestions for improving the utility of the Part II ethynlestradiol (EE2) case example; and 4) Providing guidance on implementing the recommendations.

The Executive Summary is rather long (10 pages), but it reflects quite accurately the discussion and recommendations found in the body of the report. Regarding EPEC’s review of Part II, the case example on EE2, I might add that the Agency

could probably benefit from the exercise of Part II with several other CECs of differing modes of action such as polybrominated diphenyl ethers (PBDEs), bisphenol A, and perfluorinated octynyl sulfonate (PFOS). These are also problematic and controversial CECs that have raised questions in the mind of the public over the capability of EPA's risk assessment procedures and aquatic life criteria to protect health and the environment. Also, these chemicals which differ from the stated concern in the White Paper over pharmaceutical and personal care products entering the aquatic ecosystem from wastewater treatment plants. But they are nonetheless important and instructive case studies that might shed new light on revising the outdated 1985 Guidelines document. The EPEC Report recognizes this possibility in several parts of the report (p. 29, lines 29-35 and lines 40-44). I applaud the call to rely more on risk-based considerations, weight-of-evidence, and increased use of judiciously chosen expert panels to improve the 1985 Guidelines document and procedures.

***Response - The following text will be inserted on page 29, line 35. "Other CECs with differing modes of action such as polybrominated diphenyl ethers (PDBEs), bisphenol A, and perfluorinated octynyl sulfonate (PFOS) could be considered. These are problematic and controversial CECs and concerns about these chemicals differ from the stated concern in the White Paper over pharmaceutical and personal care products entering the aquatic ecosystem from wastewater treatment plants. They are nonetheless important and instructive case studies that might shed new light on revising the 1985 Guidelines."***

Some specific comments on the EPEC Committee Report follow.

Page xvii, lines 29-30) on vitellogenin as a biomarker. The fourth point here is a little unclear and not quite the way that the point is expressed in the main text of the Committee's report. I believe the report is overly cautious and subtly varies in its recommendations regarding vitellogenin as a biomarker (see page 22, lines 31-32, and page 23 lines 14-16). Gender alteration is listed as an important biological effect in Figure 1 on page 25. Certainly, if the sex ratio in humans changed due to chemical exposure, it would be an endpoint of considerable concern (not simply a biomarker of exposure). "Evidence of absence" of population change is not the same as "absence of evidence", and our techniques for detecting population changes *in situ* may not be sufficiently sophisticated for endocrine disruptors. In this case, a more precautionary approach may be recommended.

***Response - The statements in the executive summary and main body of the report (page 23, lines 8-16) reflect the Committee's view that vitellogenin is a biomarker of exposure but its linkage to population effects is limited. We would prefer not to revise this section of the report.***

Page xviii, lines 34-45. I whole-heartedly agree with this Committee recommendation. Grouping chemicals by their modes of action is a good research strategy for EPA. We need creative methods of simplifying the process if possible. It may accelerate EPA's ability to make ALC determinations and improve their efficiency. However, the Committee contradicts itself a bit on page 11 (lines 16-24) when it states that modes of action are not well known, and it casts some doubt on the whole exercise. In balance, I am in favor of recommending the grouping of chemicals by dominant modes of action, at least as a matter of research during the development of ALCs.

***Response - On page 11, the Committee states that modes of action are not known for some CECs, that a known mode of action may not be the only mode of action, and that different organisms may be affected in different ways as adults and juveniles. The Committee has advised EPA to consider this when deciding whether test data are needed for certain taxa. We would prefer to keep these statements in the report.***

Page xx, lines 17-27. I may have missed it, but I did not see any discussion of EC<sub>10</sub> and EC<sub>20</sub> in the main text of the report, only here in the Executive Summary.

***Response - The following text will be inserted on page 31 at the end of line 25: "The selection of a specific EC<sub>x</sub> value for derivation of an aquatic life criterion depends upon the level of protection or effect that decision-makers are willing to accept or detect in the field. However, an EC<sub>20</sub> has been used for most species and an EC<sub>10</sub> has been used for threatened and endangered species."***

Page 9, lines 8-9. One order of magnitude seems a little excessive to me in Recommendation #3. I would suggest 1-2 orders of magnitude allowing some judgment regarding the uncertainty of the data and the possibilities of unmeasured pulses of chemical discharge.

***Response - The sentence on page 9, lines 8-9 will be revised to state that: "The Committee recommends that CMCs be derived for compounds where LOECs are found to be within 1-2 orders of magnitude of LC50s."***

Page 10. References should be Brain et al., 2007 (not 2008); and Pennington et al., 2001.

***Response: This correction will be inserted.***

#### **14. Dr. Valerie Thomas**

Overall, this advisory is well written, addresses the charge questions, is clear and logical, and the conclusions are supported by the body of the report. There are a few points which could be clarified, as discussed below:

Letter to the Administrator, p. 2, second paragraph: This paragraph would be more clear if the phrase “create a conceptual model to guide development of aquatic life criteria for CECs. Such a conceptual model should” were cut. By making this cut, the second sentence of the paragraph would read “In particular we urge EPA to include consideration of probably direct and/or indirect...” I think this would improve the clarity of the paragraph message because it would emphasize the issues (topic of the first sentence of the paragraph) the Committee recommends EPA consider. If it is important to mention conceptual models, a new sentence could be added: “These issues could be incorporated through development of a conceptual model.”

***Response: This change will be included in the letter.***

Executive Summary, p. xiv, lines 15-10. “Mixtures of CECs.... Therefore research is needed.” The overall discussion of the importance of mixtures throughout the document, and in particular the discussion of the availability of approaches from pharmacology to identify the potential impact of mixtures suggests that the Committee may not have meant simply to recommend more research, but to actually recommend that the potential effect of mixtures be incorporated into the aquatic life criteria. In particular, page xviii says “As stated previously, aquatic life criteria for CECs, should take into account the fact that aquatic organisms are exposed to mixtures of these chemicals.” But, as far as I can see, this was not state previously. On page 8, lines 19-27, the Committee does state that “Consideration of mixture effects is important.... The Committee feels strongly that mixture effects of compounds ... should be taken into account.” The strength of this recommendation is not reflected in the Executive Summary.

***Response - The Committee has stressed the importance of considering the interactive effects of CECs in mixtures. However, in the absence of a specific methodology to derive criteria for mixtures, the Committee has recommended additional research in this area and noted that in the future criteria may be revised up or down to account for the interactive effects of contaminants with similar modes of action. The sentence on page xviii, line 35 will be revised as follows: “It is important that aquatic life criteria for CECs take into account the fact that aquatic organisms are exposed to mixtures of these chemicals. As more information is developed on CECs, it is possible that water quality criteria may be revised up or down for individual CECs based upon data on joint interactions. Use of such data would produce more risk-based criteria.”***

Executive Summary, p. xviii, lines 16-17. “we recommend that the Agency ... customize and update the 1985 Guidelines.” This is an excellent and key point; this should probably also be stated in the Letter to the Administrator.

***Response – The sentence on page ii, line 11 will be revised as follows: “Within the context of risk-based aquatic life criteria we recommend that EPA consider***

***a number of issues in addition to those identified in the White Paper, and that the Agency customize and update the 1985 Guidelines to address these issues.”***

Executive Summary, p. xviii, line 23: “(2) developing a robust conceptual model.” It is not clear what this really means. The implication is that the EPA currently does not have a “concept” on which the criteria are based; this five-word phrase does not make clear what will be the benefit of the model, and it is not clear what a robust versus non-robust model is. Perhaps this would be more clear if the Committee said what content would be included. For example, a phrase might go something like this (the Committee would need to develop its own content: “(2) going beyond the fate and direct effects of CECs by including, at least at the level of a conceptual model, consideration of probable direct and or indirect impacts on food webs, ecological processes and services, unique, endangered or keystone species or species of special societal value or concern.” (Text is taken from p. 24 lines 39-43.)

***Response - The conceptual model is discussed in more detail in section 4.2 of the report. We would like to reference that section for more detail. Line 23 on page xviii will be revised as follows: “...2) as further discussed in Section 4.2 of this report, developing a conceptual model that addresses more than fate and direct effects of CECs...”***

Executive Summary, p. xviii. “As stated previously, aquatic life criteria for CECs, should take into account the fact that aquatic organisms are exposed to mixtures of these chemicals.” As far as I can see, this was not stated previously.

***Response – See revision above.***

Page 8, lines 19-27, “Consideration of mixture effects is important....The Committee feels strongly that mixture effects of compounds ... should be taken into account.” This idea is again emphasized on p. 9 lines 15-17. The strength of this recommendation is not reflected in the Executive Summary.

***Response – See revision above.***

p. 24 line 39-p. 25, line 8. This is the discussion of the conceptual model approach that is heavily recommended by the Committee. This discussion does not mention how such a model might or might not be “robust.” Especially because the committee emphasizes the criteria for determination of robustness in other parts of the document, the Committee could add discussion of the robustness issue here, or drop the word “robust” from discussion of the conceptual model approach in the Executive Summary (p. xviii, line 23; also page 26 line 20).

***Response – Discussion of how the conceptual model might or might not be robust is somewhat beyond the scope of the report. Therefore, the word “robust” will be removed on line 23, page xviii and line 30, page 26.***

p. 37, lines 27-28: “As previously discussed, the Committee recommends that EPA incorporate the use of conceptual site models....” Where were “site models” previously mentioned? Is this the same as the recommendation for “robust conceptual models”? Should all of these models be site models? Is a site model a model for one type of location (site) only, and if so, how many site models would be needed for a single CEC?

*Response – The word “site” will be removed on page 37, line 28.*

#### **15. Dr. Thomas Wallsten**

I have read the three draft reviews. It appeared to me that all three adequately addressed the charge questions, were logically laid out, and provided supporting information for their conclusions and recommendations. I have three comments on the reports:

- a) The review of the White Paper on "Aquatic Life Criteria for Contaminants of Concern" mentioned the use of expert panels to provide professional judgment during criteria development (Section 4.1.6). I concur that such panels can be very useful. My question is whether EPA has, or has not considered, guidelines for how such panels should operate to assure careful, unbiased judgmental extrapolations from available data to end points of concern?

*Response: The Committee’s report provides a number of recommendations concerning the use of expert panels. Developing additional guidelines for how such panels would operate is somewhat beyond the scope of this report. Guidance has been developed by the Science Advisory Board staff office. The SAB process is mentioned in the report (on page 24, line 6) as a model to be considered.*

- b) The same white paper urges that attention be paid to the possible effects of mixtures of contaminants, not just contaminants acting alone. This point would seem to apply to the "SAB Advisory on EPA's Third Drinking Contaminant Candidate List," yet I did not see it mentioned there (although I may have missed it).

*Response: No changes needed.*

- c) Finally, only the review of "Toxicological Review of Acrylamide" included a list of abbreviations. While some acronyms are common (e.g., LOEL, NOEL, DNA), others may be unique to specific fields or topics (e.g., CEC, ROPC, WBDO). It would helpful for all reports to have a list acronyms.

*Response – A list of acronyms will be included in the report.*

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WASHINGTON D.C. 20460

OFFICE OF THE ADMINISTRATOR  
SCIENCE ADVISORY BOARD

[Date]

EPA-SAB-09-00

Honorable Stephen L. Johnson  
Administrator  
U.S. Environmental Protection Agency  
1200 Pennsylvania Avenue, N.W.  
Washington, D.C. 20460

**Subject: SAB Advisory on EPA's Draft Third Drinking Water Contaminant Candidate List (CCL 3)**

Dear Administrator Johnson,

EPA's Office of Ground Water and Drinking Water requested that the Science Advisory Board (SAB) Drinking Water Committee (hereafter, the Committee) provide advice on EPA's Draft Third Drinking Water Contaminant Candidate List (CCL 3) and the process used to derive it. EPA is required to publish this Contaminant Candidate List (CCL) every five years. This draft CCL 3 includes 93 chemicals or chemical groups and 11 microbiological contaminants that are known or anticipated to occur in public water systems. Contaminants on the CCL will be considered by the Agency for a regulatory determination.

The Committee believes that the process used to produce the draft CCL 3 represents an improvement over the former processes. While the draft CCL 3 uses a more data-driven and systematic approach, internal EPA expert panels were used to identify potential shortcomings of the data analysis, and ultimately, many decisions were still based on the expert judgment of EPA staff. The Committee views the current process as a first step toward a reformed CCL process, and acknowledges that, as recommended by EPA's National Drinking Water Advisory Council (NDWAC), the process should be designed as an adaptive process that will improve with further experience and data. The Committee's comments on the limitations of the current process should be viewed in this context.

The Committee believes that the documentation of processes that produced the draft CCL 3 still lacks transparency. EPA used professional judgments of its internal experts to revise the

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1 process in a way that was designed to change the contaminants on the list. The Committee was  
2 not concerned that the process underwent mid-course corrections, because such changes are part  
3 of the desired, adaptive assessment process. However, the Committee was concerned that these  
4 modifications by Agency staff were not readily apparent in the current documentation. The  
5 Committee expressed some concern that the lack of clarity could impede the ability of others to  
6 understand the basis for decisions about the CCL, an enunciated criterion for transparency made  
7 during the reviews by the National Research Council and NDWAC. The Committee also  
8 recommends that EPA document and justify why certain contaminants which were included on  
9 previous CCL lists were excluded from the draft CCL 3. This will improve readers'  
10 understanding of the evolution of the process as well as its transparency.

11  
12 In addition to increasing the transparency of the process, the Committee has  
13 recommendations for improving the CCL selection process. The Committee believes that the  
14 draft CCL 3 includes contaminants that should not be considered for regulation and excludes  
15 contaminants that should be considered for regulation. For example for chemicals, the  
16 Committee suggested that the EPA should evaluate whether pesticides that were about to be  
17 cancelled completely should be on the list for additional SDWA regulation. This determination  
18 could be made after some assessment of use, occurrence (transport and fate), and particularly  
19 persistence, which will help to determine if the agent as used previously would have any ongoing  
20 contamination issues. This will assist in the determination in whether the agent should be  
21 regulated or not; in some cases, these types of pesticides may not require regulation. The  
22 Committee recognizes that at least some evaluation of cancelled pesticides would be necessary so  
23 as not to be shortsighted on the Agency's part. For pathogens, the Committee noted that two  
24 globally important waterborne pathogens, *Adenovirus* and *Mycobacteria*, were excluded from the  
25 draft CCL 3 and other pathogens, *Vibrio cholera* and *Entamoeba*, were included. Rare  
26 outbreaks, and the outbreak data base in general, played a significant part in placement on the  
27 list, and the Committee has suggestions both for the use of more of the publicly available data, as  
28 well as for more comprehensive use of the databases already used in the CCL process. The  
29 Committee acknowledges that any list will have some contaminants that a panel of experts would  
30 prefer to add or to remove. Nonetheless, there was general agreement that the current process  
31 could be improved to generate a better list.

32  
33 The current process also does not evaluate some of the less direct, potential hazards of  
34 contaminants. Exposure to antibiotics may lead to antibiotic resistant pathogens. The current  
35 CCL process would not identify this impact as a threat to human health. Similarly, secondary  
36 transmission of pathogens by vectors other than drinking water would also not be expected to be  
37 detected as a problem through the current process.

38  
39 The Committee believes that the draft CCL 3 may be too large to fulfill the objectives of  
40 the Agency without prioritizing between the need for regulatory determination and the need for  
41 collection of additional data. For some of the contaminants on the list, there is already ample  
42 evidence of occurrence in public water supplies at concentrations that pose public health  
43 concerns. In some cases, failure of the Agency to make regulatory determination on these  
44 contaminants is causing uncertainty among utilities and has led to individual states setting action  
45 levels or guidelines. To alleviate some of these uncertainties and to assure protection of public  
46 water supplies throughout the entire nation, EPA needs to place a high priority on making

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1 regulatory determinations, or the collection of data critical to making final regulatory  
2 determinations, as part of the CCL process. Many other contaminants on the draft CCL 3 have  
3 been included mainly due to a lack of basic data on occurrence and toxicity. These contaminants  
4 should be included in the CCL process. However, the purpose of listing these contaminants is  
5 different and pertains mainly to the manner in which EPA allocates resources for toxicology  
6 research and the collection of occurrence data.

7  
8 Thank you for the opportunity to provide advice on this important process. The SAB  
9 Drinking Water Committee looks forward to receiving your response regarding this advisory.

10  
11  
12 Sincerely,

13  
14  
15  
16  
17 Dr. Deborah Swackhamer, Chair  
18 Science Advisory Board

Dr. Joan B. Rose, Chair  
Drinking Water Committee

**NOTICE**

1  
2  
3 This report has been written as part of the activities of the EPA Science Advisory Board (SAB),  
4 a public advisory group providing extramural scientific information and advice to the  
5 Administrator and other officials of the Environmental Protection Agency. The SAB is  
6 structured to provide balanced, expert assessment of scientific matters related to problems facing  
7 the Agency. This report has not been reviewed for approval by the Agency and, hence, the  
8 contents of this report do not necessarily represent the views and policies of the Environmental  
9 Protection Agency, nor of other agencies in the Executive Branch of the Federal government, nor  
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**U.S. Environmental Protection Agency  
Science Advisory Board  
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1 **Introduction**  
2

3 The 1996 Safe Drinking Water Act (SDWA) Amendments require EPA to publish a list  
4 of heretofore unregulated contaminants that are known or anticipated to occur in public water  
5 systems and may require regulation in drinking water in order to protect public health. EPA is  
6 required to publish this Contaminant Candidate List (or CCL) every five years. Following  
7 publication of the first list (CCL 1) in 1998, the Agency requested a review of the CCL process  
8 from the National Academy of Sciences' National Research Council (NRC), and their  
9 recommendations were published in 2001. NRC proposed a broader, more reproducible process  
10 to identify the CCL. In 2004, EPA's National Drinking Water Advisory Council (NDWAC)  
11 provided suggestions on how to implement the NRC's recommendations to be used for the CCL  
12 3. As this approach was being developed, the second list, CCL 2, was published in 2005. Based  
13 on recommendations from NRC and NDWAC, EPA developed a more data-driven CCL  
14 selection process which was used for development of the CCL 3. The Agency also requested  
15 public nominations for chemical and microbial contaminants for the upcoming CCL 3.  
16 Information regarding the CCL processes and lists can be accessed through the CCL web page  
17 at: <http://www.epa.gov/safewater/ccl/index.html>.

18  
19 Both the new process developed in response to the recommendations of the NRC and  
20 NDWAC, as well as the specific chemicals and microbial pathogens on the draft CCL 3 list,  
21 were subject to review. The charge questions posed by EPA were as follows.  
22

- 23 1. Please comment on whether the Federal Register Notice and support documents are clear,  
24 transparent, and adequate to provide an understanding of the overall processes and  
25 selection of contaminants for the draft CCL 3.  
26
- 27 2. Please comment on whether the draft CCL 3 list represents those contaminants that have  
28 the highest potential to occur in public water systems and cause adverse human health  
29 effects.  
30
- 31 3. Please provide any data that may suggest that contaminants which are currently on the  
32 draft CCL 3 list should not be listed.  
33
- 34 4. Please provide any data that may suggest that contaminants which are currently not on  
35 the draft CCL 3 list should be listed.  
36

37 The Drinking Water Committee (hereafter, the DWC or Committee) of EPA's Science  
38 Advisory Board (SAB) met in a public session on April 23 – 24, 2008 in Washington, DC, to  
39 review the draft CCL 3. The Committee held a subsequent teleconference call on August 13,  
40 2008 to discuss its draft advisory report. The first section of this report presents the general  
41 comments and overall conclusions of the Committee. The second section discusses  
42 recommendations for steps that will make the current process more transparent. The third  
43 section provides suggestions to improve the process when it is used for future CCLs. In the  
44 fourth section, recommendations with regard to specific contaminants are discussed. The fifth  
45 section highlights emerging issues and research needs.

1 **1. General Comments from the Committee**  
2

3 The Committee believes that the process used to produce the CCL 3 (EPA, 2008)  
4 represents a major improvement from the processes used to generate CCL 1 and CCL 2. The  
5 process used to generate the first two lists relied heavily upon expert opinion and best  
6 professional judgment, as well as stakeholder nominations, with the potential health risk  
7 contributing to the first part of the assessment followed secondarily by whether the contaminant  
8 occurred in drinking water. The process for the CCL 3 outlined in the Federal Register Notice  
9 (FRN; EPA, 2008) uses a data-driven, systematic approach, focusing on assessing the  
10 information, including surrogate information to identify contaminants based on both the potential  
11 or known occurrence in drinking water and their potential or known ability to cause adverse  
12 effects in people. As recommended by the NRC and NDWAC, the CCL 3 process attempted to  
13 address the Universe of contaminants and developed a Preliminary CCL (PCCL), using a more  
14 data-driven process. Expert panels were used along the way as part of the review of the  
15 approach. During the assessment, 6000 chemical contaminants and 1400 pathogens were  
16 identified. The Committee views the current process as a first step toward this data-derived  
17 CCL, and acknowledges that, as recommended by the NDWAC, the process should be designed  
18 as an adaptive process that will improve and develop with further experience and data. The  
19 Committee's comments on the limitations of the current process should be viewed in this  
20 context.  
21

22 There are numerous challenges that must be overcome when whittling the initial  
23 "Universe" of contaminants down to a CCL. EPA has documented its decision-making process  
24 and has described its attempts to identify biases in that process and to obtain expert feedback on  
25 the process. In general, the approach is scientifically justified and, particularly for the chemical  
26 list, is an intensified documented process and includes the development of models to create the  
27 chemical list.  
28

29 The Committee found that use only of the data-supported process of the CCL 3 (as  
30 described in the FRN) generated a list of contaminants that was viewed as suboptimal. Based on  
31 the changes made by EPA's panel of internal experts, the Committee infers that EPA's scientists  
32 also agreed that expert judgment was necessary at this time for developing a CCL. Therefore,  
33 EPA requested the opinions of internal experts for professional assessment of chemicals or  
34 pathogens to revise the process and therefore the contaminants on the draft CCL 3. The  
35 Committee was not concerned that, in developing the process, a review was needed and mid-  
36 course corrections were undertaken. Rather, the Committee was more concerned that these  
37 modifications (or suggestions) by Agency staff that were accepted or rejected were not readily  
38 apparent as the Committee reviewed the documentation in the FRN. In addition, the  
39 justifications for the decisions in which expert opinion was accepted or rejected were not  
40 articulated. The Committee expressed some concern that the areas of the process without full  
41 transparency could impede the ability of others to go through the same exercise as the EPA with  
42 the same results when data drove the primary outcome and with a clear understanding of where  
43 experts were used to address key decisions in the process. Such reproducibility was an  
44 enunciated criterion for transparency made by the NRC and NDWAC.  
45

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1           Moreover, this apparent lack of clarity or transparency in the process led to frustration as  
2 Committee members attempted to determine why specific contaminants were retained or  
3 removed from the group of contaminants that would become the draft CCL 3. Committee  
4 members who tried to follow the decision-making process for one or more contaminants could  
5 not do so. Some of the confusion arose from the previously mentioned role of EPA experts in  
6 the process that was not clear to the Committee. Additionally, some of the information about  
7 individual contaminants that had been organized by EPA was only available in the regulatory  
8 docket. Committee members either did not know that the docket might contain that information  
9 or had difficulty locating the docket and/or the information desired. The Committee  
10 recommends that both the FRN and the EPA web sites contain citations for all of these  
11 documents, and that the web site post the documents and/or hyperlinks directly to each  
12 document, as well as the location of the regulatory docket.  
13

14           In addition to improving the transparency of the process in the written documentation, the  
15 Committee had recommendations for the existing and future CCL selection processes. These  
16 suggestions were often based on concerns about contaminants that were either retained or  
17 removed from the evolving CCL. In particular, an explanation should be included for those  
18 contaminants that were on the CCL 1 or CCL 2 but were not included in the new list via the new  
19 process, with the appropriate justification. The DWC acknowledges that any list of contaminants  
20 would have some contaminants that each expert would prefer to add or to remove. Nonetheless,  
21 there was general agreement that the current process could be improved to generate a list that  
22 would contain fewer surprises. For example, members believed that even a cursory sensitivity  
23 analysis could be used to improve the scoring systems and justify the cut-off points that were  
24 used to retain contaminants. Also, knowledge about a pesticide's regulatory status under the  
25 Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and Food Quality Protection Act  
26 (FQPA), particularly whether or not cancellation of all or many uses has been completed or is  
27 underway (e.g., molinate, the organophosphates), might obviate retention in a process designed  
28 to determine whether regulatory action is necessary under SDWA. For example, in the draft  
29 CCL 3, all uses of nitrofen were cancelled in 1983, with use of existing stocks phased out within  
30 a few years. Depending upon Toxics Release Inventory (TRI) releases from just one site in one  
31 year as a surrogate for exposure does not constitute a rationale for considering development of a  
32 national drinking water standard. Pesticides that were no longer in use could be removed from  
33 the list after some preliminary assessment to determine whether the agent previously used had  
34 any ongoing contamination issues. This would include occurrence as well as fate and transport  
35 data, and could be used to help determine whether the contaminant needed to be regulated or not  
36 under SDWA. The Committee recognizes that at least some evaluation of cancelled pesticides  
37 would be necessary, so as not to be shortsighted on the Agency's part.  
38

39           The DWC further believes the list of chemicals on the CCL 3 is too large. Additional  
40 priority ranking based on, for example, availability of data necessary for a regulatory  
41 determination, should be undertaken before chemicals are selected for regulatory review. This  
42 list serves to guide the future safety of drinking water via regulation, to focus research into  
43 methods of water treatment, and to interface with other rules such as the Unregulated  
44 Contaminant Monitoring Rule (UCMR). It is one of the most critical and important activities  
45 within the EPA and thus certainly deserves the efforts that the Agency has devoted to it. The  
46 final list must be viewed within that context.

1  
2 The Committee members also had suggestions for the use of more of the publicly  
3 available data and for the more comprehensive use of the databases already in the CCL 3. In  
4 particular, information in the peer-reviewed, published literature could be effectively used at  
5 certain junctures of the process, especially when the list of chemicals or pathogens considered  
6 for a particular decision is sufficiently small to reduce the burden of a literature search and  
7 retrieval. Similarly, the increasing use of wastewater affected sources of drinking water suggests  
8 that databases containing information on contaminants in wastewater effluents would inform the  
9 CCL process.

10  
11 The Committee discussed specific ways in which the CCL process might need to be  
12 modified in the future. For example, general exposure to antibiotics may lead to antibiotic-  
13 resistant pathogens, but the current CCL process for chemicals would not identify this adverse  
14 effect. Similarly, secondary transmission of pathogens by vectors other than drinking water  
15 would also not be expected to be identified as a problem through the current process.

16  
17 Finally, the Committee’s discussions highlighted emerging issues and research needs for  
18 consideration by EPA for the future. This included, in particular, the identification and obtaining  
19 of data that are appropriate for decisions that are necessary for the optimal operation of the CCL  
20 process.

## 23 **2. Clarifications Regarding Steps In The Process That Will Make It More Transparent**

24  
25 Obtaining the list of contaminants for the draft CCL 3 involved development of a new  
26 contaminant-selection process. The goal of this process was to use data, not just expert opinion,  
27 to derive the list of contaminants. The developing process and the available data affect each  
28 other. Determination of the questions to be answered and the issues to be resolved identify the  
29 essential data. Selection of the databases with specific attributes can determine whether  
30 parameters are estimated directly or when surrogates must be used. Lack of readily available  
31 data can constrain the decision options within the process. The DWC considered these aspects of  
32 the CCL process, as well as their implications on the selection of chemicals and pathogens for  
33 potential regulation.

### 34 35 Models and Selection Processes

36  
37 The process of selecting the CCL 3 involved three major steps: (1) identifying the  
38 “Universe” of contaminants that might be of concern; (2) using data on occurrence and potential  
39 to cause adverse effects to obtain a “Preliminary Contaminant Candidate List” (PCCL); and (3)  
40 using data, processes, and opinions from EPA’s internal experts to refine the selection into a  
41 draft CCL. To improve transparency between CCL 2 and CCL 3, the Committee recommends  
42 that EPA list all contaminants from CCL 2 that are not included in CCL 3, and provide the  
43 reason the contaminant is not on CCL 3.

44  
45 The improvements in the selection process that were recommended by EPA’s internal  
46 experts are consistent with the theme of adaptive management recommended by NWDAC and

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1 endorsed in the FRN. Thus, the methodology for the listings can be adapted as more experience  
2 with the CCL-listing process is gained. The use of internal EPA panels of experts to modify the  
3 process, however, was not clear and transparent to the Committee members. These revised  
4 procedures that were the basis for the recommended CCL 3 need to be more fully explained.  
5 Furthermore, Committee members thought that the CCL 3 list, as modified by the internal  
6 experts, might have been more acceptable if external experts' opinions had also been sought. A  
7 schematic flowchart could be developed which shows where in the process experts (internal or  
8 external) were used (see below).

9  
10 *Chemical Contaminants*

11  
12 The discussion in the FRN regarding the methodology for moving chemicals from the  
13 PCCL to the CCL is organized in a chronological manner. This presentation imports  
14 significance to a complex and somewhat cumbersome initial methodology that was ultimately  
15 subsumed within a new methodological framework proposed by EPA's internal expert panels.  
16 This complex, initial approach was not used to determine which chemicals moved from the  
17 PCCL to the CCL. The actual approach began by dividing the chemical PCCL into three groups  
18 (high, medium, and low uncertainty) depending on the type of data available to characterize the  
19 contaminant. For each of these groups, a new decision rule was developed to determine whether  
20 or not the contaminant should move forward to the CCL. While these decision rules are  
21 indicated in the bullets in Section III.A.4. (page 9644 of the FRN), the explanations attached to  
22 each bullet need to be expanded so that the decision rules are more clearly explicated. Moreover,  
23 since the initial classification model was only used for chemicals in the medium certainty bin,  
24 EPA should "re-train" the model using only training chemicals that would fall into this bin.

25  
26 The DWC suggests developing one or more flowcharts that a stakeholder can use to track  
27 the progress of a contaminant through the system, with the appropriate references and URLs for  
28 each step. Such flowcharts would not only make the process more transparent, but they might  
29 also highlight decisions that might suggest improvements for future CCL processes. Also,  
30 parameters chosen for the models, as well as the stopping rules or specification decisions, should  
31 be provided (in more detail than is provided in Appendix E). To further improve the clarity of  
32 the process, approaches that were discarded should be moved to the end of the document,  
33 perhaps in an appendix. The training set used for calibration should be readily available in the  
34 documentation via links to the web site.

35  
36 The Committee noted that the draft CCL 3 gives equal weight to all chemicals, although  
37 some chemicals are likely to be ready for regulatory determination, while others will require a  
38 significant amount of additional research before a regulatory determination can be made.  
39 Therefore, prioritization within the current CCL is considered important. Additional data and  
40 processes should be used to priority rank those chemicals, by a method that will select chemicals  
41 that have sufficient existing information for a data-based regulatory decision. Priority ranking  
42 contaminants may also require reformulating or retraining the algorithms, since the dependent  
43 variable of the algorithm must now indicate whether a contaminant should be studied for listing,  
44 and with what urgency the contaminant should be studied.

1           The Committee also noted some deficiencies in presentation of the process. Details are  
2 lacking, for example, as to how fate parameters like the octanol/water partition coefficients were  
3 used in the evaluation. Also, all parameters should include the appropriate units, e.g., on LD<sub>50</sub>  
4 and related parameters in Exhibit 9.

5  
6           *Pathogen Contaminants*

7  
8           The process for selection of pathogen contaminants, as outlined in the FRN  
9 documentation, was overall judged a relatively transparent one, however issues emerged with the  
10 approach used that were not resolved. There was an analytical protocol employed; however, it  
11 did not discretely quantify potency, for example, in terms of dose-response relationship as it had  
12 for the chemicals proposed for CCL 3 inclusion. Nonetheless, there was much more of a  
13 quantitative underpinning that was superior to previous CCL formulations that appeared much  
14 more subjective. The sources of information and data that were used in candidate selection are  
15 clear, and the effort to be inclusive in receiving information from non-government organizations  
16 (NGOs), the public, professional organizations, and municipalities is apparent. The development  
17 of the Universe and the PCCL were data driven. However, the resolution of the details of the  
18 information that was used to assign a numerical rating to the pathogen was limited.

19  
20           The process for moving pathogens from the PCCL to CCL is not sufficiently clear. In  
21 particular, it is somewhat ambiguous as to how the ultimate pathogen scores for this process  
22 were developed. For pathogens, it appears that the internal EPA experts adjusted the scoring  
23 system. This adjustment should be presented more prominently. The Committee believes  
24 decisions regarding the selection of data sets and resolution of the information within those data  
25 sets (as discussed further in the next section) were partially responsible for the suboptimal  
26 results. The Committee believes that the relative weighting of Center for Disease Control and  
27 Prevention (CDC) Waterborne Disease Outbreaks (WBDO), “Occurrence,” and “Health Effect  
28 Scoring,” as well as data normalization, is described, but not necessarily transparent. It is  
29 recommended that the limitation of WBDO data sets be articulated clearly, for example, in  
30 regard to underestimation of waterborne disease via a passive surveillance and the percentages of  
31 outbreaks where no etiological agent is identified. Exhibit 15 shows evidence of WBDO using  
32 the CDC surveillance database. Over the more than three-decade period in question, the scoring  
33 system does not differentiate between pathogens that have caused many outbreaks and those that  
34 caused only two outbreaks. Furthermore, scoring of the WBDO data does not appear to take into  
35 account the geographic dispersion of the outbreaks. Also lacking are data on specific, identified  
36 pathogens for the majority of studied outbreaks. Furthermore, a rudimentary sensitivity analysis  
37 of the pathogen-weighting criteria would have demonstrated that the results are not robust to  
38 small changes in the scoring. For example, a change of only "1" unit in WBDO score would  
39 move some organisms on or off the list. Also, the use of “Occurrence” data does not appear to  
40 be a quantitatively robust term, i.e., the 1-to-3 ranking scale may have less utility than initially  
41 expected. An occurrence term of 3 appears only to mean that it has been found in U.S. drinking  
42 water, but not that it is found with any type of frequency or geographic distribution in U.S.  
43 drinking waters. In fact, a score of 3 may mean that it was only found once in drinking water.  
44 Outbreak data were not independent of occurrence, as an outbreak would in and of itself suggest  
45 that the organism had been found in drinking water and influenced that score. This gave the

1 WBDO a greater weight in the ranking. If it were only detected once, the exposure potential,  
2 and therefore the risk, may be quite low.

3  
4 Decisions Regarding Data Sets

5  
6 In several places EPA appears to use data that may not be optimal for its stated intent of  
7 offering equal protection to water consumers. For example, on page 9640 of the FRN,  
8 prevalence is defined as "...the percent of public water systems or monitoring sites across the  
9 nation with detections, number of states with releases..." Neither of these measures takes into  
10 account the number of people who are potentially exposed to contaminants through these  
11 drinking water systems. A contaminant that is found in two or three small states could receive  
12 greater weighting than one found in a large, populous state. The reasons for and implications of  
13 such decisions should be discussed.

14  
15 *Chemical Contaminants*

16  
17 EPA also used a hierarchical approach for data sources to indicate health effects. For full  
18 transparency, the order in this hierarchy of references should be clearly presented. Furthermore,  
19 for food-use pesticides, it would seem more appropriate to use the population-adjusted dose  
20 (PAD), i.e., the dose that incorporates the additional uncertainty factor for children under the  
21 FQPA, rather than the reference dose (RfD) in the calculation of a health reference level (HRL).  
22 Therefore, the Committee recommends that the Agency recalculate the health-concentration  
23 ratios for those pesticides on the PCCL that have PADs smaller than their respective RfDs. It is  
24 possible that additional substances may qualify for inclusion on the draft CCL 3 because their  
25 revised ratio could now be 10 or less.

26  
27 *Pathogen Contaminants*

28  
29 The data used (or more specifically, the data not used) and the resulting pathogens  
30 selected, were not necessarily the optimal set to consider for regulation. For example, a choice  
31 was made by EPA to primarily rely on national data sources and use only data sources with  
32 entries (in this case, for recorded outbreaks) for all of the organisms. This led to heavy reliance  
33 on CDC databases and lack of use of the peer-reviewed, published scientific literature. This  
34 process does not necessarily represent the "best available science." While there was general  
35 agreement that the existence of a WBDO should bring special attention to a microbial pathogen,  
36 the WBDO grading system did not appear to be able to provide a resolution regarding details to  
37 the scoring algorithm; thus, the full breadth or range of data available was not used. For  
38 example, there is no resolution between organisms which have caused outbreaks in the Marshall  
39 Islands [*Cholera*] and an organism that has caused several outbreaks in the continental U.S.  
40 [norovirus and *Campylobacter*]. The potential problems caused by highly endemic diseases that  
41 are never detected as outbreaks are not fully explained. A supplementary table containing the  
42 published, waterborne-attributed, case reports for each of the organisms would be useful. There  
43 is also a lack of data and discussion about the prevalence of organisms in sewage and  
44 wastewater. As a result, organisms such as *Naegleria* or *Vibrio* may receive a pathogen PCCL  
45 score higher than expected because of this weighting for "Occurrence," which is tied to whether  
46 there has been an outbreak. An environmental frequency or distribution score for pathogens,

1 rather than or in addition to its “Occurrence” score, is needed. The ranking and the line that  
2 separated the PCCL from the CCL seemed arbitrary and should be better described (Exhibit 18).

3  
4 Perhaps what is less clear are the effects of the information that was not used in  
5 developing candidates for CCL inclusion. As EPA is aware, the CDC represents the premier  
6 organization in reporting disease statistics and occurrence for organisms typically associated  
7 with waterborne disease. EPA has partnered well with CDC, including evaluating the likelihood  
8 of disease outbreaks, as the consequences of global environmental change become manifest.  
9 CDC also partners with many other organizations and associations in disease surveillance.  
10 Perhaps most notable are state public health offices, responsible for first response in reporting  
11 disease associated with water and food borne exposure. It is presumed that these data are  
12 directly available to the EPA. CDC accesses a boarder base of data, which may or may not be  
13 immediately available to the EPA, as data indicators for PCCL consideration. Some of these  
14 sources include United States Geological Service (USGS) well monitoring programs, or the  
15 National Environmental Health Association (NEHA). NEHA itself has many partner  
16 organizations such as the Council for State and Territorial Epidemiologists (CSTE). Other  
17 organizations such as the Bureau of Environmental Epidemiology (Florida) or the New York  
18 City Department of Environmental Protection, Waterborne Disease Risk Assessment Program,  
19 may prove useful, as other data or sentinel sources of information on outbreaks.

20  
21 At the international level the United Nations Food and Agricultural Organization (UN-  
22 FAO) and World Health Organization (WHO) monitor and report relevant outbreak and disease  
23 incidence. Significantly the European counterpart to the CDC, the European Center for Disease  
24 Prevention and Control (ECDC), continues to develop its waterborne disease and monitoring  
25 program and makes data relatively available through its Enter-net databases for waterborne  
26 disease organisms. It is likely the EPA is aware of all these sources, but it may wish to  
27 investigate whether these and other information channels could facilitate more robust and  
28 quantitative tools in assessment of PCCL consideration and CCL listing.

29  
30 Peer reviewed research articles in journals and periodicals received less attention as data  
31 sources than disease monitoring or surveillance data from other agencies, state, or municipal  
32 sources. Given the relatively limited number of microbial pathogens proposed for inclusion on  
33 the CCL, reviews of the scientific literature are desirable in addition to the sources that were  
34 used to develop this draft CCL 3. Exceptions to the process whereby journal articles were used  
35 for bacteria included publications on *Arcobacter* and *Mycobacterium avium* complex (MAC). It  
36 is likely that other organisms would change position, if outside data and internal and outside  
37 professional judgment were used. The literature may also be more current with respect to  
38 sensitivity, selectivity, and specificity than those derived from some more standard methods.

39  
40 There was discussion in the document about not using susceptibility to water treatment to  
41 guide the selection list. This may be appropriate for the PCCL as well as the CCL. However, as  
42 with the chemicals, prioritization and discussion should be addressed for the list created in regard  
43 to investment in generating more data (on methods, occurrence, and health effects) or rule  
44 development. Thus, if it is believed that the Long Term 2 Enhanced Surface Water Treatment  
45 Rule (LT2ESWTR) or the Ground Water Rule (GWR), for example, already addresses risk  
46 management for specific pathogens, this could begin to be articulated. It does not benefit public

1 health or water science to have a number of pathogens on a CCL that can just be taken off once  
2 they are “controlled” without formally establishing an MCL or treatment technique. Thus, for  
3 example, the large numbers of *Legionella* cases and the fact that no current regulatory approach  
4 can be documented to reduce this risk may place this type of pathogen in a higher priority  
5 category on the CCL.

### 7 Use Of The CCL For Regulatory Decisions

9 The CCL 3, as currently defined, serves two distinct purposes. The first is to identify  
10 unregulated chemicals that might have sufficiently high occurrence and produce adverse effects  
11 of concern that resources might be directed to obtaining more information. Toward this end,  
12 either data on occurrence or data on adverse effects could lead to development of a regulatory  
13 control. In contrast, the second goal is to select those contaminants that should be considered for  
14 imminent regulatory action. In general, such action would require the existence of, rather than  
15 the generation of, information on both occurrence and adversity. Priority setting should use this  
16 criterion, as absent this information, future CCLs will not achieve their stated goal.

18 Finally, the number of contaminants on the CCL keeps increasing in every iteration.  
19 However, regulatory determinations are only made for 5 to 10 contaminants every five years.  
20 The continued increase in contaminants on the list may give the public a sense that water quality  
21 is declining with time. EPA should consider how to address this issue of risk perception in its  
22 documents on the CCL process.

### 24 **3. Suggestions To Improve The Process For Future CCLs**

26 If EPA uses this process again, the Committee believes that it will be important to  
27 incorporate the lessons learned in generating the next CCL. For example for chemicals, the  
28 models will need to take into consideration the level of certainty, and also some measure of the  
29 ratio between the level of concern and the potential drinking water level.

31 The databases used by the EPA in the CCL 3 analyses do not include much of the journal  
32 literature that could be a rich source of information. While these sources might be difficult to  
33 search for the “Universe” of chemicals, these data could more easily be included in the PCCL to  
34 CCL process, especially for the limited number of pathogens. The use of advanced text-  
35 processing software should be investigated for this application. E-government initiatives  
36 throughout the federal government, as well as a lively and innovative academic community, are  
37 potential sources of help for EPA in pursuing this approach. Similarly, use of available  
38 computational toxicology data might improve the selection of chemical contaminants.

40 EPA should consider regulating chemicals with similar sources and mechanisms (or  
41 modes) of action and microbial pathogens with similar potency and disease endpoints (for  
42 example, diarrhea, pneumonia, or meningitis) as groups. The proposed CCL 3 list was  
43 constructed with consideration only about individual chemicals and pathogens. Grouping has  
44 been used for other drinking water contaminants (e.g., trihalomethanes and haloacetic acids)  
45 because occurrence, health effects, and treatment options are related. In the draft CCL 3, (1)  
46 perflourochemicals and (2) acetochlor, metolachlor, and their degradates are two examples

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1 where it may be helpful to list the compounds as a group. In both cases, not all of the  
2 compounds in the group are released from the same source, nor would they likely always occur  
3 together. However, within each group, users could substitute a non-regulated compound for a  
4 regulated one and escape regulatory concern if these contaminants were not grouped.  
5 Additionally, some groups of chemicals may need to be considered in different ways depending  
6 on the goal of the analysis. For example, many nitrosamines have similar toxicities and  
7 carcinogenicities. Therefore, they should be considered together when they co-occur in the same  
8 drinking water samples when evaluating risk. If they do not occur together, if they can not be  
9 used as substitutes, or if they require different treatment methods for removal, grouping for these  
10 purposes is not recommended.

11  
12 The Committee agreed that it will be important to consider information regarding  
13 wastewater concentrations on the exposure side of the assessment. This will be important both  
14 because wastewater discharges are increasingly a greater percentage of water supplies and  
15 because they are being processed into potable water. Also, wastewater contains a wide variety of  
16 contaminants including pharmaceuticals, personal care products, enteric pathogens, and other  
17 emerging contaminants. In the case of pharmaceuticals, perfluorinated surfactants, and other  
18 contaminants that are prevalent in wastewater effluent, EPA may want to consider using data  
19 obtained in wastewater effluent monitoring programs for the CCL screening process. Large  
20 water systems may be subjected to significant discharges of wastewater effluent, and it is likely  
21 that the concentrations of contaminants measured in wastewater effluent could be used as a  
22 surrogate for concentrations in raw water. An approach for predicting the role of unplanned  
23 wastewater reuse that may be appropriate for predicting concentrations in raw water sources is  
24 presented in Anderson et al. (2004).

25  
26 The listing criteria for chemicals should also consider including an element that evaluates  
27 analytical methods used to quantify the chemical contaminant concentrations in occurrence data.  
28 Without a “standard” method and an established detection limit, the quality of the occurrence  
29 data will reflect the self-documented capabilities of the laboratories doing the analytical work.  
30 There can be significant differences in the analytical capabilities of the laboratories that must be  
31 accounted for when reviewing the occurrence data. As a result, some members of the Committee  
32 cautioned against using the 90th percentile of the measured water concentrations in combination  
33 with a 10-fold ratio. It is clear that, for the very skewed distributions of contaminant  
34 concentrations in water, some water utilities could be in a zone of concern, and the chemical  
35 would still be screened off the list, using the existing criteria and algorithm.

36  
37 Significant limitations in understanding which microbial pathogens were considered for  
38 the CCL 3 list include the lack of occurrence data, very limited surveillance for most of the  
39 microbial pathogens, and the broad range of potential health effects. The CDC WBDO database,  
40 for example, is widely acknowledged to be an incomplete reflection of the true number of  
41 outbreaks, and it does not capture the burden of disease relating to endemic, lower level  
42 transmission. Thus, the Committee considers its concerns regarding the pathogens selected for  
43 the CCL 3 to be a signal for the acquisition of better data on occurrence and surveillance  
44 regarding human disease. In general, given the small numbers of pathogens, greater details from  
45 the data sets could be used as well as endemic disease rates. Data on occurrence is particularly  
46 poor, and thus the literature on surveys will require more scrutiny. The Committee recommends

1 that same exceptions made for *Aerobacter* and MAC in how a WBSO is defined should be  
2 applied to the other pathogens for which there is are high-quality, peer-reviewed reports.

3  
4 Some contaminants that may be considered in the future may need a different algorithm  
5 for the selection process. For example, concern about general exposure to antibiotics includes  
6 the development of antibiotic-resistant pathogens that would not be measured in the current score  
7 for adverse effects. Similarly, secondary transmission of pathogens and that effect on burden of  
8 disease might require additional considerations. While the index case might be due to exposure  
9 from drinking water, subsequent transmission might be by a variety of vectors. This issue is  
10 neither discussed in the document nor addressed in the current process.

11  
12 We recommend that EPA to include the DWC earlier in the process. Requesting advice  
13 from the DWC throughout the process, and not just at the end, would allow EPA to take better  
14 advantage of the expertise of the DWC.

#### 15 16 **4. Contaminant-specific Recommendations**

17  
18 The Committee members were surprised by some of the chemicals and pathogens that  
19 made the list, and by some that did not. The members acknowledge that any procedure would  
20 likely include contaminants that individual experts believe should or should not be included in  
21 the CCL. Furthermore, the members did not attempt to recreate the CCL process. Nonetheless,  
22 the Committee recommended reconsideration of certain aspects of the process that might  
23 enhance the utility of the CCL.

24  
25 The Committee experts in pathogens had not expected to see *Entamoeba histolytica* and  
26 *Vibrio cholerae* on the CCL list, and they were surprised not to see *Adenovirus* or *Mycobacteria*.  
27 As discussed earlier, the weighting of documented outbreaks on health effects, and the approach  
28 used regarding occurrence ranking, moved *Entamoeba* and *Vibrio* higher on the list. If endemic  
29 disease, numbers of outbreaks, and geographic locations and venues, as well as better assessment  
30 on occurrence had been used, these two globally important waterborne pathogens would have  
31 moved off the list for the U.S. Information on endemic disease and occurrence in water, based  
32 on the literature, would have moved the *Adenovirus* and *Mycobacteria* on to the list. Expert  
33 opinion, both internal and external, would likely have questioned *Vibrio* and *Entamoeba* on the  
34 CCL. Other countries' environmental agencies look to the EPA's CCL. Thus, when the system  
35 that is used reveals pathogens that are no longer considered waterborne disease risks in the U.S.,  
36 the reasons for this should be addressed and the data based numerical approach should be  
37 investigated and corrected. Health effect scoring should distinguish acute from chronic effects.  
38 The potential for pathogen occurrence in ambient waters could be considered based on  
39 contaminants occurrence in wastewater (as described in the previous selections). Thus, the  
40 Committee believes that the data sets selected, the scoring process used, and the poor occurrence  
41 information may have significantly influenced these results and it is clear that the process can be  
42 improved.

43  
44 The Committee experts in chemicals had not expected to see pesticides for which all uses  
45 had been cancelled on the CCL (e.g., nitrofen; see earlier comment). Similarly, they questioned  
46 the value of considering, for additional SDWA regulation, those pesticides for which

1 cancellation of all or many uses is in progress (e.g., molinate, the organophosphates). The  
2 isomers of hexachlorocyclohexane that were on or off the list did not appear appropriate, and  
3 other pesticides that did not appear on the CCL 3 that were mentioned as potentially worthy of  
4 listing included some for which information was provided to EPA by public commenters. The  
5 absence of data on the occurrence of pharmaceuticals in surface waters was also noted, and it  
6 was thought that use of the data from the USGS, or any of the numerous studies in the peer-  
7 reviewed literature, would have included these chemicals. Also, is a consensus among experts  
8 that N-nitrosodimethylamine (NDMA), methyl tertiary butyl ether (MTBE), perchlorate, and  
9 perfluorooctanoic acid (PFOA) should be a high priority for consideration by the Agency,  
10 because there is a higher degree of certainty about their toxicity, occurrence, and treatability. In  
11 contrast, proposed CCL chemicals such as germanium, hexane, and quinoline appear to be on the  
12 list mainly because they scored highly in one category (e.g., production volume for hexane and  
13 toxicity for germanium). The Committee believes that these chemicals may be of a lower  
14 priority for regulatory action at this time.

## 17 **5. The Future: Emerging Issues and Data Needs**

18  
19 As discussed in the previous sections, the Committee concluded that the CCL 3 is a major  
20 improvement on the previous CCL process. While some of the limitations may be overcome by  
21 using existing data more effectively, the Committee recognizes that additional data would serve  
22 to increase the effectiveness of selection of contaminants both for priority research and/or  
23 possible regulation. Key areas to improve the process must be explored and addressed in the  
24 future include: sensitivity analysis, data uncertainty, and data quality.

25  
26 There are also some clear categories of contaminants that need special attention. These may  
27 be on the PCCL or in the Universe. These include pharmaceuticals, personal care products,  
28 endocrine disruptors, antibiotics, and algal toxins. Opportunistic pathogens (e.g., *Serratia* and  
29 *Pseudomonas*) should also be addressed in the future, as waterborne disease in hospital settings  
30 has been documented.

## 33 **References**

34  
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37 Schwab, B. W. 2004. Screening analysis of human pharmaceutical compounds in US surface  
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39  
40 Environmental Protection Agency (EPA). 2008. Drinking Water Contaminant Candidate List 3.  
41 Draft. Federal Register 73:9628-9654.

## ATTACHMENT Q

### SAB Comments on Draft Drinking Water Contaminant Candidate List 3 Panel Report -UPDATE

#### 1. Dr. LD McMullen:

I had the opportunity to be part of the process in developing the first CCL as part of the National Drinking Water Advisory Council. We also helped in developing some of the ideas for the development of the second CCL.

I have read the document and have found it to be well organized and easy to follow. I believe it answers the charge questions that were presented to the committee.

On page 9 first paragraph, I think an example might helpful such as an addition or removal. This could be helpful to the agency and make sure that the point is not missed. This is done very well in the second paragraph on page 9.

The direction of the last paragraph on page 9 I agree with. However, I got a little lost in the process proposed. There are several different types of data needed for regulation. It seemed that the message was not to put anything on the list until all or most of the data was available. I don't think that was the intent of the CCL. We may want to talk about that.

On page 10 second paragraph, I think I agree with the intent of the paragraph. However, it could be made a little more clear, by stating it was pathogens in the water that have been exposed to antibiotics in the water, or maybe I don't understand the point correctly.

On page 15 last paragraph and on to the top of page 16, I agree with the idea if we are sure that the science is there to support the idea. I did not follow the discussion of substitute a non-regulated compound for a regulated one. An example might be of help.

On page 16 first fall paragraph, I agree with the statement that in some areas wastewater discharges can make up a significant portion of a water treatment plant raw water source. However, there are many areas of the country where that is not the case such as the Midwest and Great Lakes area. We may want to qualify the paragraph a little more. Also, do we know that the NPDES monitoring results have emerging contaminants? I don't think it is common for municipal discharges.

#### 2. Meryl Karol:

a) *Are the original charge questions to the SAB Panel adequately addressed in the draft report?*

Yes

b) *Is the draft report clear and logical?*

The draft report is logical and, in general, clear. However, the following lines would benefit from some careful editing:

p. 2 lines 23-31

p. 8 lines 40-43

p.12 lines 17-18

c) *Are the conclusions drawn, and/or the recommendations made supported by information in the body of the report?*

Yes

d) *Errors/omissions*

p. 2 line 20 change as follows: .....in the determination ~~in~~ of whether....

p. 11 line 7 The flowchart was not included

### **3. Dr. James Sanders:**

Are the charge questions adequately addressed?

Yes, the Committee addressed the charge questions adequately. While this draft report is brief, each of the questions is discussed, and the comments herein should help to improve the process for listing contaminants in the future.

Is the report clear and logical?

For the most part, the report is clear. There are some typographical errors, and some wording that is not clear to me. For example:

p. 11, line 13. Does the committee mean “impart” instead of import?

p. 11, line 24. What are training chemicals?

p. 14, line 12. Broader, not boarder.

Are the conclusions supported?

The Committee has provided appropriate comments and recommendations. Their efforts should improve the process in the future.

### **4. Dr. Thomas Wallsten:**

I have read the three draft reviews. It appeared to me that all three adequately addressed the charge questions, were logically laid out, and provided supporting information for their conclusions and recommendations. I have three comments on the reports:

- a) The review of the White Paper on "Aquatic Life Criteria for Contaminants of Concern" mentioned the use of expert panels to provide professional judgment during criteria development (Section 4.1.6). I concur that such panels can be very useful. My question is whether EPA has, or has not considered, guidelines for how such panels should operate to assure careful, unbiased judgmental extrapolations from available data to end points of concern?

- b) The same white paper urges that attention be paid to the possible effects of mixtures of contaminants, not just contaminants acting alone. This point would seem to apply to the "SAB Advisory on EPA's Third Drinking Contaminant Candidate List," yet I did not see it mentioned there (although I may have missed it).
- c) Finally, only the review of "Toxicological Review of Acrylamide" included a list of abbreviations. While some acronyms are common (e.g., LOEL, NOEL, DNA), others may be unique to specific fields or topics (e.g., CEC, ROPC, WBDO). It would be helpful for all reports to have a list of acronyms.

## **5. Dr. Terry Daniel:**

*The original charge questions to the SAB Panel are adequately addressed in the draft report, the report is clear and logical, and the conclusions and recommendations are supported by the information in the body of the report.*

Some suggestions for extensions to some sections for the CCL3 review are presented below.

The Federal Register Notice implies that the lists of candidate contaminants are intended for both technical audiences (e.g., scientists and water utilities managers) as well as concerned citizens. An alphabetically arranged list with little or no information about the relevant characteristics (viz. criteria for drinking water safety) seems less than optimal for either audience. The SAB Committee noted in several places that it was difficult for readers to determine the reasons for inclusion of a chemical/pathogen on the list or to get any sense of the urgency, severity or priority for regulation of one candidate over others. The Committee suggested that organizing the list even roughly on the basis of priority for consideration for regulation would be helpful. In particular it was suggested that the listing should first identify contaminants that are well researched and are known to have both significant occurrence and health risks. A second category for contaminants where adequate data is currently lacking could be divided to distinguish those for which occurrence data, health risk data or both is insufficient. This second group identifies contaminants for which there is a need for monitoring and for targeted research to close the indicated data gaps. Finally there are many nominated contaminants about which relatively little is known, so that this category calls for a broader and longer term program of research.

In addition to priority-based classifications, the Committee recommends that contaminants be grouped according to mode of action, occurrence, health effects and/or other relevant factors. Any meaningful grouping and prioritization would be an improvement over an alphabetically arranged list of 93 chemicals and 11 pathogens. However, the noted difficulty for readers seeking to determine why a given contaminant is on the list would need to be extended to include questions about

why it has been assigned to a given priority class and why it is included in one or another grouping. One approach to addressing such questions is to include relevant information about each contaminant directly in the listing. That is, the list could be presented as a matrix, where priorities and groupings are explicitly designated, along with summary indicators of critical criteria, such as potency/concentration ratio, occurrence, mode of action, health effects, source, model scores, expert panel conclusions, etc. The committee also suggested that citations of government documents and other sources relevant to the evaluation of each candidate contaminant be more readily accessible for readers. Including all of the desired information in a printed listing would be unwieldy, so there would have to be constraints on the size of the suggested matrix. Of course, an electronic version of the matrix would be less restricted in this regard, as the reader could follow hyperlinks (in the matrix) to find additional information relevant to their questions about a particular contaminant.

#### **6. Dr. Rogene Henderson:**

I found it difficult to follow the advisory without having seen the write-up of the process on which advice was being given. However, I thought the report addressed the charge questions in a logical and rational manner and I think the report would be clear to someone familiar with the process by which the CCL3 was developed. The tone seemed appropriate; it was helpful and not derogatory.

#### **7. Dr. David Allen:**

- Page 5: There appears to be a header missing after "Other SAB Members"
- Page 11 Lines 7 and 8: The language led me to expect to see a flowchart, which was not included
- Page 17, line 2: grammatical error

#### **8. Dr. Duncan Patten:**

**General Comment.** In all three cases, the SAB review committees have offered excellent review and advice to EPA. The reviews are comprehensive and in sufficient detail to allow EPA staff to reconsider their positions on topics of concern and to rewrite or rework the materials presented in the white papers.

In order to fully assess the responses of the SAB review committee, one would have to be more expert in the particular field of science than I am. Thus my comments are more general, but specific in some cases.

An aside comment on Cumulative Effects and Synergism relevant to two of the reviews.

One question that comes to my mind as I read the reviews, and thus responses to EPA questions, especially those for "Aquatic Life Water Quality" and "Drinking Water Contaminant Candidate List" deals with the concepts of "cumulative effects" and "synergism" in effects of contaminants. Why aren't these concepts considered more critically in testing or selecting contaminants of concern? Only in the Aquatic Life Water Quality review is the concept of synergism (page 11) even considered, and apparently only in passing. Are not the synergistic interactions as well as cumulative

effects among and within contaminants of importance in selection and testing of toxic effects?

Comments specific to Contaminant Candidate List (CCL3):

The response of the SAB committee was quite thorough but some of its statements in response to EPA questions need more detail.

When the committee mentions that it acknowledges that the process should be “an adaptive process” (page 8, line 18) is the committee clear, or does it understand what this means? It should ask for goals and outputs to be identified in this process that will help the improvement of the report.

In the development of “models” for the SAB report, the committee should address how good the model development was (page 8, line 26).

Bottom of page 8 the committee emphasizes “transparency”. Is articulating the decisions by experts primarily the improvement needed to gain more transparency?

Top of page 9. Committee members could not follow the decision making process for some contaminants. It is uncertain whether putting the information on the web site and developing hyperlinks will solve this. Better guidelines of how the process proceeds might be in order.

After page 9, line 37 there should be some statement that emphasizes longevity of pesticides in ecosystems which would be a criterion for cancelling or keeping a pesticide.

Part 2 on clarification regarding steps... that will make it more transparent is probably one of, if not the, most critical commentaries in the review. Clarity is one thing, but transparency of process and expert inputs for example, may be most important to acceptability of the CCL3 report.

Decisions Regarding Data Sets...(paragraph lines 6-14, page 13) Emphasis on large populous states seems imbalanced. The committee should recommend some emphasis on geographic distribution (not necessarily within state boundaries but perhaps watersheds).

Page 13 (line 33)... should point out clearly how literature has appropriate data on outbreaks, etc.

Page 15, line 22. Good statement on consideration of “risk assessment”.

Page 18, lines 13-14. Does the committee believe “these chemicals may be of lower priority..” because the assessment approach was wrong. Needs to be clear.

## **9. Dr. Bernd Kahn:**

I have read the three draft Reviews and consider them to be well written.

## **10. Dr. Timothy Buckley:**

This report seems more problematic in that it is not organized around the charge questions. It may very well be that the charge questions have been addressed, but it is very difficult to tell the way this report is currently organized. I also have a few editorial suggestions that can be taken or left.

Letter, Page 2. Lines state that “The Committee expressed some concern that the lack of clarity could impede the ability of others to understand the basis for decisions about the CCL, an enunciated criterion for transparency made during the reviews by the National Research Council and NDWAC.” **I would break this up into two sentences and replace “enunciated” with “stated.”**

Letter Page 2, Line 31 **replace “better” in “to generate a better list” with “more scientifically credible”.**

Letter Page 2, Line 43, “make regulatory determination on” **Can you just say “regulate” here or “develop regulations.”**

Report Body, Page 8, Line 14: **Consider replacing “data-driven” with “evidence-based.”** Same Page, Line 44: **“stated” instead of “enunciated.”**

## **11. Dr. Jerald Schnoor:**

I have read the 18 page report from the Drinking Water Committee (EPEC) of the SAB reviewing the EPA’s Draft Third Drinking Water Contaminant Candidate List (CCL 3) Report, and I find it to be a good report. It speaks to the charges provided by EPA and answers the questions posed. (I did not read the original Federal Register Notice and support documents for the draft CCL 3.) My comments on the DWC report are summarized below.

If EPA is going to use internal (or external) expert panels in the future to generate or scrutinize CCL lists (which is currently the conventional wisdom in the U.S. and Europe and with which I agree) then we will be challenged to provide better communication about the deliberation and results of the panel. The discussion on the top of page 9 in this regard is highly relevant and important to getting the process right.

I especially liked the recommendation that the Drinking Water Committee made to be involved during the document development process and not just at the end (Page 17, lines 12-14), and the entire discussion on page 17 (see point #4, lines 18-46).

My overall impression is that the report is surprisingly positive considering the overall conclusions that it is still not an adequately transparent process and that the DWC Committee still does not understand why certain pathogens and chemicals

appear on the list and why others do not. This illustrates the problem and confusion for the public when some chemicals are scrutinized in some countries and not in others, why some states have set MCLs and others have nothing, and why some experts express dismay and concern over potential exposures and others do not. Somehow we need to get to a point of agreement by simplifying the process, increasing its transparency and reproducibility, and improving our risk communication to the public and water utilities.

## **12. Dr. Steve Roberts:**

The panel report is very well written and provides thoughtful comments and recommendations that should be valuable to the Agency. Each of the charge questions was addressed clearly in the report, with supporting rationale and examples. This is an excellent report – I have no suggested changes.

## **13. Dr. David Dzombak:**

*(a) Are the original charge questions to the SAB Panel adequately addressed in the draft report?*

The SAB Drinking Water Committee has addressed all of the charge questions, but at different levels of depth, and not in a systematic manner. The Committee has provided extended, very useful commentary on the process used to develop the CCL3 list, which relates mostly to Charge Question 1, but has addressed Charge Questions 2, 3, and 4 directly only in a brief manner at the end of the report. I recommend that the report be more direct and transparent about addressing the charge questions by organizing it around the charge questions. I don't think this will require a great deal of revision, but it will do much to clarify the responses of the committee to the charge questions.

*(b) Is the draft report clear and logical?*

The report is logically organized but focuses almost entirely on the process used to develop the CCL3 list. This discussion pertains to Charge Question 1, which asks if “the Federal Register Notice and support documents are clear, transparent, and adequate to provide an understanding of the overall processes and selection of contaminants for the draft CCL3.” Charge Questions 2, 3, and 4, which ask whether the listed contaminants have the highest potential to occur in public water systems and cause adverse human health effects, and for recommendations about contaminants that should be removed or added to the list, are answered directly only in brief statements in Section 4, near the end of the report. I recommend that the report be reorganized to have individual sections of the report for each of the charge questions, and that direct responses to Charge Questions 2, 3, and 4 be given in the sections corresponding to each of those questions.

The organization of the letter to the Administrator would also benefit by a sequential and direct addressing of all of the charge questions. The emphasis of the importance of improving the process for determining the CCL list is fine, but Committee responses to Charge Questions 2, 3, and 4 should be more clearly stated. In the letter, the opening sentence of the paragraph on the bottom of page 2 (lines 39-41) is not

well stated. The corresponding language in the report body (page 11, last paragraph) is clear and discusses prioritization among contaminants, but the “prioritizing between” statement in the letter refers to two approaches and is confusing.

*(c) Are the conclusions drawn, and/or recommendations made, supported by the information in the body of the draft SAB report?*

The conclusions drawn by the Committee for Charge Question 1 are well supported in the report. The limited conclusions related to Charge Questions 2, 3, and 4, however, are discussed only briefly and warrant some additional discussion. Separate sections for each of the charge questions would make apparent where more support discussion is needed.

#### **14. Dr. Judy Meyer:**

I found this to be a readable report. The charge questions are addressed, although I found relatively little reference to additional data that could be used to either add or remove a chemical from the list (charge questions 3 and 4). The report is clear and logical. The recommendations are supported by the text of the report.

I have a couple additional comments:

Is there not an Executive Summary? I recognize that section 1 provides a broad overview, but an Executive Summary would also include the highlights of conclusions from the other sections. I don't recall seeing any other SAB report without an Executive Summary.

pp. 15-16: I was pleased to see the recommendation on grouping compounds by mode of action. A similar recommendation was part of the report EPEC produced on Aquatic Life Criteria for Contaminants of Emerging Concern.

p. 17, line 13: I have some misgivings about including the DWC “throughout” the process. That recommendation strikes me as being pretty vague. Furthermore, if the DWC is involved throughout the process, then its ability to be an objective reviewer of the final list is compromised. I suggest the committee identify a couple specific points in the process where input from the DWC would be sought.

I also found some typos:

p. 15, line 11: should be “of concern so that resources”

p. 15, line 29: “concentration” would seem to be a more appropriate term here than “level”

p. 17. line 1: should read “that the same exceptions”

p. 17. line 12: should read “We recommend that EPA include the DWC”

p. 17. Line 49: should read “in the previous sections”

p. 18, line 7: should read “Also, there is a consensus”

p. 18, line 23: should read “to improve the process that should be explored” – note in addition to adding “that” I changed “must” to “should” which seems more appropriate for a report like this.

## 15. Dr. Valerie Thomas:

The Committee has worked carefully through the CCL3 and appear to have developed a useful set of recommendations for the Agency. These recommendations would be more readily adopted by the Agency if the draft advisory were revised to increase its clarity. In particular, the draft advisory does not clearly or directly address all of the EPA's charge questions. Charge question 1 is clearly answered ("no"). Charge question 2 is also implicitly answered "no", although the response to this question could be made more clear in the letter to the Administrator and in the body of the report. Charge question 3 and 4 are not answered: no data are provided, although the document does say that some contaminants on the list should not be listed and some not listed should be. The Committee could come closer to providing these "data" by providing references and a clearer and more organized statement, backed up with data or references to the extent feasible, of which contaminants or types of contaminants should or should not be listed. Alternatively, it would be legitimate for the Committee to not answer some of the charge questions; in this case the Committee should clarify that it is providing advice that diverges from the charge, and explain why.

The Advisory diverges from the Charge Questions in a way that suggests that discussion with the EPA during the Advisory process may have suggested to the Committee a different charge. The letter begins by saying that the EPA asked for advice on the Process, and the Advisory contains a substantial section on how the EPA could improve the process in the future. However, the charge questions do not ask for comments on the process, and they do not ask for comments on future CCLs; they ask for comments on the list itself that is being used for this CCL3.

I think that the Committee could usefully revise the Advisory to more directly and unambiguously address the written charge questions.

Detailed Comments:

Letter to the Administrator, p. 2, lines 15-23. This statement is not clear.

Line 15: remove the words "example for": this is not an example; it is a main point of the advisory.

Line 16: Change "suggested" to "suggests" – present tense.

Line 16: Change "were" to "are".

Line 23: cut "so as not to be shortsighted on the Agency's part."

Lines 33-37. Does the Committee recommend that these contaminants be included in the CCL?

Line 35: Change "would" to "does", and similarly revise the next sentence.

Lines 40-41: The meaning is not clear. Does the Committee means to say that EPA should identify those contaminants ready for regulatory determination and those for which more data are required? The phrase "prioritize between" is confusing.

p. 8 line 26. What does "intensified" mean here? Should this word be deleted?

p. 9, line 16: change "were" to "are"

- p. 9, lines 28-37. This is confusing.
- p. 9, lines 28-30. Does the Committee mean to say “For example, all uses of nitrofen were cancelled in 1983, yet nitrofen appears in the CCL3.”?
- p. 9, lines 30-32. This sentence does not seem to be related to anything else in the paragraph. Is there a chemical listed in the CCL3 for which EPA proposed a national drinking water standard based on a TRI release from one site?
- p. 9, lines 32-37: Here again the discussion of canceled pesticides is unclear.
- p. 9, line 40: It seems that “for example” should be cut. The prioritization based on data availability is a key finding of the Committee, not an example.
- p. 10, line 3. Change “in” to “used to develop”
- p. 10, lines 11-15. This paragraph is written as a report of what the Committee discussed. Does the committee want to recommend that the CCL process might indeed need to be modified in the future in these ways, or does the Committee simply want to say, as written, that the topic was discussed?
- p. 10, lines 17-20. Again, does the Committee, as written, simply want to record that these issues were discussed? Or does the Committee in fact identify any emerging issues or research needs?
- p. 10, line 23. Clarify the heading. Perhaps “Improving the Transparency of the CCL Process.”?
- p. 10, lines 41-43. This is a clear recommendation. Should it be included in the letter to the Administrator?
- p. 11, lines 5-6. This statement is not clear. Is the Committee saying that expert opinions would (why is the word “might” used here?) have been more acceptable than internal expert opinions? Did only some members conclude this, or the entire Committee? Is the Committee saying that external expert opinions need less transparency than internal expert opinions?
- p. 11, line 24. This is not clear.
- p. 11, line 43: Is the word “listing” correct? Or is “regulatory determination” meant here? At this stage in the process, the contaminant is already listed; the context implies that the algorithm would refer to the readiness for regulatory determination.
- p. 12, line 1. The word “additional” might be inserted before the word deficiencies.
- p. 13, lines 6-13. This paragraph suggests that the Committee might be saying that people living in small states should be less protected than people living in highly populated states. There is a good point embedded here; however the paragraph should be carefully revised to avoid misinterpretation.
- p. 13, lines 36-37: “did not appear to be able to provide a resolution regarding details to the scoring algorithm.” This statement is unclear.
- p. 14, line 4. Change “effects” to “potential effects.” Unused data couldn’t have had effects.
- p. 14, line 5. Change “represents” to “is.”
- p. 14, line 12. Change “boarder” to “broader.”
- p. 14, line 14. Change “or” to “and.”
- p. 15, lines 24-p. 17 line 14. Overall, the purpose and implication of section 3, “Suggestions to improve the process for future CCLs” is not clear. The charge to

the committee relates to this CCL, not to future CCLs, and the items included in the suggestions for future CCLs are also included in the previous discussion of this CCL. Why does the Committee recommend these changes for future CCLs rather than this one?

p. 15, lines 26-29. It would be helpful if the Committee would list the lessons learned. Two examples are given in this paragraph. Is this the complete list? If not, what are the other lessons learned?

p. 18, line 7. The subject of the sentence (“there” or “it”) needs to be added.

p. 18, lines 35-38. There is only one Committee-provided reference. The EPA’s charge specifically requested data; if the committee cannot specifically identify useful data, that should at least be stated clearly in the report.