National Toxicology Program
Board of Scientific Counselors

June 21-22, 2010

National Institute of Environmental Health Sciences
Research Triangle Park, NC

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I. Frequently Used Abbreviations and Acronyms

ADC	adenocarcinoma
AML	acute myeloid leukemia
ATSDR	Agency for Toxic Substances and Disease Registry
BSC	Board of Scientific Counselors
CEBS	Chemical Effects in Biological Systems
CERHR	Center for the Evaluation of Risks to Human Reproduction
CoWC	cobalt-tungsten carbide: powders and hard metals
DERT	Division of Extramural Research and Training
DIR	Division of Intramural Research
DSP	Draft Substance Profiles
EPA	Environmental Protection Agency
EU	European Union
FDA	Food and Drug Administration
FDH	formaldehyde dehydrogenase
FEMA	Federal Emergency Management Agency
FISH	fluorescence *in situ* hybridization
FOIA	Freedom of Information Act
GWF	glass wool fibers
HHS	Health and Human Services
IARC	International Agency for Research on Cancer
ICH	International Conference on Harmonisation
ITIA	International Tungsten Industry Association
µm	micrometer
LHC	lymphohematopoietic cancers
MCL	mononuclear cell leukemia
MTD	Maximum Tolerated Dose
NAS	National Academy of Sciences
NAIMA	North American Insulation Manufacturers Association
NCI	National Cancer Institute
NPC	nasopharyngeal nancer
NICHD	National Institute of Child Health and Development
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute of Occupational Safety and Health
NTP	National Toxicology Program
OSHA	Occupational Safety and Health Administration
ppm	parts per million
RCC	Research and Consulting Company
RoC	Report on Carcinogens
ROS	reactive oxygen species

SCC	squamous-cell carcinoma
SNC	sinonasal cancer
SPF	Special Purpose Fibers
$WT_{1/2}$	weighted half-life

II. Attendees

Members in Attendance:

Tracie Bunton, Eicarte LLC
Russell Cattley, Amgen
David Eastmond, University of California
Elaine Faustman, University of Washington
Stephen Looney, Medical College of Georgia
Mitzi Nagarkatti, University of South Carolina School of Medicine
Raymond Novak, Wayne State University School of Medicine (Chair)
Ruthann Rudel, Silent Spring Institute
James Sherley, Boston Biomedical Research Institute
Gina Solomon, Natural Resources Defense Council
Justin Teeguarden, Pacific Northwest National Laboratory

Members not in Attendance:

Edward Carney, The Dow Chemical Company
Janan Eppig, The Jackson Laboratory
William Janzen, University of North Carolina at Chapel Hill

Pending BSC Members

Miguel Fernandez, University of Texas Health Science Center at San Antonio
Dana Loomis, University of Nevada, Reno [present only on June 22, 2010]
Melissa McDiarmid, University of Maryland School of Medicine
Richard Miller, GlaxoSmithKline
Judith Zelikoff, New York University School of Medicine

***Ad Hoc* Reviewers:**

Joseph Landolph, University of Southern California
Andrew Olshan, University of North Carolina at Chapel Hill
Margaret Quinn, University of Massachusetts, Lowell

Other Federal Agency Staff

Paul Howard, Food and Drug Administration (FDA)
Mark Toraason, National Institute for Occupational Safety and Health (NIOSH)

National Institute of Environmental Health Sciences Staff and Contractors:

Stanley Atwood
Eddie Ball
Linda Birnbaum
John Bucher
Jennifer Fostel
Sally Fields
Paul Foster
John French
Sanford Garner
Robbin Guy
Kembra Howdeshell
Marc Jackson
Gloria Jahnke
Ruth Lunn
Robin Mackar
Barry McIntyre
Cynthia Rider
Andrew Rooney
Thaddeus Schug
Michael Shelby
Keith Shockley
Diane Spencer
Kristina Thayer
Raymond Tice
Michael Waalkes
Nigel Walker
Vickie Walker
Lori White
Kristine Witt
Mary Wolfe

Public:

Melvin Anderson, The Hamner Institutes for Health
Jeff Cossman, United States Diagnostic Standards (by telephone)
Angus Crane, North American Insulation Manufacturers Association
George Cruzan, ToxWorks
Reshan Fernando, RTI International
Robinan Gentry, ENVIRON International Corporation
Jonathan Gledhill, Policy Navigation Group
Robert Golden, ToxLogic
Ken Gould, Owens Corning
Mark Gruenwald, Hexion Specialty Chemicals
John Hadley, Owens Corning
Thomas Hesterberg, Navistar, Inc.
Stewart Holm, Georgia-Pacific Chemicals LLC
Meetu Kaul, Arnold & Porter LLP
Gary Marsh, University of Pittsburgh
Roger McClellan, Consultant
Scott Miller, Knauf Insulation
Betsy Natz, Formaldehyde Council, Inc.
Catherine Price, RTI International
Bruce Ray, Johns Manville
Aymon de Reydellet, Saint-Gobain
Joseph Rodricks, ENVIRON International Corporation
Ivan Rusyn, The University of North Carolina at Chapel Hill
Barbara Shane, Barbara Shane Consulting
Thomas Shaw, Sandvik
James Swenberg, The University of North Carolina at Chapel Hill
Rochelle Tyl, RTI International

June 21, 2010

III. Introductions and Welcome

The National Toxicology Program (NTP) Board of Scientific Counselors (BSC) met June 21-22, 2010, in Rodbell Auditorium, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina. Dr. Raymond Novak served as chair. He welcomed everyone to the meeting and asked the BSC members and attendees to introduce themselves. Dr. Lori White read the conflict of interest policy statement. She mentioned Dr. Teeguarden had a potential conflict of interest related to the BSC's consideration of the Draft Substance Profile on

Formaldehyde, and would not participate in that portion of the proceedings. She also acknowledged the presence of *ad hoc* reviewers Drs. Quinn, Landolph and Olshan.

IV. Report of the NTP Director

A. Presentation

Dr. Linda Birnbaum, Director of NIEHS and NTP, welcomed attendees to the meeting, where important recommendations would be made regarding listing or delisting substances from the 12th Report on Carcinogens, as well as proposed concepts for future NTP projects. She said her report would encompass both an update on the overall activities of NIEHS and NTP and information about NIEHS activities related to the Gulf oil spill. She reported the effort to establish NTP as a separate division within NIEHS is progressing more slowly than anticipated, but would certainly happen.

She identified what she believes to be the priority areas for research in the environmental health sciences, several of which have direct relevance to NTP: (1) low dose, (2) windows of exposure, (3) toxicology screening, (4) mixtures, (5) clinical research, (6) emerging hazards, (7) human health effects of climate change, and (7) green chemistry

She reported progress in efforts to put new permanent NIEHS staff in place: Scientific Director; Director of the Division of Extramural Research and Training (DERT); NIEHS Deputy Director; and a senior-level toxicologist to be based in the Institute's Bethesda office. Once the new permanent hires are in place, the process of creating a new strategic plan for NIEHS for the next five-year period, from 2012-2016, would begin. She is also seeking to ensure all staff appreciate the importance of their NIEHS/NTP colleagues' work, by focusing on filling in the statement, "My work helps us understand how the environment can impact public health because…"

Dr. Birnbaum reported several NIEHS and NTP staff members had been working diligently to ensure the institute contributes its expertise and resources to the federal government's efforts related to the Gulf oil spill. For example, Chip Hughes, the director of the Worker Education Training Program, was on site within 48 hours of the spill to assess the hazards being faced by workers involved in cleanup activities and to coordinate worker safety training in association with Coast Guard and BP officials, representatives of other responding federal agencies such as the National Institute of Occupational Safety and Health (NIOSH) and the Environmental Protection Agency (EPA), and local and state agency officials. His group quickly published and distributed 5,000 copies of the booklet, *Safety and Health Awareness for Oil Spill Cleanup Workers*. The booklet has been printed in Spanish and Vietnamese as well as English, in order to be accessible by many of the workers on site. NIEHS trainers are training the BP worker trainers, who as of June 10 had conducted approximately 30,500 training sessions using the NIEHS worker training materials. NTP has also been involved with oil spill efforts, participating in the interagency working group that has been established, and creating a website for the group

that serves as a central repository of toxicological data and publications relevant to the oil spill. NIEHS Senior Medical Advisor Aubrey Miller has testified at two congressional hearings recently about institute efforts on human health effects of the oil spill, and spearheaded NIEHS activities to create the Interagency Oil Spill Health Monitoring and Research Workgroup. The Workgroup, initially consisting of NIEHS, NIOSH, Agency for Toxic Substances and Disease Registry (ATSDR), Substance Abuse and Mental Health Services Administration (SAMHSA) and Health and Human Services/Assistant Secretary for Preparedness and Response (HHS/ASPR), is intended to coordinate and facilitate public health monitoring for human health effects of exposures related to the oil spill. It continues to expand quickly as other stakeholders are added, with six working committees already.

Dr. Birnbaum added that NIH Director Dr. Francis Collins, testifying recently before the House Energy and Commerce Committee, announced he is providing $10 million from the Common Fund and his discretionary fund to NIEHS to initiate a ten-year prospective cohort study in New Orleans. Dr. Dale Sandler of NIEHS is beginning work on the study, which will be closely coordinated with other agencies such as EPA, NIOSH, OSHA, and ATSDR. NIEHS has developed a detailed plan for proposed oil spill research, including studies within DERT and the Division of Intramural Research (DIR), as well as NTP studies to include a mixture of literature evaluations, analytical chemistry activities, toxicity pathway screens, and targeted testing in rodent studies to confirm and extend understanding of the hazards presented by the complex materials comprising the oil spill. DIR will establish a cohort of workers for assessing short- and long-term health effects related to exposures, with collection of biological samples and survey data. DERT will engage in a comprehensive research portfolio including monitoring, exposure assessment, risk assessment, communication, and outreach, utilizing the NIH research infrastructure. Response worker training will also continue, with additional funding coming from the Coast Guard, as Superfund funding has for the program has been depleted.

Dr. Birnbaum concluded by noting NIEHS personnel would be taking part in a meeting later that week among several governmental agencies and other stakeholders called *Assessing the Human Health Effects of the Gulf of Mexico Oil Spill*, sponsored by the Institute of Medicine.

B. BSC Discussion

Dr. Faustman outlined to Dr. Birnbaum her concern about the lack of a cohesive sampling plan for the oil spill, and that there seemed to be no overall experimental design to the sampling efforts being undertaken. Dr. Birnbaum said the four NIEHS/National Science Foundation (NSF)-sponsored Oceans and Human Health Centers were going to be important elements, and that NIEHS is working with EPA and other groups to coordinate research efforts, along with various state and local health agencies in the affected areas.

Dr. Solomon asked how the cohort study was beginning to shape up, and whether it would be restricted to studying the workers, or if local residents, particularly children and pregnant

women, would also be included. She also inquired about the level of community-based involvement planned in the study. Dr. Birnbaum answered that as leaders in community-based participatory research, it would certainly be a key part of the study. However, the plan for the study had just emerged within the past several days, and much work remained regarding the design of the study, which is on a very fast track compared to typical prospective longitudinal cohort studies. She welcomed Dr. Solomon's suggestions, and urged everyone to "stay tuned" as details of the study emerge in the near future.

Dr. Novak asked if anyone had considered the possibility of a Gulf hurricane driving the exposures farther inland. Dr. Birnbaum said there were groups looking at that, and if it did occur, the nature of the cohort would be changed accordingly. Dr. Sherley asked whether other institutes would be involved in the oil spill response and research. Dr. Birnbaum answered that she would be briefing other institute directors at a meeting later in the week, and would anticipate there would be opportunities for them to become involved. She closed her comments by mentioning how proud she was of the NIEHS leadership in the response and research efforts.

V. Center for the Evaluation of Risks to Human Reproduction: Proposed Evaluation Concept: Cancer Chemotherapy during Pregnancy

A. Presentation

Dr. Kembra Howdeshell, Center for the Evaluation of Risks to Human Reproduction (CERHR), presented the proposed evaluation concept: Cancer Chemotherapy during Pregnancy. This was nominated internally by the staff of CERHR, and was developed in consultation with experts from the National Cancer Institute (NCI), the National Institute of Child Health and Development (NICHD), the Food and Drug Administration (FDA) Center for Drug Evaluation and Research, and the National Comprehensive Cancer Network. Interest was originally piqued by an article that appeared in the *New York Times* in August 2008, *With Child, With Cancer* by Pamela Paul, relating the treatment experiences of two pregnant women who had been diagnosed with breast cancer. Contrary to past medical opinions, current research found that pregnant women with cancer have a similar prognosis to non-pregnant women with cancer.

Dr. Howdeshell related the background and rationale for the concept. She said 1-in-6000 to 1-in-1000 pregnant women are diagnosed with cancer; based on six million pregnancies per year, approximately 1000 to 6000 pregnant women are diagnosed with cancer annually in the United States. The frequency is expected to increase as women postpone having children to later ages. Chemotherapy is commonly used to treat cancer, and most chemotherapeutic agents are FDA Pregnancy Category D, indicating positive evidence of human fetal risk. In general, medical opinion is that chemotherapy should be avoided during the first trimester, the period of major organogenesis, but treatment during the second and third trimesters presents minimal risk to the fetus. She said a thorough, systematic assessment of pregnancy outcomes following chemotherapy has not been published, although there are some reviews that are generally limited

to specific cancer types or chemotherapy agents. A large literature of more than 500 papers on more than 50 agents exists.

Dr. Howdeshell said the proposed NTP Monograph would review the evidence for developmental effects of exposure to cancer chemotherapy *in utero*, with the main focus on clinical data in humans, to be supplemented with biomedical and toxicological literature in animals. The goal of the monograph would be to provide clinicians, patients and researchers with a comprehensive review of the incidence and types of adverse effects seen in humans exposed *in utero* to cancer chemotherapeutic agents. The monograph would not be intended to be a clinical guidance document. The key objectives of the document would be to (1) identify the complete published scientific literature on chemotherapy during pregnancy in humans, focusing on the most common cancers occurring during pregnancy; (2) critically evaluate the strength and consistency of the literature on embryo, fetal, and postnatal outcomes in humans by cancer type, chemotherapeutic agent, and trimester of exposure; (3) develop weight of evidence conclusions on the occurrence of adverse effects at different gestational stages, by agent; and (4) identify data gaps and research needs for evaluating the effects of exposure to cancer chemotherapeutics *in utero*

The proposed approach to the monograph, said Dr. Howdeshell, would include a review of the published literature in the area, including primary reports and secondary sources. This would allow the development of summary tables by chemotherapy agent, including trimester of exposure, pregnancy complications, and pregnancy outcomes. The tables would also include known information regarding placental transfer of an agent and the known or proposed mechanism of action involved in causing adverse effects. Weight of evidence conclusions would be developed on the occurrence of adverse effects at different gestational stages by agent. Scientific input would be obtained from technical advisors such as oncologists, obstetricians/gynecologists and pediatricians, the public, and from interagency review. The monograph is tentatively scheduled to be peer reviewed in the summer of 2011, with public comment and a public peer review meeting of an *ad hoc* expert panel, which would include a BSC member. Following peer review, the monograph would be finalized.

In terms of significance and expected outcomes, Dr. Howdeshell said the proposed monograph would provide a thorough survey and critical scientific evaluation of pregnancy outcomes of women treated with cancer chemotherapy during gestation, and it would be useful to physicians, their patients, and researchers. It would highlight efforts to establish registries of pregnant cancer patients and would include follow-up studies on offspring exposed *in utero* to cancer chemotherapy agents. It would also identify needs for further research in the area.

Dr. Sherley asked if there was a sense of how many individuals are affected by this problem, given that it is one of the key elements of evaluating the worthiness of the proposed monograph. He said it was his impression it is a relatively small population, which would call into question the appropriateness of devoting NTP resources to it. Dr. Howdeshell said this monograph would

provide the most comprehensive review to date on the topic. Dr. Bucher added that one of the elements considered by NTP in the nomination process are orphan drugs, i.e., drugs serving too small a population to be of major commercial interest. He said the population potentially affected by cancer chemotherapy during pregnancy is actually larger than the typical orphan drug population.

Dr. Zelikoff urged that placental toxicants be looked at as well as chemicals that actually pass through the placenta itself, and said she approved of the intention to study long-range outcomes, asking how long the studies would last and what types of disease outcomes would be addressed. Dr. Howdeshell replied there were at least three clinical trials in progress that include attention to long-range outcomes, and NTP would comb the literature carefully for longer-term evaluations of individuals exposed *in utero*.

Dr. Solomon asked whether the proposal had been initiated by CERHR. Dr. Howdeshell reiterated that the idea had emerged from CERHR staff. Dr. Thayer added that in CERHR's outreach efforts, they had been told by the outside parties they had contacted that this project would fill a niche for them and be useful in helping inform clinical practice. Dr. Howard said the FDA is also very interested in the project, and believes it will fill gaps in knowledge and help the process of evaluating future drugs.

B. Public Comment

Mr. Bruce Ray of Denver, CO addressed the BSC. He suggested several elements he felt should be incorporated into the study, particularly intravenous anti-emetics, since they are often co-administered to cancer patients undergoing chemotherapy. He said other co-administered agents should be added, such as prophylactic antibiotics, anti-virals, and anti-fungals, as well as immune system boosters and anti-clotting agents—all targeting co-morbidities often experienced by cancer patients, particularly neutropenia.

C. BSC Discussion

Dr. Nagarkatti, first lead reviewer, rated the proposal a medium-to-high priority for NTP. She felt the rationale was very well articulated in the proposal, with clear objectives, and strongly justified the project. She said there was a considerable need for the review, which would address a major public health problem, but expressed concern that the review would only be looking at the problem on a scientific basis, without giving an opinion on the level of concern involved, as the NTP does for other agents. She also recommended taking into consideration the exposure dosimetry faced by the progeny of the cancer patients, as well as the possibility that perinatal exposure to chemotherapy agents through lactation may cause further effects, and effects seen later in life through fetal basis of adult disease mechanisms. She also recommended expanding the scope of the review to include documentation of genetic and epigenetic effects, as well as reproductive effects. She recommended consideration of involving other NIH institutes in the

project, as their fields of specialty may speak to specific cancers. Responding to Dr. Nagarkatti's concern about not using the level of concern approach, Dr. Howdeshell said the chemotherapy agents in question are clearly established cytotoxicants, and so the weight of evidence approach was more appropriate in this case. Dr. Thayer said the document is not structured to generate clinical treatment guidelines, and so the language that would accompany a level of concern approach might be confusing to clinicians. Dr. Faustman said she supported the approach outlined in the proposal.

Dr. Sherley, second lead reviewer, said that although the proposed monograph addresses a very important patient population deserving of more attention, he nonetheless rated the project a very low priority. He believed the topic was already well-covered by the biomedical profession and the agents involved are well-known to be toxic, so it is difficult to justify the expenditure of NTP resources, given that the project is unlikely to add significantly to knowledge or to the resources already available to clinicians. In response, Dr. Howdeshell emphasized CERHR's unique resources to thoroughly comb the literature and coordinate with other federal agencies, to ensure that the project would generate the type of medical information they would most need. She said the evaluation would also be very useful to the medical community, because cancer during pregnancy is much rarer than cancer in general and many clinicians are unaware of the resources available for specialized treatments, resulting in many pregnancies being terminated due to clinicians' reluctance to administer chemotherapy during pregnancy. Dr. Zelikoff added that the report would be important in documenting effects seen in gestational trimesters, thus adding to doctors' knowledge.

Dr. Looney, third lead reviewer, felt that the clarity and validity of the proposal were clearly described. He said it was directly relevant to NTP goals, but it was unclear from the document what type of research synthesis would be performed. He said a meta-analysis would have the greatest impact on the medical community. He wondered what type of weight of evidence conclusions would be presented. He felt that he could not judge the public health importance of the project due to lack of information about the proportion of pregnant women treated with chemotherapeutic agents, and the timing in terms of trimester of those treated. Being unsure about the public health importance, he rated the proposal as a moderate priority.

Dr. Fernandez, fourth lead reviewer, echoed the concerns of other reviewers regarding the small population involved. He was also concerned about the lack of information in the proposal about the demographics of the study population, which could limit its relevance. He supported the idea of looking at adjunctive therapy to be complete in the monograph. He rated the project a moderate priority. Dr. Howdeshell alerted BSC members to a recent editorial in *Nature* discussing under-studied populations in biomedical science, pregnant women being prominent among them. She added the proposed monograph would be one way to address that dearth of knowledge. Dr. Fernandez characterized the proposed goal of evaluating perceptions in the medical community regarding cancer chemotherapy during pregnancy as "very odd."

Dr. Faustman said there is a tremendous need among clinicians for the kind of information in the proposed monograph, and CERHR is in a unique position to perform the review, particularly due to its proven track record of including all available information in its reviews, most particularly animal experimental data, which would be of value to inform clinical practice in this area. She also pointed to the importance of going beyond just information regarding dose to include pharmacokinetic and pharmacodynamics data, particularly as they relate to administration at the different stages of pregnancy. She noted the lack of a toxicologist, a teratologist, or a neurodevelopmental specialist in the list of experts to be consulted in the project. She felt the collection of the proposed information in one easily accessible location would be a valuable addition to the available resources. She said the complexity of the therapeutic materials being given should be added to the tables. She expressed strong support for the proposal, endorsing a high priority designation. Dr. Howdeshell reminded the BSC that the proposal was a broad overview document without much detail about how the project would handle toxicological or mechanism data. She clarified the language in the proposal about the technical advisors who would be consulted, in that it was not presented as a complete list, and that certainly toxicologists and other pertinent advisors would be consulted.

Dr. Solomon expressed her confusion about some differences she perceived between the written document and the proposal presented at the meeting. She felt the review described in the document was limited in scope (and value), but the subsequent description, including the addition of animal data and other material, would broaden the scope and be more interesting and useful, especially if it looked at other mixtures and additional drugs as raised by public comments and not just the chemotherapy agents themselves. Dr. Howdeshell said the project is still in the planning stage in terms of how broad the review would be.

Dr. Bunton recommended organizing the tables not just according to the agent, but also according to the mechanism of action involved. Dr. Bunton asked for more information about the repositories of information on pregnant women with cancer. Dr. Howdeshell said there are three registries in the United States, one in Canada, and one in Germany. There are also three ongoing clinical trials directly addressing pregnancy outcomes of pregnant women with cancer. Several other clinical trials had pregnancy outcomes and offspring follow-up as a secondary goal.

Dr. McDiarmid felt there is widespread misunderstanding on the part of clinicians who use these drugs about their appropriate use during pregnancy, both by unnecessarily suggesting termination of pregnancies or by endorsing unrestricted use after the first trimester, which is also inaccurate. Common cancers treated for women of reproductive age are treated with multiple drugs, thus it will be challenging to identify individual agent effects. It would be necessary for the review to contain clinically accessible helpful information to be of sufficient value. She agreed with other BSC members that reproductive toxicity endpoints (i.e., subsequent pregnancies) should be included in the review along with the developmental toxicity data. She

said the impact of the chemotherapeutic agents on breastfeeding should also be included, since most of the drugs are known to enter breast milk. She also recommended that certain occupational populations exposed to the drugs during pregnancy be included.

Dr. Novak summarized the discussion to that point, identifying organization as one of the key concepts for the document. Secondly, he noted the complexity of the protocol, since the cancers involved are generally treated with multiple drugs, including hormonal therapy for hormonally responsive cancers. He mentioned the dynamicism of the protocols themselves and how they have changed over time and based on outcomes. His perception was there was high to moderate enthusiasm for the monograph among BSC members, and that the document, if prepared correctly, could be useful to clinicians and to individuals facing the issues involved.

Dr. Howard asked for clarification regarding the target audience of the proposed monograph. Dr. Thayer replied that clinicians developing treatment guidelines would be the most important target audience, but an executive summary in plainer language would also likely be included.

VI. Peer Review of Draft Substance Profiles for the 12th Report on Carcinogens: Process and Charge

Dr. Wolfe reviewed the Report on Carcinogens (RoC) the process used to evaluate substances for the forthcoming 12th RoC, and the charge and format for this meeting's peer reviews. The RoC became a congressionally mandated biennial report in 1992. It is designed to alert the public and regulatory agencies about potential cancer hazards, listing substances as *known* or *reasonably anticipated human carcinogens*. The current 11th RoC has 246 listings – 58 *known* and 188 *reasonably anticipated* substances. The report consists of *substance profiles*, which identify the listings, summarize relevant information that supports each listing, and provide information on properties of the substance, its use and production, and current Federal regulations and guidelines to limit exposures. The Secretary of HHS has delegated preparation of the report to the NTP, which uses a multi-step process, the current version of which was released in April 2007. Specific criteria are used to evaluate the scientific evidence on a substance to determine whether or not it should be listed. To be considered a *known human carcinogen*, there must be sufficient evidence of carcinogenicity from studies in humans. To be listed as *reasonably anticipated*, three different levels of evidence may be considered:

- Limited evidence of carcinogenicity from studies in humans
  OR
- Sufficient evidence of carcinogenicity from studies in experimental animals
  OR
- Less than sufficient evidence of carcinogenicity in humans or experimental animals, but the substance belongs to a well-defined, structurally related class of substances whose members are listed in the RoC, or there is convincing evidence that the substance acts through mechanisms indicating that it would be likely to cause cancer in humans

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information (these apply to both *known* and *reasonably anticipated*).

After substances are nominated for inclusion in the RoC, there is a scientific review of the candidate substances. When a final background document has been released, the next step is peer review of the draft substance profiles (DSP) by the BSC. Dr. Wolfe reminded the BSC that it had already considered five of the eight candidate substances under consideration for the 12th RoC, and in this meeting they would be reviewing the remaining three. She reviewed the format of the DSP for the BSC, which uses the same format as an RoC substance profile, provides NTP's preliminary policy decision on listing status for the substance, and summarizes the scientific information supporting the listing recommendation as well as information on potential for exposure, properties, use and production of the substance, and current Federal regulations.

The NTP will consider the BSC's peer reviews and public comments in finalizing the DSPs, with the NTP Director making a final decision on listing recommendations. The NTP will then prepare the draft RoC. The report goes to the NTP Executive Committee and to the Secretary of HHS for review and approval, and ultimately is released to the public and transmitted to Congress. At the same time, NTP documents responding to the expert panel peer review, the BSC peer review, and the public comments received since the final background document are released. Dr. Wolfe went over the format for the peer review, starting with RoC staff presenting NTP's preliminary listing recommendation and supporting scientific information. Public comments follow, and then peer review comments by BSC reviews and *ad hoc* reviewers. Then additional BSC comments are solicited, and finally there is BSC discussion. Dr. Wolfe concluded her presentation by reviewing the BSC's charge, which was to determine whether the scientific information cited in the draft substance profile for a candidate substance is technically correct, clearly stated, and supports the NTP's preliminary policy decision regarding its listing in the RoC.

VII. Peer Review of Draft Substance Profiles for the 12th Report on Carcinogens: Glass Wool Fibers

A. Presentation

Dr. Quinn joined the table as an *ad hoc* reviewer for this portion of the meeting. Dr. Gloria Jahnke presented the DSP on glass wool fibers (respirable) as a class, defining glass wool fibers (GWF) as "amorphous fine glass fibers resembling wool; silicon dioxide is the primary chemical component." The physical properties and chemical composition of the different fibers vary, and are controlled during manufacturing. The fibers break cross-wise (unlike asbestos, which breaks length-wise). Commercial fibers are produced as bulk materials that contain a range of fiber

dimensions. The nominal diameter (not a median or average diameter, but what the manufacturer represents the diameter of a product to be) of fibers is typically:

- Insulation glass wool: 1 to 10 µm (nearly all >3 µm)
- Special purpose glass fibers: 0.1 to 3 µm

Fiber diameters may overlap, as they may fall within a range of diameters. Respirable fibers can penetrate into the alveolar region of the lung upon inhalation. The World Health Organization and the U.S. EPA have standard specifications defining what they consider to be respirable fibers. Major uses of GWFs include applications for insulation purposes such as building insulation, and as filtration media, which are the special purpose fibers (SPF), the largest use of which is for battery separator media.

Routes of exposure can be inhalation, ingestion, dermal or ocular. Occupational exposure can occur during manufacture or installation/removal. Environmental exposure can occur in indoor air. More than three billion pounds of fiberglass were used in commercial and residential building insulation in 2000, with 6 billion pounds of all glass fiber types used in the U.S. in 2002. SPFs comprise approximately 1% of that amount.

The NTP has proposed that GWFs (Respirable) as a Class be listed in the RoC as *reasonably anticipated to be a human carcinogen*, based on sufficient evidence from studies in experimental animals for fibers as a "class," and supporting mechanistic evidence. However, it is noted in the DSP that "not all glass fibers in the class are carcinogenic," and the dividing line between carcinogenic and non-carcinogenic fibers is not clear.

Dr. Jahnke said the fiber properties that influence carcinogenicity are called "the three Ds:" dose, dimension, and durability. Tumor incidence increases with increasing dose, and lesion severity increases with cumulative fiber burden. Tumor incidence also correlates with fiber size and shape, with longer, thinner fibers being more carcinogenic. Fiber durability is described by both *in vitro* dissolution rate, measured as K_{dis}, and biopersistence, measured as weighted half-life ($WT_{1/2}$) or *in vivo* weighted fiber half-life in days by inhalation or intratracheal instillation. In terms of the mechanisms of their carcinogenicity, fibers can either affect target cells directly, or can cause a chronic inflammatory response through their biopersistence, with reactive oxygen species production and particular signaling pathway activations. This can act as a wound, causing fibrosis along with carcinogenesis. GWFs are genotoxic, having shown positive results in both *in vitro* and *in vivo* tests. Longer, thinner fibers have been shown to be more cytotoxic and genotoxic than shorter, thicker fibers in mammalian cells.

In studies in experimental animals, routes of exposure have included inhalation and intratracheal instillation and intrapleural, intraperitoneal, and intrathoracic implantation, depending on the specific study protocol. Dr. Jahnke said there is sufficient evidence in experimental animals for fibers as a class, with tumors found in multiple species (rats and hamsters) and by multiple

routes. Regarding the strengths of the animal data, she reported that a range of carcinogenic responses was observed across fiber types, with the carcinogenic response stronger in SPFs than in insulation fibers, although there were some positive results in studies involving insulation fibers. The experimental M fibers were not found to be carcinogenic at all. The positive data are strongest for the specific SPFs E glass and 475 glass. Dr. Jahnke summarized results from several of the positive chronic cancer animal studies, including studies reporting lung tumors, mesotheliomas, mononuclear-cell leukemias, and sarcomas. The NTP had determined there was inadequate evidence of carcinogenicity in humans. Although there was a small excess of lung cancer in studies of glass wool manufacturing workers, the magnitude of the risk estimates could be explained by confounding by co-exposures to tobacco smoke, and there was a dearth of any clear positive exposure-response relationships. Thus, risk estimates for glass wool are for the class, as some manufacturing plants in the U.S. cohort also made special application fibers.

Dr. Jahnke presented data from several human studies, including cohorts in the United States, Europe, Canada, and France, along with a case-control study from Russia (the only one that controlled for smoking) and a meta-analysis of the four cohorts. The U.S. study of more than 10,000 workers was judged to be the most informative. She also described a nested case-control study by Marsh and colleagues (from the U.S. cohort) of lung cancer among male glass wool manufacturing workers that found, after adjusting for smoking, negligible elevation in risk and no significant association with cumulative exposure, average exposure or duration of exposure to respirable glass fibers, with 186 cases considered. However, a study involving female glass wool workers, using glass filament workers as a reference, found a three-fold increase in relative risk, although women were exposed to lower levels of glass wool than men. However, the power of the study was low, with only 6 cases. Risks increased with increasing employment duration and latency, but not with cumulative exposure. Looking at other cancer sites, some reports of cancer of the upper respiratory tract and alimentary tract were noted; mesothelioma data were inadequate to evaluate.

Dr. Jahnke discussed why GWFs were being considered as a class and that individual fibers of the class vary in physicochemical properties. Only a subset of fibers has been tested for carcinogenicity, and commercial bulk material can contain potentially carcinogenic fibers. Addressing the question of how carcinogenic and non-carcinogenic fibers can be differentiated while accurately predicting the carcinogenicity of untested fibers, she said studies have demonstrated that dose, dimension, durability, and biopersistence are key factors in determining carcinogenicity, and that different review groups have divided fibers into separate hazard categories based on different parameters to assess biopersistence or durability.

One parameter is commercial application, or use of the fiber. In general, SPFs are more durable than insulation fibers, and so commercial application can be used as a surrogate marker for biodurability. The International Agency for Research on Cancer (IARC) has rated insulation glass fibers as not classifiable as to carcinogenicity in humans (Group 3), while it has rated SPFs

as possibly carcinogenic to humans (Group 2B). Concerns with commercial application as a parameter include: there is some overlap in physicochemical characteristics, products with the same use may have different compositions, and use and physical-chemical characteristics are time-dependent, in that technology and use can change.

Another parameter is *in vitro* dissolution rate and size. K_{dis} is a mathematical model showing that a fiber's dissolution rate can be related to tumor formation and fibrosis; fibers with a K_{dis} >100 are unlikely to cause fibrosis after inhalation. The RoC Expert Panel has recommended that special fibers of concern, which are ≥15 μm in length with a K_{dis} ≤100, be listed as reasonably anticipated to be human carcinogens. Concerns about this parameter include: other factors may also be important in biopersistence, the assay is not standardized, it is unclear whether the most relevant assay is at pH 7.4 or 4.5, and K_{dis} has not been adopted by regulatory agencies in the United States, the European Union (EU), or Germany.

$WT_{1/2}$ is another parameter to be considered. $WT_{1/2}$ tests were modeled from biopersistence and fibrosis results observed after 2-year inhalation exposure in rats. It was found short-term biopersistence scores could be used to predict average collagen score, reaching significance with a $WT_{1/2}$ ≥10 days by inhalation or ≥ 40 days by intratracheal installation. The EU and Germany use $WT_{1/2}$ tests in fiber exoneration criteria. Although the EU and Germany classify all synthetic vitreous fibers (as a class) as possibly or probably carcinogenic, individual fibers could still be exonerated on a case-by-case basis. The EU will exonerate if a fiber passes one of four tests used; in Germany a fiber is exonerated if it passes one of three tests used.

She provided an example of the use of the three parameters she had outlined to assess carcinogenicity, showing results for the respirable fractions of two similar insulation fibers, MMVF 10 and MMVF 11. Since both are insulation fibers, both were considered non-carcinogenic according to the commercial application parameter. However, under the K_{dis} parameter, MMVF 10, at 300, would be classified as non-carcinogenic, while MMVF 11, at 100, would be considered carcinogenic. On the other hand, looking at the $WT_{1/2}$ parameter, MMVF 10, at 14.5 days, would be judged carcinogenic, while MMVF 11, at 9 days, would be non-carcinogenic. Thus, different conclusions can be reached depending on the parameter used to assess biodurability, making final assessments difficult.

In summary, Dr. Jahnke reiterated that the proposed RoC listing for respirable GWF as a class is *reasonably anticipated to be a human carcinogen* based on sufficient evidence from animal studies and supporting mechanistic evidence. However, the DSP acknowledges that not all glass fibers are carcinogenic, and that the dividing line between carcinogenic and non-carcinogenic fibers is unclear. A range of carcinogenicity has been observed, with individual fiber properties influencing carcinogenicity. Thus, fibers should be tested *in vivo* on a case-by-case basis, as is done in the EU.

B. BSC Questions

Dr. Nagarkatti asked whether animal studies had been conducted in species other than rats and hamsters, and if so, why they were not included. Dr. Jahnke replied that there had been studies in guinea pigs, as well as inhalation studies in monkeys, that had been negative. She explained that she did not include negative results in her presentation, as it is the practice to only report studies that support the listing recommendation.

Dr. Teeguarden asked about any correlation between the carcinogenic properties seen *in vitro* and the results reported *in vivo*. She answered there is some correlation in terms of the production of reactive oxygen species (ROS) and they discussed whether ROS production and fibrosis lead to tumor formation. Dr. Bucher added that in terms of genotoxicity, a positive result would indicate the positive potential for a response, but that the concept could not be reversed.

Dr. Sherley asked if there was any way to draw a line in the carcinogen vs. non-carcinogen determination—whether it was simply difficult, or impossible. Dr. Jahnke replied that clearly durability is a factor, and that element is what the industry has been addressing with the development of less bio-durable fibers. She said the question is a moving target, as new products are constantly being developed and old ones removed from the market. Dr. Lunn elaborated that the listing must apply also to new fibers that have not been tested.

Dr. Solomon said the listing of PCBs had been used as an example and wondered if that was an analogous situation in that not all PCBs are carcinogenic. Dr. Lunn agreed that the PCBs listing is probably the closest to this GWF consideration in the NTP's history.

Dr. Zelikoff asked if there were any differences in production of the fibers, other than size, and whether other chemicals were added as part of the manufacturing process. Dr. Jahnke said it depends on the end use—that some insulation fibers have a binder added, for example. Also, some fibers have different chemical make-ups or different sizes depending on application, and that is determined in the manufacturing process. Dr. Zelikoff questioned whether the production process itself had been looked at as a parameter in terms of the carcinogenesis. Dr. Jahnke replied that the use of various oxides in production was in fact part of the carcinogenesis index that is one of the factors considered.

Dr. Teeguarden asked about the challenge of classifying materials yet to be produced. Dr. Lunn said there is a benefit to classifying based upon physical and chemical characteristics, allowing more generalizable approaches as opposed to testing one fiber at a time, while still allowing for new developments. Dr. Bucher added that the listing is based upon two factors. First of all, the recognition that none of the current methods of classification are quite "ready for prime time," thus the listing of GWFs as a class, while still indicating as much information as possible as to the critical chemical and biopersistence properties that would lead one to believe that the fibers

would be falling more toward the non-carcinogenic end of the class. He said it is clearly a communication challenge.

Dr. Quinn acknowledged the potentially wide variability in diameter of glass fiber products, and said the ability to define which ones are carcinogenic and which are not is still being worked on, in evaluating the results of toxicological studies. Dr. Cattley asked Dr. Jahnke whether leukemias had ever been found from the non-inhalation routes of exposure. She confirmed that leukemias had never been described through non-inhalation routes of exposure.

C. Public Comments

Mr. Angus Crane, North American Insulation Manufacturers Association (NAIMA), described NAIMA's petition to delist insulation glass wool, which was originally filed in 2002, based on IARC's 1987 decision to change its classification of GWFs from Group 2B, possibly carcinogenic, to Group 3, not classifiable. That reclassification concluded that the human data remained "inadequate," and the animal data were no longer "sufficient," but "limited." He noted that in 2009, NTP's expert panel on glass wool had unanimously recommended to delist insulation glass wool. Mr. Crane stressed the fact that there were actually two separate nominations in this case—NAIMA's nomination to delist glass wool insulation and NIEHS' nomination to list SPFs. He noted that NTP had acknowledged that these were separate nominations, which are reflected in the IARC classifications. Thus, NAIMA's petition asks NTP to recognize that GWFs do not meet NTP's criteria for listing in the RoC, noting that they represent about 99% of the total commercial volume of glass fibers. Asking that the NTP follow the advice of its expert panel, he noted that the DSP acknowledges, "not all fibers within this class cause cancer." He said the DSP as it stands would make the United States the only jurisdiction in the world where glass wool insulation fiber would need to be labeled as a carcinogen, creating a dichotomy that prevents a globally harmonized system of classification and labeling. He reported that NAIMA believes there is a solution to the problem, by mirroring the EU's system of classification, which creates a "bright line" differentiation between insulation glass wool and SPFs, using the four tests described earlier by Dr. Jahnke. He pointed out that many international and U.S. authoritative bodies differentiate the fibers, and that industry practice provides a clear delineation of glass wool insulation from SPFs in terms of biosolubility/duration, uses, and diameter. In conclusion, he asked that the NTP delist glass wool insulation and that at a minimum NTP should recognize the differentiation between insulation glass wool and SPFs of concern.

Mr. Bruce Ray, Johns Manville, supported Mr. Crane's assertion that SPFs of concern should be differentiated from insulation fibers, noting that such a differentiation has long been the practice of health effects researchers, scientific bodies (NTP Expert Panel, IARC, NAS, ATSDR), manufacturers, end users, and product stewardship professionals. He said SPFs are sold to manufacturers, not to consumers, for use in high performance liquid and air filters, and battery chamber separators. They are used in nuclear power plants, semiconductor manufacturing clean

rooms, electric transportation, and continuous power backup products. His company believes that the RoC should reflect actual hazards, and there is scientific evidence to support the differentiation between insulation fibers and SPFs in terms of potential cancer hazard. He urged NTP to reflect that differentiation in the RoC, and to follow the recommendation of the Expert Panel. He acknowledged that older, more durable SPFs such as JM 475 should be listed as "reasonably anticipated," but added that insulation fibers have no animal cancer hazard by inhalation, the most relevant route of exposure, and should not be listed. He cited JM 475 as a case study in the practices of his company—it became clear years ago that its durability and biopersistence were key to its toxicology, and the company designed a new fiber to replace it, with JM 481 matching 475 in durability, but not being biopersistent, with a K_{dis} of 250. Similarly, the JM 753 aerospace/filtration fiber designed decades ago, with durability clearly related to its potential hazard (K_{dis} = 15-30) was discontinued and replaced by an inherently safer fiber, JM 902, with a K_{dis} of 150. He concluded by enumerating the reasons why lumping together all GWFs as potential carcinogens is bad policy and would have negative consequences (1) less incentive to conduct product hazard research, (2) less incentive to develop and manufacture safer products, and (3) it sends a strong and scientifically incorrect signal that biosoluble fibers have the same hazard as biodurable fibers

Dr. Quinn asked whether during the testing of the more biosoluble fibers, length and diameter distributions had been accounted for in addition to comparisons between less soluble and more soluble particles. Mr. Ray said they were taken into effect, and had been painstakingly addressed in the studies done by the RCC (Research and Consulting Company, Füllinsdorf, Switzerland) in the 1990s. Dr. Quinn asked if there were data available regarding comparisons of the size distributions and the doses delivered in the applicable studies. Mr. Ray replied that the RCC tests on the JM 475 fibers had delivered intact fibers to the animals for inhalation, and that the later tests on the replacement JM 481 fibers had followed the EU and German protocols, which require size-separated fibers.

Dr. Solomon asked Mr. Ray whether the "greener" fibers he had mentioned were classified as SPFs or insulation fibers. He said the JM 481 fiber was classified as neither by IARC, but was designated to be a "newly developed, more biosoluble fiber." Dr. Solomon found that confusing given Mr. Ray's request to NTP to differentiate between insulation fibers and SPFs. He replied that that situation was well covered in the recommendations of the Expert Panel.

Dr. Birnbaum asked whether, in the context of green chemistry, a full life cycle analysis had been performed with any of the fibers in question. Mr. Ray replied that in his industry, there is a passion for understanding the hazards associated with their products and making ever-safer products. Dr. Birnbaum expressed support for that concept, but was concerned about what happens to the products once they have reached the end of their useful lives and are either recycled or dumped somewhere. Mr. Ray said their trade association is actively engaged in a full quantitative life cycle analysis for all of their products.

Dr. Zelikoff asked whether the newer, more soluble fibers had been tested in animals, the JM 902 for example. Mr. Ray replied that the 902 fiber had been tested according to the EU protocol, and had passed the intratracheal installation exoneration test. He added that all of his company's new products will be soluble, with a K_{dis} above 100, and that anytime a newer, soluble fiber can replace an older, less soluble fiber, the older product would be discontinued. Dr. Solomon asked about the typical K_{dis} of an insulation fiber. Mr. Ray said his company's 901 fiber, its "workaday" insulation fiber, has a K_{dis} of approximately 300.

Ms. Rudel asked what U.S. regulations would require labeling as a carcinogen as a result of being listed in the RoC. Mr. Ray said under the OSHA Hazard Communication Standard, a cancer hazard must have a label indicating such a hazard. The cancer hazard is determined by either data showing the substance to be a hazard, or the fact that an authoritative body has determined the substance to be a cancer hazard, with the NTP listed as one of those authoritative bodies, along with IARC and OSHA itself. He said a requirement to put a hazard label on a substance known to be a non-carcinogen would "turn the hazard communication and product stewardship principles on their head," and that is the essence of their petition to delist, in hopes of having the RoC provide accurate information for appropriate labeling. Ms. Rudel asked whether a cancer bioassay had been performed on the JM 902 fiber. Mr. Ray replied that it had been done according to the EU protocol. Ms. Rudel pointed out that that did not include pathology, which Mr. Ray acknowledged. Ms. Rudel said there was still some question as to how predictive the EU protocol tests are, and that conducting longer-term tests might be more convincing evidence if the products still proved negative in terms of carcinogenicity. Dr. Roger McClellan said part of the EU's approach is driven by animal welfare concerns, and that there is a high degree of confidence in the predictive quality of the biosolubility tests, thus avoiding extensive experimental animal use.

Dr. John Hadley, Owens Corning, discussed why biopersistence is important in this context. He said it is because of the aerodynamics involved, including the anatomy of the lung and the fibrous shape of fibers. It is known that the fibrous shape is the only geometry in nature with the ability to penetrate to the lower regions of the lung. Thus, the long fibers are particularly important, as is dissolution rate, because the body has no natural way to rid itself of long fibers; continued exposure will result in chronic inflammation and disease. This puts the critical role played by K_{dis} in perspective. He provided examples of the dissolution rates of several materials, demonstrating the very wide range involved. Quoting from the charge to the BSC, he said the DSP does not meet the criteria in terms of providing enough information for a reader to judge either the quality or the relevance of the available data. He then quoted a passage from the DSP questioning the predictive ability of K_{dis} as an example, pointing out that the dosage difference between an insulation fiber study and a SPF study was not discussed, nor was a companion paper in which K_{dis} was specifically recognized as one of the principal determining characteristics. He said the DSP had omitted some important science, specifically the well-established principle that the dissolution rate is a function of composition. Fiber composition links directly to dissolution

rates *in vitro* and *in vivo*, to long fiber clearance *in vivo*, and ultimately to presence or absence of chronic lung disease. The predictive value of the dissolution rate *in vitro* has allowed the EU, he asserted, to design short-term, animal-sparing protocols that allow manufacturers to obtain clearance to produce special compositions of wool that have guaranteed solubility. This relationship between composition and K_{dis} is what allows manufacturers to control the biopersistence of the fibers they produce. Dr. Hadley said omission of this point was a major shortcoming of the DSP. He concluded by pointing out that 19 international fiber science experts had voted unanimously to remove insulation wool fibers from the IARC list of possible carcinogens, and 8 NTP-selected fiber experts on the Expert Panel had done the same, whereas the opposite conclusion was reached by two anonymous governmental committees of non-fiber scientists. He reiterated his main points about the critical role of biopersistence in the toxicology of fibers, the fact that (1) the science is well-established, (2) multiple panels of experts had recommended removal of insulation glass wools from lists of possible carcinogens, and (3) the DSP preliminary recommendation is the only exception.

Dr. Birnbaum asked Dr. Hadley how he knew that the scientists on the anonymous government panels were not fiber experts. Dr. Hadley replied that in his twenty years of experience in the field, he had never seen a publication on fiber biopersistence by a government scientist.

Dr. Solomon asked whether K_{dis} was being used as a surrogate for dose, and whether something with a high K_{dis} was deemed to be non-carcinogenic, or non-carcinogenic at a dose-equivalent level. Dr. Hadley confirmed that a substance with a high K_{dis} is in fact deemed non-carcinogenic, and that K_{dis} can be used as a surrogate for dose.

Dr. Lunn questioned the strict K_{dis} cutoff at 100, pointing out that at that level, one of the insulation fibers would still be listed. He answered that in the mathematical model, the actual cutoff for fibrosis seemed to be approximately 80 to 85, and that the K_{dis} =100 insulation fiber she was asking about had been through an inhalation test with no evidence of fibrosis.

Dr. Teeguarden asked for elaboration on the K_{dis} mathematical model paper, in that K_{dis} was being directly related to disease incidence. Dr. Hadley replied that it was perhaps inaccurate to call it a mathematical model, in that the parameters do not change. He said for smaller fibers, the rate of clearance is all the same, based on uniform mucociliary activity. Thus solubility is not an issue in that instance, but it applies to the larger fibers. Dr. Teeguarden asked if this model was the closest thing that exists to a lung dosimetry mode for partially soluble fibers. Dr. Hadley said he wasn't that familiar with what had been done with asbestos, and that this was just a simple attempt to use the concept of biopersistence to reconcile the data on hand from many studies.

Dr. Birnbaum said it had recently emerged that asbestos exposures can lead to adverse effects at non-pulmonary sites, so she questioned the focus on inhalation studies exclusively, relative to protection of public health. Dr. Hadley said there was no indication of fibers being detected in

non-pulmonary tissues, except for occasional detections in the lymph nodes and pleura. He said he was unaware of any means of transport of the fibers outside of the pulmonary circulation.

Dr. Quinn asked how the target tissue, especially for human lung cancer, was accounted for when evaluating K_{dis}, and whether the alveolar region is what is being considered to be the target tissue. Dr. Hadley confirmed that the alveolar lining layer was the target tissue. Dr. Quinn asked whether the model included accounting for exposures in the tracheal/bronchial region, as that is apparently the area where most lung cancers are formed. Dr. Hadley said total removal of fibers from the lower lung was the standard, including some of the bronchi, but none of the trachea.

Referring to a figure in Dr. Hadley's mathematical model paper, Dr. Teeguarden clarified that the bright line cutoff point of K_{dis}=100 was designed to protect against fibrosis, not lung cancer *per se*. Dr. Hadley agreed. Dr. Sherley pointed out that much weight was being placed on the mathematical modeling, which had been published several years ago, and asked if any empirical testing had been done since its publication. Dr. Hadley replied that it had been used to test durable and soluble fibers, but had not been individually tested. He explained the model simply uses biopersistence to adjust the dose rate, and added that it is actually mechanistically conservative, particularly in that a specific dissolution rate in a rat, say three months, would be the same in a human, and would constitute a much smaller segment of the lifespan.

Dr. Thomas Hesterberg, from Navistar and a consultant to NAIMA, restated that the DSP was in disagreement with the Expert Panel, IARC, and EU conclusions by classifying all glass fibers as reasonably anticipated to be human carcinogens. He noted that a robust body of science does not provide evidence of carcinogenicity of GWFs, including epidemiological studies and high concentration animal inhalation studies. The low biopersistence of GWFs provides a mechanistic basis for their lack of carcinogenicity. Speculating as to why the NTP's DSP is in conflict with the Expert Panel and the IARC, he said the NTP appears to have given more weight to intracavity injection studies, despite the fact that most fiber scientists have concluded that those techniques are not appropriate for evaluating human health hazards. He said that approach is contrary to a vast body of scientific research, and that there are numerous problems associated with this non-physiological method of exposure. He said injection of fibers bypasses the natural defense mechanisms of the lung, with the possibility of injecting large, non-respirable fibers that would not normally get to the lung. Also, large quantities of fibers are concentrated at the injection site, and the normal defense mechanisms of the lung may be overwhelmed, leading to the induction of promotion of cancer that may otherwise not have occurred. He provided a list of several scientific panels that have concluded that the intracavity tests are not useful for hazard assessment of fibers. He also pointed out shortcomings associated with *in vitro* cell culture studies of glass fibers, including many of the same shortcomings as the intracavity studies. They also produce numerous false positives and have little value for glass fiber hazard assessment. He said the gold standard is the well-conducted chronic inhalation bioassay, characterized by

respirable fibers in exposure aerosols with a large proportion of long fibers in sizes representative of occupational exposures. The aerosolization system does not destroy the fiber geometry, and multiple exposure levels can be achieved, with the highest exposure at the Maximum Tolerated Dose (MTD), while avoiding lung overload and non-specific pathology. He displayed the data from several inhalation studies from his laboratory, with different fibers ranked according to pathogenicity. He strongly urged the NTP to follow the advice of its own Expert Panel and the IARC regarding the inclusion of insulation glass wool in its proposed listing.

Dr. Roger McClellan, engaged by NAIMA to review the documents pertinent to the BSC's considerations, pointed out that a decision not to list or to delist a substance has important ramifications to the public and regulatory agencies. He noted an important distinction between evaluation of specific chemicals, which do not change, although evidence of carcinogenicity may change over time, and the physical properties of glass fibers, which can be changed by altering production methods to produce safer, biosoluble fibers without carcinogenic potential. He characterized the sound scientific basis for evaluating the carcinogenic hazard of glass fibers—epidemiological evidence, *in vitro* and implantation/injection evidence (which he characterized as crude screening tools lacking in specificity), and animal inhalation evidence, particularly post-1985 research using inhalation bioassays. Describing the participants as "world class experts," he related the conclusions of the 2002 IARC Expert Panel evaluation, which endorsed separate classifications for insulation glass wool and SPFs. Similarly, he related the decisions reached by the 2009 NTP Expert Panel, which recommended delisting GWFs and listing SPFs. He then presented a corrected statistical analysis for Mitchell *et al.* (1982), a paper cited in the DSP, which showed no evidence of exposure-related effects for insulation glass wool, contrary to the conclusions shown in the uncorrected version cited by NTP, which found mononuclear cell leukemia (MCL) in the spleen of exposed rates. Dr. McClellan urged the NTP to follow the "scientifically sound advice" of its Expert Panel.

Responding to a question from Dr. Faustman, Dr. McClellan said he felt that the bright line K_{dis} cutoff of 100, although a subjective professional judgment, was just about on target in his opinion.

D. BSC Discussion

Dr. Cattley, first lead reviewer, noted that the background document distinguishes between insulation glass wool and other synthetic vitreous fibers, and between insulation glass wool and other SPFs, according to a variety of criteria that include size, metal oxide content, and durability. He said there would be value in maintaining these distinctions in the profile, especially since insulation glass wool comprises 99% of production, while SPFs account for just 1% and only appear in highly specialized circumstances. Differences in biopersistence and carcinogenicity appear relatable to the differences between the materials. He noted that while the distinctions do appear in the profile, the extension of those distinctions into the summary

section on carcinogenicity seems limited. He recommended extending the distinctions into the listing section, and making that consistent with the Expert Panel's report. He mentioned that in the profile the potential for exposure among workers in insulation wool is well documented, but additional estimates for the number of workers exposed to SPFs and upper limits for airborne fiber levels would be of value. He questioned the apparent over-reliance in the DSP on a single study implicating insulation wool in an increase in MCL in F344 rats, given the high background rate of that neoplasm in that rat strain. He felt that the mechanistic data suggest differences between glass wool and SPFs with respect to biosolubility and biopersistence, and that those are likely critical factors when comparing the carcinogenicity of the different fibers, but the relationship of those factors to carcinogenicity should be more explicitly addressed in the profile.

Dr. Quinn, second lead reviewer, expressed support for treating the respirable GWFs as a class. There is evidence for separation by K_{dis}, but that is not the only dimension of fiber exposure that may be related to carcinogenicity and other adverse outcomes. Fiber length and diameter are not well characterized in animal or human epidemiology studies. The scientific evidence does not support limiting the scope of the DSP only to respirable fibers, and that "inhalable" might be more appropriate terminology. She said "respirable" might limit consideration to fibers that deposit efficiently in the alveolar area of the lung, whereas the target tissue in most lung cancers is the tracheobronchial region, and should be explicitly included in studies of fiber toxicity. The exposure section of the DSP should include a description of indices of exposures that have been considered to be biologically active, including fiber length, diameter, and biopersistence. This would be useful to interpret the animal studies and human exposure assessments. To fully understand fiber dimensions it is useful to look at fiber variability in terms of fiber length and diameter. She added that although biopersistence is certainly important, the link between it and cancer outcomes in animals is not yet fully established. She disagreed with the conclusion in the DSP that there is "inadequate" evidence of human carcinogenicity, averring that it should instead be considered "limited." She cited the language of the RoC listing criteria, "there is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias, or confounding factors, could not adequately be excluded." There is consistency of findings across the published studies, in that nearly all reported a modest elevation in lung or respiratory cancer risk. While some findings were statistically significant and some were not, it is notable that nearly all the studies were in the same direction, showing a relative risk of one or greater. This is common and expected pattern in occupational cancer studies with the healthy worker effect. One would expect to find it less than one in at least some of the studies if there were no carcinogenic effect. Exposure assessments were generally of limited quality, likely resulting in substantial misclassification of the study populations by exposure status. In addition to the typical limitations of historical exposure data, studies of glass wool fiber exposures have the potential to be misclassified because the most commonly used metrics may not be the most biologically active. This error will, on average, bias resulting relative risks towards the null. The DSP appears to place considerable weight on lack of a "dose-response" pattern as a reason to discount a study's

positive findings. This criterion of dose-response should be cautiously interpreted when exposure assessments are of limited quality. Random misclassification of exposure measures will often result in inconsistent response patterns over different exposure levels. The pattern of consistently elevated risks, with limitations due to inconsistent exposure characterization and limited investigation of confounding, fits well the situation described by the NTP criteria for "reasonably anticipated to be a human carcinogen.

Dr. Lunn responded that the "inadequate" designation did not mean negative. Dr. Quinn elaborated, describing the technical aspects of the exposure assessments carried out by her research group and others, which she said could in fact lead to misclassifications, potentially significantly reducing the power of the study.

Dr. Jahnke responded to Dr. Cattley's comments about MCLs in inhalation studies. She said when the study in question was conducted, separating males and females was not typically done. She also reported that the statistical calculations had been re-done. She said the authors had included quantitation of fibrosis, and that the inhalation study of insulation fibers showed the lymph nodes to be positive. She said she would add that information to the document to clarify why those data were considered to be significant. Dr. Cattley replied that plausibility was the issue, in that in all of the other studies, including intracavity injections, there was no other evidence of leukemia.

Dr. McDiarmid, third lead reviewer, agreed with Dr. Cattley regarding the clarity of the identity and descriptions of the fiber classes needing to be tightened. She felt that the DSP should be understandable to people who want to read it, but is not at this point, and it is difficult to follow the argument in the document in terms of the nomenclature. She made several specific suggestions for editing changes to the DSP, and generally suggested moving glossary information into the body of the document to help the reader understand the wording.

Dr. Teeguarden, fourth lead reviewer, said he felt the issue of the inhalation study involving MCLs was very significant, and wondered how NTP would deal with the issue based on BSC comments. He also wondered in terms of the mechanistic questions whether a single finding of tumor development in an animal strain with a very high background, of statistical questionability, is really plausible, emphasizing that the fate of the material seemed to hang in the balance, and so the issue deserves a very high level of scrutiny and a very strong justification from the NTP. He was not currently convinced that such a justification existed. He asked where NTP was going with this, given the BSC's opinions. Dr. Bucher said he was not sure exactly where NTP would go and that it is still in the process of gathering information. He said the BSC discussions on MCL were similar to recent NTP internal discussions on MCL in preparation for the meeting, and that the NTP was listening. Dr. Teeguarden pointed out that dose should have been considered in the incorporation of the study implicating SPFs in mesothelioma (Miller *et al.* 1999). Despite discussions that there were other important factors than biopersistence to consider when evaluating the carcinogenicity of fibers, it should be kept in mind that

biopersistence is in fact a very strong factor that seems to correlate with *in vivo* responses. The class issue is the most important element of the entire assessment. He reported that in conversation with NTP staff, they had said it would not be appropriate to classify the fibers by use, nor by physical/chemical properties. In the DSP, he noted, it seemed quite clear that there was vacillation regarding whether there is even a class in this situation. Looking at the statement on page one of the DSP regarding the fact that not all of the materials are carcinogenic, he said it begs the question as to whether there is a class at all in this situation, of which he was not convinced. He said he could not agree that the DSP in its current form is technically correct or accurate regarding classification. The insulation wool fibers and the SPFs could not be swept together into a single classification; it really isn't appropriate to classify fibers by use, but they should not be classified by physical/chemical characteristics alone. New fibers designed for biosolubility would still be labeled under this proposal, even if they were clearly non-carcinogenic. He noted some apparent reluctance in the DSP to accept K_{dis} as a parameter of carcinogenicity, but that the same level of scrutiny had not been applied to the other *in vitro* studies, such as the genotoxicity studies and transformation studies. He said the K_{dis} does make sense, and urged it be more recognized in the document to provide some needed balance and fairness. He suggested the addition of a short mode of action statement in the mechanistic section to discuss development of mesotheliomas.
Dr. Jahnke responded regarding the statistical methods used in the MCL study, clarifying that the analysis had ruled out the possibility of chance leading to the positive conclusions. She asked what he would recommend doing regarding the class issue, knowing the currently available information. He responded that two major expert panels had already made a decision about the issue, and that he had picked up the fact that there was a major problem within the first two paragraphs of the DSP—that there just wasn't a basis for a class. He recommended the NTP try to classify the materials according to the properties most likely to correlate with carcinogenicity, which would set the appropriate stage for today's materials as well as tomorrow's, and would be most useful for all concerned parties.

Dr. Zelikoff agreed with Dr. Teeguarden's comments regarding the issue of class in the document. She also agreed with Dr. Quinn's assertion that "inhalable" would be a better term in the document than "respirable." She asked Dr. Bunton whether fibrosis always leads to cancer. Dr. Bunton said many different things could cause fibrosis, but in this situation it was being used as an indicator of something that would go on, appropriately or not. Dr. Cattley said fibrosis was being used as a surrogate for ongoing injury and continuous macrophage activation, which are believed to be the pathogenesis for many types of fiber carcinogenicity. Dr. Zelikoff questioned the use of fibrosis as an indicator for lung cancer. Dr. Cattley explained it not necessarily an indicator, but some of the characteristics of fibrosis, such as release of cytokines and generation of ROS have been implicated in carcinogenesis; this is a reflection of biology occurring that is relevant for cancer. Dr. Zelikoff said she was not yet convinced of the correlative value of the K_{dis} metric.

Dr. Eastmond asked for an explanation for the difference between the recommendations of the Expert Panel and the conclusions of the DSP. Dr. Lunn replied there were many points in the DSP that had been incorporated from the Expert Panel's report. She said NTP was uncomfortable with accepting the K_{dis} standard. The NTP had sought to follow the EU's idea of listing as a class, but then allowing individual substances to be exonerated. Dr. Eastmond said perhaps "biopersistent" should be added to the document's title, but left ambiguous, to facilitate the use of the EU approach, the German approach, or another approach, thus pointing to the importance of the single general property, but without going into great detail. Dr. Bucher replied that that is what NTP thought it had done, by classifying the whole class as *reasonably anticipated* while recognizing that there were fibers with particular general characteristics that would not be likely carcinogens. Dr. Faustman questioned whether regulatory agencies would appreciate and recognize that subtle distinction. Dr. Bucher said he was unsure, but that most regulatory agencies have discretion to interpret within this area. Dr. Toraason said he was unsure how NIOSH would deal with the distinction. Dr. Sherley said he felt uncomfortable with some of the language in the DSP. He suggested replacing "not all fibers within this class cause cancer," with "not all fibers within this class are reasonably anticipated to be a human carcinogen," because the language in the former is too strong for what is known about GWF.

VIII. Peer Review of Draft Substance Profiles for the 12th Report on Carcinogens: Cobalt-Tungsten Carbide: Powders and Hard Metals

A. Presentation

Drs. Olshan and Landolph joined the table as *ad hoc* reviewers for this portion of the meeting. Dr. Lunn presented the DSP on cobalt-tungsten carbide: powders and hard metals (CoWC), which are made up of composites of tungsten carbide particles with a metallic cobalt powder binder. The hard metals are produced by pressing the composites into a compact solid at high temperature by a process called sintering. This listing is specific to hard metals, powders and dusts that contain both cobalt and tungsten carbide. The materials are primarily used for cutting tools, with the hard metals characterized by extreme hardness, abrasion resistance and toughness.

Dr. Lunn reported that there is considerable production in the United States, with 2004 domestic output estimated to be 6,080 tons. The most significant exposure, primarily via inhalation or dermal absorption, occurs in occupational settings such as hard metal manufacturing, recycling of hard metal products, and grinding and sharpening of hard metal tools. End users receive negligible exposure. There is also some evidence of exposure in the general population living in the vicinity of hard metal production or maintenance facilities.

Exposure studies have been conducted at all stages of the production process: pre-sintering, sintering, and post-sintering. Higher exposure was seen during pre-sintering and sintering operations than in post-sintering, when the hard metals are ground into the finished tools. The NTP is recommending that cobalt-tungsten carbide: hard metals and powders be listed as

reasonably anticipated to be human carcinogens, based on limited evidence of carcinogenicity from studies in humans, along with supporting mechanistic evidence. Dr. Lunn delineated the limited evidence in humans:

- Consistent findings of excess lung cancer mortality among cobalt-tungsten carbide hard metal manufacturing workers across studies
- Positive exposure-response relationships
- Risks not likely to be explained by confounding from tobacco smoking
- Limited number of studies; only one well-conducted study of an independent population

Dr. Lunn cited several details of the four studies cited in the DSP, including a Swedish multi-plant study involving more than 3,000 male workers, a French industry-wide study in 10 plants involving more than 7,500 male and female workers, and two smaller studies conducted at individual plants in the French study. She described the French studies in more detail, noting that the larger study had used a job exposure matrix to create a semi-quantitative exposure scale for exposure to CoWC, based on four exposure metrics: level, duration, unweighted cumulative dose, and frequency-weighted cumulative dose. She noted that the two studies conducted in independent populations, the Swedish study and the French industry-wide study, both reported approximately 30% excess mortality from lung cancer. In the Swedish study, there was a significant increase in mortality in men who had worked in the plant for ten years, with a 20-year latency. In the French study, individuals who had been exposed had an approximately two-fold increased risk. In the four exposure metrics, the individuals with the highest exposures had between two- and four-fold increased risk. She added that the smaller French studies generally supported the findings of the larger study. In the French study, risk estimates and trend values were not substantially changed by controlling for tobacco smoking or exposure to IARC carcinogens. The other studies reported similar results regarding potential confounding.

Dr. Lunn said although the mechanism for carcinogenesis resulting from exposure to CoWC is unknown, and there are two proposed potential mechanisms. In one, CoWC undergoes solubilization to cobalt, which itself can cause cancer. It has also been shown that tungsten carbide increases the bioavailability of cobalt, and that cobalt has been detected in the urine, lymph node, lung and other tissues of hard metal workers. Inhaled cobalt sulfate has been shown to cause lung cancer in mice and rats, and there is a body of evidence relating cobalt ions to key events associated with carcinogenicity, including the production of ROS leading to oxidative stress, DNA repair inhibition, genotoxicity, disruption of cell signaling pathways, the regulation of genes involved in the response to hypoxia, modulation of apoptosis, etc. The other possible mechanism involves CoWC itself, which has shown greater cytotoxic and toxic effects than either cobalt or tungsten carbide alone in *in vivo* and *in vitro* studies. There is greater production of ROS, which occurs via a surface reaction and this increased ROS is thought to result in oxidative stress, which may contribute to genotoxicity and other effects. Dr. Lunn enumerated some of the evidence of CoWC genotoxicity in animal, cell, and human studies.

In conclusion, she reiterated the DSP preliminary listing recommendation to list CoWC: hard metals and powders as *reasonably anticipated to be human carcinogens*, based on limited evidence from human studies that demonstrate an association between lung cancer and exposure to CoWC, and based on mechanistic data that demonstrate plausibility and support the findings in humans.

B. Public Comment

Mr. Thomas Shaw, International Tungsten Industry Association (ITIA), introduced himself as Environmental Health and Safety Manager for Sandvik Tooling, a major manufacturer of cemented carbide cutting tools, and as speaking on behalf of the ITIA. He said the ITIA had been involved in the RoC listing process since 2004, when CoWC hard metals and powders were first nominated for inclusion.

ITIA's position was that the epidemiological data on hard metal exposures and cancer is extremely limited, and is insufficient to base a determination of hard metals' carcinogenicity in humans. No cancer bioassays have been conducted on CoWC powders, leaving the NTP to rely mainly on *in vitro* studies specific to cobalt, and not involving CoWC preparations and products currently on the market. Most importantly, he noted, in light of the paucity of scientific information, there are several multi-million dollar studies now underway that will substantially enrich the scientific understanding of the toxicity of CoWC powders, including the first and only chronic cancer inhalation bioassay of CoWC powder, which is being conducted by the NTP. That study is complete and results are being analyzed. The NTP is also conducting the first and only chronic cancer bioassay evaluating oral exposure to tungsten. He said a multinational epidemiological investigation of more than 25,000 workers at 18 facilities in five countries is also currently being conducted and is expected to be completed in 2013 or early 2014. With what he characterized as the overall lack of critical data on the carcinogenicity of CoWC powders and hard metals, and the ongoing studies scheduled to be completed in the near future, he said the ITIA would ask the NTP to delay the listing until the new information can be completed and reviewed by the scientific community.

Dr. Toraason asked Mr. Shaw what exposures are being targeted in the epidemiological study he mentioned. Mr. Shaw replied that to his knowledge the study is targeting all relevant exposures.

C. BSC Discussion

Ms. Rudel, first lead reviewer, said she thought the document was extremely clear, well-written, and made its case very well, including its frank description of the lack of independence of the epidemiologic data. She also felt that it made good use of the mechanistic data. She wondered about the typical co-exposures to IARC carcinogens in the occupational studies, as well as whether the exposure levels being described in the epidemiologic studies exceeded standards.

She did not think the listing needed to wait for new data, as the existing data are adequate in her opinion.

Dr. Olshan, second lead reviewer, began his review by stating that he would like to have seen more data on the potential for exposure patterns to change over time in the DSP general background section on exposures. He agreed with Ms. Rudel that some information on other occupational exposures would be useful in terms of potential confounding. He said he felt that the evidence presented in the DSP supports the listing recommendation. The studies showed a suggestive association of CoWC exposure and an elevated risk of death from lung cancer. Some studies indicated a positive exposure-response pattern. However, potential confounding by tobacco smoking and other workplace exposures, potential exposure misclassification, random error, and other methodologic limitations do not allow a conclusion regarding the causal nature of the association to be drawn. The DSP adequately describes the epidemiologic studies and their strengths and weaknesses, with the exception of some wording changes to better describe the imprecision of the effect estimates. Studies that attempted to adjust for smoking could not rule out residual confounding by smoking, but even with those limitations, the effect estimates could not be totally explained by smoking. Exposure misclassification should not bias away from the null, but some effects might have been stronger with improved exposure assessment.

Dr. Landolph, third lead reviewer, said he agreed with much of what had been said by the two previous reviewers. He suggested adding chemical structure data about how the preparations would change with varying amounts of cobalt. He felt the DSP sections on use, production, and exposure were clear and presented useful information. He also agreed with the listing recommendation. He noted that if the animal carcinogenesis data would be coming soon, it might be prudent to wait for it, so that the ultimate decision would be irrevocable and would stand the test of time. He felt that there were more epidemiologic data in the document than he had expected, and that it was good evidence. He also found the mechanistic data of great interest, although he suggested adding reference to any phagocytosis of CoWC, if such data are available.

Dr. Zelikoff, fourth lead reviewer, said she felt that the information provided was accurate and technically correct. She found the production and use sections to be adequate and asked that information be added to the exposure section regarding particle size. She expressed concern that the reference to the Fallon, Nevada study in the exposure section overstated the authors' conclusions regarding the likelihood that a hard metal facility in the town was a candidate source of airborne particulates. She agreed that it would be prudent to wait for the carcinogenicity studies if they would be available soon. She felt the four epidemiological studies should be broken out within the profile document and treated as discrete entities, to aid understanding by the lay public. In terms of the sufficiency of the evidence in support of the listing recommendation, she said she found the Moulin and Wild studies most convincing due to their consideration of cigarette smoking, a major confounder in lung cancer investigations. By the

same token, she found some of the other studies less compelling due to the lack of consideration of cigarette smoking. Although personal monitoring data, dose-response relationships, and additional independent studies would have strengthened the conclusion of sufficient evidence, she nonetheless found the existing evidence sufficient to agree with the listing recommendation. She felt the data were completely insufficient to conclude any kind of causal relationship for other types of cancers. She found the mechanistic data to be very convincing, "where all of this hangs together." She found the genotoxicity and inhalation/intratracheal installation studies in rodents to be the most relevant support for the human cancer studies, and recommended more emphasis on those studies in the document, along with the studies showing that cobalt associated with CoWC was more bioavailable than that associated with pure cobalt particles alone. She asked for better explanation of the association of gene changes and cancers. Some of the negative genotoxicity data should be included for balance, despite the approach in the DSP of only presenting information supporting the conclusion. Dr. Lunn agreed that more information on particle size should be included. She also agreed that some of the language in the document should be clarified in order to be understandable to the educated lay reader.

Dr. McDiarmid noted that since there were no data on cancer studies in experimental animals in the DSP, the NTP might want to consider looking at Dr. John Kalinich's work at the Armed Forces Radiobiology Research Institute regarding tungsten-nickel-cobalt. Although Kalinich's work was with a different alloy, the animal results were "blockbuster," with all experimental animals dead within five months of administration.

Dr. Nagarkatti asked whether the NTP would be considering the effects of cobalt alone and tungsten carbide alone, as distinct from those seen with CoWC. Dr. Lunn explained that the listing was driven by the human exposures, which are to the mixture CoWC.

Dr. Faustman questioned studies that brought up the issue of smoking as a potential confounder, in that it may call into question the sensitivity of the study to pick up cancers in the first place. Dr. Lunn clarified that Dr. Faustman was referring to the human studies where smoking-related diseases were not found. She explained that in the study, the effects of smoking were not the subject of investigation, only whether the workers had more smoking-related illnesses than would be found in the general population. Thus, smoking would not be considered to be confounding in the cohort. This assumes that the rate of smoking within the cohort would be the same as the control.

Dr. Bunton asked for the standard practice when carcinogenesis data are so close to being ready, as in this particular circumstance. Dr. Lunn explained that, in this case, the NTP felt that there were sufficient data to proceed without a need for additional data. Dr. Bucher said the NTP knows the outcome of the impending studies, and if they become available in time during the process, they would include pertinent information from them.

Dr. Miller said he supported the overall conclusion of the listing, and accepted the mechanistic data, but suggested listing as a *known human lung carcinogen*.

Dr. Zelikoff said she disagreed with Dr. Bucher's statement, and considered the human data questionable in this case. She felt that it was important to include the experimental animal carcinogenesis data if they became available. Dr. Solomon felt that given the NTP criteria, new animal data would be highly unlikely to result in a change in the listing, and since the current listing recommendation is adequately supported by the presently available database and there is a timeline involved in terms of delivering the RoC, it is reasonable to proceed.

IX. Chemical Effects in Biological Systems (CEBS): An Integrative Data Management System for the NTP

Dr. Jennifer Fostel of NIEHS/NTP briefed the BSC on the CEBS database. She noted that the database houses much more than just responses to chemicals, including studies of chemicals themselves, studies of environmental agents such as ozone, studies of genetic changes such as knockout animals, and studies of the effects of physical agents such as magnetic fields. CEBS was originally developed by the NIEHS DIR to house data of interest to toxicologists and environmental health scientists. Although it started as a toxicogenomics and proteomics database, it has since expanded to include public microarray datasets developed by industry and academic labs. Thus it has a flexible design and is open to a variety of study types, she said.

Because of this flexibility, said Dr. Fostel, CEBS moved from DIR to NTP, in that it was well suited to house all NTP data in a single database. Data in CEBS are integrated, thus they can be queried by study or by compound. CEBS can also be used to perform cross-study searches and analysis of NTP data, to provide NTP data to the public, and to permit NTP data to be integrated with other reference datasets. CEBS includes (1) protocol details, (2) microarray data, (3) clinical pathology/histopathology data, (4) reproductive toxicity data, (5) study conclusions, and (6) integrated data. With the aim of housing all public NTP data in CEBS, the task will include (1) collecting and loading NTP legacy data into CEBS, (2) setting up processes to load data from ongoing studies, and (3) modifying the CEBS user interface to highlight special features of NTP studies, particularly bioassay data.

Dr. Fostel reported on the current status of the project. NTP microarray data have been entered into CEBS, with three studies published at this point. Regarding the NTP legacy data, clinical pathology data and immunotoxicology data have been loaded, with genetic toxicology and developmental and reproductive toxicology in progress. Loading bioassay data is next. In terms of loading data from ongoing studies, she said the collection of high-throughput screening data from the NIH Chemical Genomics Center has been completed to date, and that a process has been put in place to align with Project Officers to collect interim and final data from labs.

She provided the BSC members with detailed examples of how to use CEBS, including how to query the database according to a variety of parameters, along with examination of several typical pages from the database, including a study's "dashboard," an index page with links to a wide variety of information available. Searches can be conducted from the dashboard page according to several characteristics. This could be used to access desired information from a particular NTP study. Conversely, the database can be used to arrive at an intersection of multiple parameters to discover pertinent datasets from several different studies, all of which can be accessed individually for further inquiry. The database will search both participants and controls if desired, and allows for various methods of statistical analysis based on the accessed datasets.

In the future, Dr. Fostel reported, NTP has plans for CEBS to (1) create a more meaningful data display of bioassay-scale studies, (2) capture and highlight NTP conclusions, (3) provide a mechanism for the CEBS user to start with a study conclusion and then visualize the underlying raw data, (4) provide the user with a list of chemicals and conditions that produce a particular phenotype, and (5) provide a mechanism to compare and subset lists of chemicals.

Dr. Teeguarden asked whether there was a plan to include chemical structure data in CEBS. Dr. Fostel said that was being considered, including a user interface to allow viewing of structural images. Dr. Nagarkatti wondered whether pathway analysis would be included with the microarray data. Dr. Fostel said that was planned, and that CEBS would like to get input from the NTP to be able to build toxicology-specific pathways information. Dr. Howard asked whether CEBS is integrated with the NCI's databases, caBIG and caBIG Nano. Dr. Fostel replied that there are technical issues involved with that integration, but efforts are ongoing to align CEBS with the NCI databases. Dr. Howard noted that NTP studies several years ago were often very similar in structure and protocol, but that today they are very different, and wondered whether the database has the flexibility to handle those differences. Dr. Fostel replied that flexibility is one of the most attractive elements of CEBS. Dr. Miller asked whether mechanistic data, *in vitro* data, enzyme binding, or receptor binding data are part of the datasets. Dr. Fostel replied that those datasets are planned to be included, although it has been a challenge since the high throughput material doesn't follow a typical pathway and is more accurately described as assay data. Dr. Sherley asked whether there would be curation of the NTP legacy data and how the citations were done. Dr. Fostel confirmed that curation was taking place, and mentioned CEBS' ability to search according to a wide variety of aliases and synonyms would aid incorporation of older data or methodologies. A paper has been published regarding citing CEBS and that paper is accessible on CEBS. Dr. Sherley asked if the system would alert a user if the user were conducting a search that had already been done, such as capturing a search to track common searches. Dr. Fostel said NTP plans to add that capability in the new CEBS interface, to include a Frequently Searched Questions section. Dr. Fernandez asked if there would be any linkage to antidotes or clinical therapies related to studies. Dr. Fostel said that

would be a second-order development; it was planned and would emerge from an ability to communicate with other databases.

June 22, 2010

X. Peer Review of Draft Substance Profiles for the 12th Report on Carcinogens: Formaldehyde

A. Presentation

Dr. Lunn presented the DSP on formaldehyde, describing it as a simple aldehyde and a reactive gas at room temperature. It is in equilibrium with its hydrated form, methylene glycol. It has many different uses, with the production of resins used in wood adhesives, pulp and paper products, plastics, and textiles accounting for more than 50% of its use. It is also used as a chemical intermediate, in agriculture as a preservative and a fungicide, and in chelating agents such as EDTA. Other uses (in medicine, in embalming, and in consumer products such as cosmetics) account for about 5% of its use.

There is significant U.S. exposure to formaldehyde, which saw production of more than 6 million tons in 2007. Exposure can occur in the occupational setting, or there can be environmental exposure, as well as endogenous exposure. The highest levels are seen among workers exposed to formaldehyde, particularly formaldehyde and resin production workers, plastics workers, and embalmers. There is considerable environmental exposure, as formaldehyde is ubiquitous in the environment and is a common metabolite produced endogenously. The primary source for exposure in the general public is indoor air, through off gassing of construction and home products and consumer goods. It has also been detected in outdoor air, food, cigarettes, and water.

The NTP proposes that formaldehyde be listed in the RoC as *known to be a human carcinogen*, due to sufficient evidence of carcinogenicity from human studies and mechanistic studies that support the findings in humans. The conclusion of sufficient evidence in human studies is based on consistent findings of increased risks of nasopharyngeal cancer (NPC), sinonasal cancer (SNC), and myeloid leukemia among individuals with the highest exposure to formaldehyde, with those associations not explicable by chance, bias, or confounding.

Dr. Lunn noted that several types of studies comprise the evidence from human cancer studies, including cohort and nested case-control studies of industrial workers, or professional groups (such as pathologists, funeral directors or embalmers), population-based case-control studies, and numerous meta-analyses. Exposure patterns vary between industrial and professional workers. Industrial workers are usually continuously exposed to formaldehyde, whereas professionals typically have high-level exposure periods alternating with low/no level exposure periods.

The DSP refers to three large cohort studies of industrial workers: an NCI study of more than 25,000 workers at companies that use or produce formaldehyde, a NIOSH study of more than 11,000 garment workers, and a British cohort study of more than 14,000 chemical workers. The NCI study had several advantages, including quantitative exposure-response analyses of peak, average, and cumulative exposures along with exposure duration. It also utilized internal analysis, with the lowest-exposed workers as the reference, whereas most of the studies used the general population as the reference population to determine the expected number of cases. The NIOSH study used a more limited exposure analysis, reporting standardized mortality ratios (SMRs) by latency, duration of exposure, and year of first exposure for selected tissue sites. The British cohort study also was limited in its exposure analysis, reporting SMRs for ever exposed, highly exposed, with a bit more detailed examination for stomach and lung cancers. In addition to those four studies, said Dr. Lunn, several smaller cohort studies and nested case-control studies were also considered, although they were judged to be less informative for the evaluation due to smaller size and more limited exposure analysis.

Six small cohort studies of professional groups were considered, with exposure assessed by license or membership in a professional society. Most informative was the 2009 Hauptmann *et al*. nested case-control study using data from three of the small cohort studies involving embalmers. It was a large study of lymphohematopoietic cancers (LHC), and among its advantages over the smaller studies was the fact that it included quantitative exposure analyses, assessing peak exposure, intensity of exposure, and cumulative exposure, as well as risk calculated by number of embalmings and duration of working in embalming jobs.

Dr. Lunn provided background information on NPC, a rare cancer. In evaluating the potential association of NPC with formaldehyde exposures, the NTP found the collective body of case-control studies to be most informative, along with the NCI cohort study. Other large cohort studies were low in statistical power. Among the more useful studies, there were consistent findings of increased risk of NPC among individuals with the highest exposure. Those elevated risks remained after consideration of confounding by tobacco smoking or wood dust or other occupational exposures. Statistically significant trend tests for exposure-response relationships further strengthened the association, she said.

SNC is another rare cancer, presenting in two major histological subtypes—adenocarcinoma (ADC) and squamous-cell carcinoma (SCC). Here again, the NTP found the collective body of case-control studies to be important, particularly a pooled analysis of 12 case-control studies conducted by Luce *et al.* in 2002. Due to low statistical power, the NTP found the cohort studies less informative in this case. The case-control studies reported consistent findings of increased risks for SNC, with higher risks found among individuals with higher exposure. Importantly, increased risks were found after controlling for wood dust exposure, a known cause of ADC, and among individuals with little or no exposure to wood dust. Also, there was some evidence of a synergistic effect ascribed to co-exposures to wood dust and formaldehyde.

LHCs are a heterogeneous group of tumors classified by tissue distribution at the time of clinical presentation as either lymphoma (in the lymphoid tissue) or leukemia (in bone marrow and blood). They can also be classified by stem cell origin, lymphoid or myeloid. They are common cancers in terms of incidence. In its evaluation of the literature, the NTP found excesses of all LHCs combined and leukemias combined in all six cohort studies of professional workers and some of the industrial cohorts. In the NCI study, there was positive exposure-response with peak exposure. In the nested case-control study of embalmers, increased risk for non-lymphoid LHCs was seen. The strongest association was seen with myeloid leukemia, with positive association in the most informative studies, which was unlikely to be explained by confounding, due to the variety of exposure scenarios involved. A 2009 meta-analysis by Zhang *et al*. reported a positive association among workers with the highest exposure. The strongest findings among the informative studies were in the nested case-control study among embalmers by Hauptmann *et al.* 2009, which found statistically significant (or approaching significance) risk estimates among individuals in the highest category (compared to the lowest category) of (1) employment duration, (2) number of embalmings, and (3) cumulative exposure to formaldehyde. Positive trends were found for employment duration, and peak and average exposure to formaldehyde. A positive trend with peak exposure was also observed in the NCI study among industrial workers; the greatest risk of myeloid leukemia with peak exposure was found among individuals with 15-25 years after the first known exposure to formaldehyde. The magnitude of the risk estimate decreased in the 2004 follow-up compared to the 1994 follow-up, a pattern seen with other leukemogens. In the NIOSH study, a statistically significant risk for myeloid leukemia was found among individuals with longer employment duration.

Dr. Lunn said there was weaker evidence of cancer at other sites such as head and neck cancer or brain cancer, and that there was inconsistent evidence related to lung cancer. She summarized that there was sufficient evidence of carcinogenicity in humans, noting again the consistent findings of increased risks of NPC, SNC, and myeloid leukemia among individuals with higher exposures to formaldehyde.

Regarding the animal evidence, Dr. Lunn reported that the NTP had concluded there was sufficient evidence of carcinogenicity from animal studies, which had found that formaldehyde causes tumors at multiple tissue sites (nose, forestomach, muscle [leiomyosarcoma] of intestines and stomach, and testes) in rats and mice, by exposure through inhalation and drinking water. The strongest evidence was for nasal tumors, with evidence that formaldehyde is a promoter of lung and stomach, but not urinary bladder tumors in rats. Nasal tumors were observed in six studies in rodents, most of which were SCCs, which are very rare in mice and rats.

Dr. Lunn detailed the metabolism of formaldehyde, which is rapidly metabolized and shares a common metabolic pathway in all species. She said formaldehyde is highly reactive, readily bonding to DNA (forming DNA adducts), protein (forming protein adducts), and forming DNA and protein crosslinks. Formaldehyde is known to be a direct-acting genotoxic compound,

which is suspected to be one of the major modes of action related to its carcinogenicity. Numerous studies have suggested that formaldehyde can also cause cancer through other modes of action, including glutathione depletion, epigenetic effects, oxidative stress, and cytotoxicity-induced cellular proliferation. Among the potential mechanisms for nasal tumors is inhalation exposure causing genetic damage in the nasal tissue of animals and humans, airway deposition, and cytotoxicity-induced cellular proliferation, which correlates with tumor incidence in rats.

LHCs and leukemia arise from damage to blood stem cells. In the case of LHCs, the different types occur depending on the level of development of the major progenitor cells, the myeloid and lymphoid stem cells. In leukemia, damage is done directly to stem cells in the bone marrow. The major question regarding the plausibility of formaldehyde-induced leukemia, stated Dr. Lunn, is whether it can reach the bone marrow, or cause toxicity, genotoxicity, or cancer at distal sites. There are several reasons to question that plausibility, foremost the high reactivity of formaldehyde, but toxic effects have been observed at distal sites after inhalation exposure in experimental animals and humans. There has also been some evidence of chromosomal aberrations in bone marrow in experimental animals. Dr. Lunn presented a diagram of possible mechanisms for distribution of formaldehyde to distal sites. She noted there have been some alternate mechanisms proposed for formaldehyde to cause leukemia without reaching bone marrow, including damage to circulating stem cells in the blood, and damage to stem cells in nasal tissue. If there is damage to hematopoietic or progenitor stem cells, evidence of hematological toxicity would be expected, she said presenting some examples from the literature in support of the concept. She stated the mechanisms are unknown, but that the available evidence does not indicate that such mechanisms are implausible.

Dr. Lunn concluded by reiterating that the NTP recommendation is to list formaldehyde as a *known human carcinogen*, based on sufficient evidence of carcinogenicity in humans, mechanistic studies supporting the finding in humans, and studies showing that formaldehyde causes cancer in experimental animals.

B. BSC Questions

Dr. Nagarkatti asked Dr. Lunn to clarify a perceived distinction between data she had presented about Chinese studies that showed decreases in white blood cells and red blood cells, but other studies had shown increases in the incidence of hematopoietic cancers. Dr. Lunn explained that theoretically the decreases would result from damage to the bone marrow. Dr. Sherley pointed out that much of the discussion about formaldehyde centers on the question of whether it reaches the bone marrow in terms of the leukemias that form, and asked Dr. Lunn about the saturability of the enzymes responsible for its metabolism. Dr. Novak pointed out that the equilibrium Dr. Lunn had shown earlier would speak to that issue; he believed the enzymes are likely to be high capacity and are unlikely to achieve saturation, even at a high dose.

Referring to the item Dr. Lunn had mentioned in her presentation about formaldehyde dehydrogenase (FDH) being ubiquitously expressed in all human tissues, Dr. Eastmond asked if she had any information regarding FDH levels in nasal passages or the nasopharyngeal region humans. Dr. Lunn said she was not aware that anyone had measured those levels in humans, although it had been shown that there were polymorphisms involved, implying varying susceptibility. Dr. Faustman noted that the background document did contain reference to the enzymatic turnover in rats and humans, showing that it was in fact efficient at high concentrations. Dr. Fernandez asked if there had been any studies in glutathione-depleted rats. Dr. Lunn said there had been studies showing that glutathione depletion may be a mechanism of carcinogenicity.

C. Public Comments

Dr. Jeffrey Cossman, U.S. Diagnostic Standards, Inc., commented by telephone, stating that he would restrict his remarks to commenting about the Zhang *et al.* paper regarding formaldehyde and leukemia, the study of Chinese workers looking at chromosomal changes using a special type of DNA test. He said his expertise was in molecular diagnosis of leukemia, but that he has no special expertise in formaldehyde.

He felt that it would be a mistake for the Zhang study to play any role in influencing the decision to conclude that formaldehyde is a cause of acute myeloid leukemia (AML), for two primary reasons. First, the technology used to detect chromosomal abnormalities, called fluorescence *in situ* hybridization (FISH), is quite difficult, and the Zhang laboratory has not sufficiently demonstrated its proficiency in the technique. With the findings of the study entirely dependent on the accurate performance of the FISH test, the results are called into question. Secondly, he said there is no evidence that the FISH test can predict the development of leukemia, and has never been shown to be associated with increased risk of leukemia. He noted that if the test were in fact predictive of leukemia, it would be "a monumental breakthrough" in leukemia and oncology, allowing early intervention in a fatal disease. He reiterated that the FISH test has been in general use for ten years, but has never been shown to predict the development of leukemia. Therefore, he said, it would be wrong to allow the Zhang study to influence any decision associating formaldehyde with the development of AML.

Dr. Eastmond asked Dr. Cossman how familiar he was with the work of Martin Smith's lab over the past 20 years, using FISH to detect chromosomal abnormalities in cancers, particularly leukemias. He pointed out that the lab had considerable experience and many publications in this area, in contrast to Dr. Cossman's characterization. Dr. Cossman said a major problem with the FISH test had emerged in recent years, in association with a high rate of mistakes regarding HER-2 and breast cancer, resulting in misuse of the drug herceptin. Thus, he said, accurate use of the FISH test is not simply a matter of experience. He also questioned the chain of custody of the samples involved in the tests reported in the Zhang study, as well as the limited use of available probes designed to detect chromosomal abnormalities associated with AML. Dr.

Eastmond responded that it had been a preliminary study designed to generate data for a grant application, and so it was clearly not as full-blown a study as one would expect, thus accounting for some of the gaps in the paper's reporting pointed out by Dr. Cossman. He agreed with Dr. Cossman that the FISH test has problems, but defended Zhang and her colleagues' use of the specific probe reported in the paper, as it had been associated specifically with chromosome loss associated with cancer.

Dr. Nagarkatti pointed out that Dr. Eastmond was a co-author of the paper in question. Dr. Eastmond corrected her assertion, noting that he frequently interacts with the Zhang research group and is a co-author of another paper likely to be discussed, but not of the paper in question.

Dr. Melvin Anderson, Hamner Institutes for Health Sciences, said formaldehyde is present in all cells in substantial concentrations. He provided details regarding the metabolism and other biochemical characteristics of formaldehyde in tissue. In the absence of any external formaldehyde exposure, it is present in the nasal epithelium at a rate of approximately 12,600 parts per billion. As such, it is present in exhaled breath. He said tissue formaldehyde concentrations are well controlled by efficient biochemical mechanisms, and as such glutathione levels are not decreased. There is efficient uptake of inhaled formaldehyde from air into epithelial tissues, with most absorption taking place in the most proximal portions of the airways, where toxicity is observed at concentrations above several parts per million (ppm). However, inhaled formaldehyde is not expected to increase concentrations in tissues away from the site of contact, because high FDH and glutathione levels in the respiratory tract limit such diffusion. He presented soon-to-be-published data on the pharmacokinetics of formaldehyde, showing that high concentrations of formaldehyde are required for toxicity and carcinogenicity with non-linearities affecting the dose-response curve. In summary, Dr. Anderson said at inhaled formaldehyde levels below the irritancy threshold of 1 ppm, contact site tumors would not be expected, and there would be no feasible way to produced increased methanediol (the form of formaldehyde found in tissues) at more distant sites. He said the endogenous dosimetry of formaldehyde needs to be considered when examining epidemiological associations postulated for formaldehyde carcinogenicity.

Dr. Birnbaum asked Dr. Anderson to comment on the fact that, although the modeling predicts that inhaled formaldehyde is taken up in the nasal epithelium, there are reports of it being present at multiple tissue sites in animal species. He said there is a time and dose dependency of the formaldehyde genomics within the nose, and that might have some impact on what might be happening in other tissues. He speculated that the early, intense immunological response in the nose might be associated with the release of cytokines, which may appear in other tissues remote from the nose.

Dr. Eastmond noted that in Dr. Anderson's presentation, there were 18-20 studies in humans reporting different DNA damage in lymphocytes. He asked why this would be seen in humans but not in rodents. Dr. Anderson replied that the rodents are a well-defined population, with

experimental control of their exposures and known associations. The human population, he pointed out, is not so well defined, and guessed that the phenomenon may be due to confounding or secondary exposures.

Dr. James Swenberg, University of North Carolina at Chapel Hill, reported on a study he recently conducted which concluded that distribution and molecular dose of inhalation-derived and endogenous formaldehyde DNA adducts support the induction of nasal carcinoma, but not leukemia. In the experiment, rats were exposed to 10 ppm [$^{13}CD_2$]-formaldehyde for 6 hours per day for 1 or 5 days, and sacrificed within 2 hours. The nasal mucosa, lung, liver, spleen, thymus, and bone marrow were collected for DNA adduct analysis, which was carried out with extremely sensitive technology. In fact, said Dr. Swenberg, this is the only study in the literature to be able to distinguish between endogenous and exogenous adducts. The results showed exogenous adducts, the product of inhalation exposure, only in the nasal tissues, whereas endogenous adducts were found in all of the tissues examined. There was no evidence that the formaldehyde reached distant sites in active form, and no evidence that methanediol transports formaldehyde to distant sites. The data, he said, do not support a causal role of inhaled formaldehyde in the induction of leukemia, but do support both a cytotoxic and genotoxic mode of action for nasal cancer. He reported that similar studies would be conducted in primates this summer, as primates and humans are oral and nasal breathers, as opposed to rats, which are obligatory nasal breathers. Other ongoing studies will shed light on the *in vivo* half-life of formaldehyde DNA adducts and the expected nonlinear relationship between inhalation-derived and endogenous adducts in exposure-response studies. Summarizing what he saw as the shortcomings of the DSP, Dr. Swenberg said to date there is only one chemical-specific study of formaldehyde adducts at site of contact and distant sites, with the rest of the data cited in the report not chemical-specific, and mixing endogenous and exogenous adduct effects. None of the methanediol references in the report demonstrate transport of reactive formaldehyde in biological systems, noting that his study had shown the opposite. He felt that the DSP gave more weight to minor studies in this area than major studies funded in the tens of millions of dollars, such as Conolly *et al*. 2004. He disagreed strongly with a conclusion reached on page 10 of the DSP, a reference from Zhang *et al*. 2010, which stated, "a subset of workers showed an increased frequency of aneuploidy of chromosomes 7 (monosomy) and 8 (trisomy)." He said the observation upon which this conclusion was based was completely inaccurate.

Dr. Sherley wondered whether Dr. Swenberg's study might have been too myopic, looking to disprove one specific hypothesis, and also asked about the possibility that there might be other ways than adduct formation for formaldehyde to cause cancer in distant sites. Dr. Swenberg replied that his colleague Dr. Kun Lu had developed a method to conjugate any formaldehyde in distant tissues, and had found none.

Dr. Eastmond asked Dr. Swenberg about the detection of exogenous adducts in the lung. Dr. Swenberg replied that none had been found, but the upcoming primate study would help to

answer that question, with some expectation of detecting adducts lower in the respiratory tract. Dr. Faustman asked Dr. Swenberg how he might approach looking at the issue of the possible involvement of cytokines in remote site impacts. He said he felt that cytokines could come from anywhere, and their presence would not necessarily indicate that formaldehyde got to a distant site. Further, since the fraction of exogenous to endogenous adducts was so tiny, he did not believe there were enough exogenous adducts present to drive the biology in terms of carcinogenesis. Dr. Faustman asked whether formaldehyde exposures could lead to NPCs. Dr. Swenberg replied that it does, at a rate of about 1% in mice and 50% in rats, and he believes it also occurs in humans.

Dr. Miller asked Dr. Swenberg about the cause of damage to the distant tissues seen in the bioassays included in the DSP. Dr. Swenberg replied he was not convinced that there was damage to distal tissues; his group did not find distant site issues or toxicity of any kind in the 40 tissues that were bioassayed.

Dr. Howard found Dr. Swenberg's mass spectroscopy data to be of great interest, but wondered about the consistency in levels of endogenous adducts found in all of the tissue types. Dr. Swenberg explained that the levels in white blood cells and bone marrow were actually lower, as would be expected.

Dr. Gary Marsh, University of Pittsburgh, speaking on behalf of Georgia-Pacific Chemicals, LLC, noted that he had been much involved in reanalysis of the NCI cohort study, which has been the most influential study for assessing human health risks from formaldehyde exposure. Results from the NCI study for NPC and myeloid leukemia, he reminded the panel, had weighed heavily on the IARC's 2004 reclassification of formaldehyde as a known human carcinogen, and the proposed reclassification by the NTP. He stated that his group and others have challenged NCI's findings, questioning the validity of its leukemia findings on grounds of biological implausibility, inadequate or questionable methods of data analysis, and errors found in NCI's 1994 follow-up. Further, they felt that NCI's findings for NPC were driven entirely by anomalous findings in one of 10 study plants, and that uncorrected data from the 1994 follow-up for solid tumors have misinformed recent risk assessments. He shared the citations for his group's re-analyses of the NCI cohort data, and noted that they were not cited in the NTP DSP, as they should have been. Of four other publications that reanalyzed the data from the solely positive Plant One of the cohort, only one was cited by NTP. He also alluded to several methodological problems with the NCI leukemia data, including miscalculations in the exposure-response analyses. When the statistics were corrected, significance disappeared, and the evidence reverted to lack of an association. He also pointed out a misinterpretation of the NCI leukemia findings for myeloid leukemia in the DSP. There were also problems with the NCI NPC data, he said, particularly because the findings for NPC were driven solely by anomalous findings for Plant One – 6 of 10 deaths. An independent study by his group found no association with formaldehyde, but strong association with prior work in silversmithing and the brass plating

industry. Also, in the 1994 update, the NCI missed nearly 1,000 deaths, mainly among the unexposed, and has not provided corrected data to compensate for that. Dr. Marsh also felt that the DSP had misinterpreted his group's findings regarding the high risk associated with prior work in silversmithing, having dismissed it as irrelevant and not a confounder of formaldehyde exposure. Instead, he said, it should have been treated as an independent risk factor for NPC. In summary, Dr. Marsh reiterated that (1) the results of the NCI cohort study weigh heavily on all evaluations of the potential carcinogenicity of formaldehyde, (2) reanalyses of the NCI cohort data and independent study of NCI's Plant One cast considerable doubt on the validity of the NCI findings for leukemia and NPC, (3) the NTP Draft Report is incomplete and contains inaccurate and misleading inferences about findings for myeloid leukemia and NPC, and (4) the findings of the NCI cohort study and the independent study of Plant One do not support reclassifying formaldehyde as a human carcinogen

Dr. Loomis asked what other evidence exists of an elevated risk of NPC from participation in silversmithing. Dr. Marsh replied there is considerable literature stating that several of the elements used in the trade had been linked with cancers at several sites in the upper respiratory tract. Dr. Loomis asked about the NTP stance on the silversmithing issue, and Dr. Marsh elaborated that due to the strength of the association, it should have been treated as a main effect, and thus an effect modifier of formaldehyde exposure, not simply as a potential confounder. He said this spoke to making some sense of the anomalous deaths in Plant One, which had analogous formaldehyde exposure levels—"If it's [formaldehyde] a carcinogen, why wouldn't it be elevating the risks in all of the plants?" Dr. Eastmond noted that Dr. Marsh had focused on one particular study associating formaldehyde exposure with NPC, and asked if he had comments on the others. He said he had focused on the NCI data because it seemed to be most important to any weight of evidence analysis done of the issue. He said there was no consistent evidence of an association from the two other largest studies either.

Dr. Robert Golden, Formaldehyde Council Inc., said based upon the same data, formaldehyde was judged to be a cause of myeloid leukemia, NPC, and SNC by the NTP, whereas in the recent EPA/IRIS assessment of formaldehyde, it was judged to cause all types of leukemia, Hodgkin's lymphoma, and NPC. He pointed out that numerous federal agencies had criticized the report, and that the National Academy of Sciences (NAS) is currently reviewing the approaches and conclusions in the EPA/IRIS document. He asked that the BSC not endorse the DSP, at least until the NAS has concluded its review. He added that there is a critical need for involvement by experts in hematology and oncology, since the idea that formaldehyde is a cause of any LHCs is highly uncertain. He cited data from the three largest cohort studies as showing no indication that formaldehyde exposure was associated with increased risk of leukemia. He indicated that the DSP does not present a convincing case that formaldehyde induces myeloid leukemia, and that its treatment of transport to distal sites ignores the fact that formaldehyde is already present in those tissues as methanediol. Recommending that this discussion be deleted from the document, he said it relies on inappropriate studies and convoluted logic to explain distal site

toxicity. He also noted that although the DSP relies on conflicting reports of damage to stem cells in bone marrow as an underlying commonality to the mechanisms proposed for leukemia caused by formaldehyde, the weight of evidence in the literature suggests that there is no conflict and that it favors no effect on blood or bone marrow. He described the common properties of well-known leukemogenic chemicals, including benzene, and presented several points regarding formaldehyde that stand in contrast to those properties, thus questioning the biological plausibility of formaldehyde causing leukemia. He mentioned the ongoing study on formaldehyde adducts in non-human primates, with data expected soon, using benzene exposures as a positive control, to determine the potential effects of labeled formaldehyde on blood and bone marrow.

Dr. Birnbaum suggested the study Dr. Golden mentioned should consider longer-term endpoints to model effects of chronic formaldehyde exposures. Dr. Golden thought it was an interesting idea and promised to consider it. Dr. Swenberg agreed, and pointed out that in the Hamner studies on genomics, a version of that model was included. Dr. Birnbaum reiterated that the protocol she was suggesting would focus on the question of formaldehyde transport to distal sites, on a longer-term, chronic basis.

Dr. Zelikoff asked if Dr. Golden was aware of any studies of epigenetic changes, whether in the nose or in distal sites. He was not aware of such studies, although Dr. Lunn had had a reference in her presentation. She alluded to one epigenetic study included in the background document on changes in histones. Dr. Swenberg also described a recent work from his group involving test tube demonstrations that formaldehyde does bind to the lysines on histone, affecting acetylation.

Dr. Joseph Rodricks, ENVIRON International Corporation, commented on whether the DSP presents a clear line of reasoning on the question of whether formaldehyde is causally related to myeloid leukemia. Citing the profile's conclusion of "consistent findings" of increased risks of NSP, SNC, and myeloid leukemia, he suggested that looking at the evidence related to myeloid leukemia would lead to another conclusion—that formaldehyde exposure is not causally related to myeloid leukemia. He said the DSP's assertion of a consistent pattern of strong associations in the epidemiology studies is not supported by a thorough examination of all of those studies. He stated that most of the findings in the four studies most emphasized in the DSP are not statistically significant, and that those that are significant are spread through various types and measures of exposures, with no consistent pattern. The many additional studies had failed to find an association, and although they were lower-powered than the more recent studies, they still should not have been ignored in the overall evaluation. He asserted that to move a substance into the causal category in the DSP, it should be supported by "extremely convincing" experimental evidence, which is not the case in this particular evaluation. He cited Dr. Lunn's use of the term "not implausible" regarding the mechanism involved, which he said is not a strong endorsement. He urged the BSC to advise the NTP to re-examine the evidence for an association between formaldehyde and myeloid leukemia, which he said is very different from

the evidence for an association between formaldehyde and nasal tumors, requiring more clarity in the lines of reasoning in the DSP than is currently the case. Given the importance of the questions involved, he also supported the idea of waiting for the NAS report on the EPA/IRIS evaluation, which would address these same questions.

Assuming that Dr. Rodricks was advocating a listing in the DSP that would not include reference to an association with leukemia, Ms. Rudel asked what the implications of such a listing would be. He said it is an important question that deserves separate treatment by NTP; otherwise there would be public confusion about what formaldehyde actually causes. Ms. Rudel clarified that she was asking about potential implications for the formaldehyde industry itself. Dr. Rodricks replied that there would certainly be product liability implications, as well as broader public health questions.

Dr. Birnbaum reminded attendees that the RoC is mandated to list known or suspected carcinogens and as such is not a risk assessment document, as the EPA's IRIS documents are. She reiterated that the substance profile is designed to provide evidence in support of the conclusions that have been reached, and not to include all of the negative information as well. Dr. Rodricks said he was aware of those points, but that the charge given to the BSC included consideration of weight of evidence and causation issues as part of the broader review.

D. BSC Discussion

Dr. Solomon, first lead reviewer, found the identity and description of formaldehyde in the DSP to be clear and technically accurate, particularly appreciating the inclusion of structural formulas. She also found the information on use, production, and human exposure to be clear and technically accurate, but cited inconsistencies in the document's treatment of food as a route of exposure. She suggested improved contexts for the discussion of total daily doses, to make the material more understandable to the lay reader. She also asked for mention of the fact that skin absorption is not a primary route of exposure for formaldehyde, and for more information on the potential for absorption through the GI tract. She found the information on carcinogenicity to be technically accurate and clearly presented, but felt there should have been more discussion of the fact that many of the studies were mortality studies as opposed to incidence studies, especially for the LHCs. Dr. Solomon suggested including data from the NCI cohort on Hodgkin's lymphoma. She also pointed out a discrepancy in discussions of latency related to leukemia. She found it useful that the issue of peak exposures was mentioned, and felt it could have been fleshed out more in the document. She felt the animal data should have received more emphasis in the DSP, particularly the data on oral exposures. The case made in the DSP was quite clear for airway cancers and "pretty strong" for leukemias, and the information was there to support the document's conclusions. She felt that the discussion of the mechanistic data needs to be tightened up and clarified, acknowledging that there are various hypotheses, but that currently the mechanism involved, particularly with the LHCs, remains an open question. She said that is acceptable, given that there is sufficient evidence in the epidemiology to support the listing.

Dr. Lunn said there was more information regarding food in the background document, and that high levels may not translate into high exposures, given bioavailability. She agreed with Dr. Solomon's points about the use of mortality studies in the examination of leukemia, and that there should be more information provided about other LHCs. She also agreed to expand the discussions of peak exposures and animal studies, and to clarify the section on mechanistic data.

Dr. Eastmond, second lead reviewer, said in general the DSP achieved the goals of being technically accurate and clearly stated, and supports the NTP's listing recommendation. He found the identity and description of the candidate substance, and the use and production information to be clear and technically accurate, but found the human exposure data to be limited in that it did not cover some common sources of human exposure, such as 20% of cosmetics sold in the United States and Europe. He mentioned that levels of human formaldehyde exposure were probably underestimated in the Federal Emergency Management Agency (FEMA) trailers, as the readings were taken two years after manufacture and during the winter, both of which would lead to lower levels of off-gassing. He found the information on NPC and SNC to be accurate and clearly presented, but the information on leukemia and LHCs to be more problematic, as different types of neoplasias were combined in different ways in the various studies. He recommended using the subheader "lymphohematopoietic cancer" instead of myeloid leukemia and recommended discussing some of the other types of LHCs. He said the information in the draft makes a strong case for the recommended listing, while suggesting several specific edits to the document: Change the statement about latency in the NCI study to, "This pattern is consistent with a follow-up lasting longer than a peak or optimal latency period, as has been seen with other leukemia-inducing agents," and include the Silver et al. 2002 study. Change the statement on the Murrell study to: Murrell *et al.* (2005) found that the olfactory epithelium of the nasal passages of rats contained multipotent stem/progenitor cells that were able to repopulate the hematopoietic tissues of irradiated rats and to form progenitor cells of multiple lineages. Change the statement about plausibility of myeloid leukemia to, "While the mechanisms by which formaldehyde causes myeloid leukemia in humans are not known, a number of plausible mechanisms have been advanced."

Dr. Lunn agreed with Dr. Eastmond's suggestions regarding common sources of exposure and updated FEMA trailer information. She also agreed that other LHCs should be included, and that the discussion of latency should be clarified.

Dr. Loomis, third lead reviewer, was happy with the information provided on the candidate substance, as well as the information on use, production, and human exposure, although there was a limitation on current occupation exposure to formaldehyde, with only older data included. With formaldehyde production having increased in recent years, he wondered whether the data presented represented current exposures. He found the information on carcinogenicity to be clear and technically accurate. He said the human data did support the listing recommendation for formaldehyde, with sufficient evidence from epidemiologic studies. He cautioned that the

reference to case-control studies being more informative may have been true for the one study cited, but that the general impression should not be left that that is the case. He found the information regarding NPCs and SNCs to be sufficient, but was less comfortable with the discussion of LHCs, finding it to be "a bit unbalanced." He agreed with other reviewers' comments that for balance there should have been reference to Hodgkin's lymphomas, multiple myelomas, and other cancers within the group, in addition to the attention given to myeloid leukemias. He did not find the evidence for a strong association with myeloid leukemia to be convincing, and said the evidence for association with any of the LHCs is "less well-established" than with NPCs. He also took issue with the treatment of peak exposures in the document, given that in some instances, such as the NCI study, the investigators estimated the exposures, with no real quantitative data beyond their expert judgment. The mechanistic data supported the listing recommendation, but more strongly for nasal cancer than for the LHCs. He found sufficient evidence of carcinogenicity in experimental animals in the profile.

Dr. Lunn said in considering evidence from the NCI embalmer study, which included analyses for LHCs, both lymphoid and non-lymphoid LHC, and myeloid leukemia, the excess mortality from all LHCs can be explained by myeloid leukemia and there was no evidence for an association with lymphoid type LHC. In addition, studies that reported risk estimates for all leukemia and myeloid leukemia found higher risk estimates for myeloid leukemia.

Dr. Faustman, fourth lead reviewer, agreed with other reviewers' comments about a need in the document for more information on exposures, including information that had been included in the background document. She emphasized that formaldehyde is one of the few chemicals for which there is quantitative dose-response information and dosimetry in animal studies that actually support location-specific and tumor types, and felt that point should be made more distinctly in the profile. The human data were sufficient to support the listing recommendation in terms of NPCs and SNCs, but found the information insufficient to conclude causality with respect to leukemias. She agreed with Dr. Loomis' comment regarding the DSP's inappropriate treatment of the peak exposure data from the NCI study as representing quantitative dose-response relationships, and recommended softening the language in the discussion of that information. She also felt that the tone of the section relating what she called the exciting, emerging mechanistic information on formaldehyde and leukemia was too strong, and may overpower other, more solid mechanistic information to support the listing recommendation.

Dr. Miller, fifth lead reviewer, said the identity and description of the candidate substance was clear and technically accurate, as was the information on use, production and human exposure. He suggested adding information about anticipated future human exposures to formaldehyde and agreed with other reviewers that the information and mechanistic data on respiratory tract cancers was compelling, but less so for leukemia. He suggested splitting the section on mechanisms to reflect that, particularly given that there is strong mechanistic data on the upper respiratory cancers. He felt that the evidence regarding LHCs needed to be better organized and

more cogently presented. He said the mechanistic description in the report of stem cells being damaged in the mucosa and then traveling to distal sites was weak, although he could understand why it was included. He suggested that since that idea is very hypothetical at this time, it should perhaps be de-emphasized. He recommended rewording the section on latency, as was suggested by other reviewers.

Dr. Faustman said she was struck by the lack of comment by reviewers on the studies regarding circulating lymphocytes and distal sites, wondering if it was because they were not believed. Dr. Eastmond said the issue really comes down to mechanism, and that the mechanism involved in the myeloid leukemias is not understood. He added that formaldehyde does not appear to be a typical leukemia-inducing agent, leading to the discussion of other sorts of hypotheses, one of which is damage to circulating stem cells in the blood, with enough reports of damage to circulating lymphocytes in the blood to make that scenario at least plausible. Dr. Miller said he wanted to see the mechanistic section of the document better organized to reflect the plausibility of a systemic effect. He was struggling with the fact that there was so much concordance between the animal and human data regarding the upper respiratory tract tumors, but that it is "lousy" with regard to the HPCs. Dr. Eastmond added that there are a number of reports of decreased blood cell counts in formaldehyde-exposed individuals, mainly from the Chinese literature, and of unclear reliability, which weighs into the plausibility argument.

Dr. Zelikoff noted that drinking water had been included in the profile as a potential source of exposure, and given that drinking water was the chosen route of exposure in several of the animal experiments, there should be more information in the profile regarding levels of formaldehyde in drinking water, as there is for ambient air levels. She recommended correlating those environmental levels with the concentrations used in the animal experiments. She wanted to see more information included from the *in utero* rat study, which she felt was passed over quickly despite being the only one of its kind.

Dr. McDiarmid wanted to see exposure data related more closely to OSHA Permissible Exposure Limits (PEL), to put it in a more understandable context in terms of occupational exposures. She agreed with other panelists that the section on myeloid leukemia should be re-organized.

Dr. Solomon mentioned a study included in the background document regarding the addition of formalin to cow feed, with the possibility that it could get into cow milk and subsequently into humans, suggesting it might be useful to include a reference to that in the profile's mechanism section. Dr. Lunn said she would look more into the study and consider adding a reference to it.

Dr. Loomis said the evidence on leukemia should be characterized as "strongly suggestive" as opposed to "sufficient," but that with that strong suggestion comes a distinct need for further research in that area.

Dr. Toraason agreed with other BSC members' comments regarding an excess of repetition in the profile, particularly in the treatment of the mechanisms of toxicity. He recommended adding language to the initial paragraph on Cancer Studies in Humans to immediately differentiate between the methods used to evaluate the upper respiratory tract cancers and the LHCs, to prepare the reader for the more detailed descriptions to come. He also suggested the inclusion of an opinion on the epidemiological studies included, rather than simply reporting on them.

XI. Report of the NTP Associate Director

A. Presentation

Dr. Bucher updated the BSC on the status of the NTP, covering staff additions, program initiatives, and upcoming meetings. He welcomed new NTP staff members Dr. Cynthia Rider (Toxicology Branch) and Danica Andrews (Office of Policy, Liaison and Review), and introduced Dr. Elizabeth Maull (Biomolecular Screening Branch) and Laura Hall (Program Operation Branch) who are detailed to NTP from their current branches for several months.

In terms of the NTP's responsibility for scientific and public health context in the information it generates, Dr. Bucher noted that new challenges include high content data, high throughput screening data, genomics, and the increasing complexity of toxicology in the 21st century. The NTP must also derive new criteria for non-cancer endpoints for substances such as bisphenol A, and meet increasing societal expectations. He said solutions will emerge from internal and BSC discussions, as well as Executive Committee deliberations, resulting in expected changes in organizational structure and programmatic expectations. Progress will be achieved through (1) new hires; (2) new processes, products, and scope for CERHR; (3) streamlining the RoC review process; (4) new partners in Tox21; (5) targeted testing; (6) herbals/dietary supplement coordination with FDA; and (7) the memorandum for International Cooperation on Alternative Toxicological Methods (ICATM).

Dr. Bucher said the intended outcome of these new developments is improved public understanding. Part of that effort will be to improve public health communications by making the language used more precise and more consistent, particularly regarding terms such as "weight of evidence." That effort may take the form of a workshop in the near future, devoted to improving the NTP's hazard/risk communication and ensuring that NTP's use of language and terminology is in harmony with other organizations. Under consideration is changing the existing use of the CERHR 7-point hazard identification scale, *Weight of Evidence for Adverse Effects*, which considers animal and human data separately on a case-by-case basis, and ultimately with consideration of exposure coalesces into a single *level of concern*. The NTP uses a 5-point scale to assess individual NTP studies. Harmonization of these systems is needed, and a workshop would allow detailed considerations of several systems for potential adoption by the NTP. The plan is for draft descriptions to be prepared during the summer of 2010, to convene a working group in the fall of 2010 to address the CERHR weight of evidence and level of concern

descriptors, possibly in separate steps, and by winter of 2010 link the new framework with RoC listing criteria and listing categories as part of revisions to the RoC listing process.

Dr. Bucher reported that the NTP is currently engaged in several formal interagency interactions. He briefly described the current nomination process. There is an interagency group that considers nominations to the testing program, after which NTP staff develop draft research concepts that are presented for public comment and then come to the BSC. He said the study design or protocol evolve as they go through the process, on occasion eventually no longer meeting the needs of the original nominating body. The hope is to avoid that situation by strengthening the concept of having an NTP point of contact with the various agency partners, to increase interagency communication, interaction, and cooperation, thereby accelerating the process. It would also facilitate interactions beginning very early in the RoC or CERHR nomination/selection processes. Similarly, once substances are selected for testing or literature-analysis, agency points of contact would help coordinate participation in design of studies, review of draft NTP documents, and communications about potential regulatory impact of NTP findings.

Dr. Bucher detailed the meetings scheduled in the near future for NTP committees, including the BSC, CERHR, and the BSC Technical Reports Review Subcommittee.

B. BSC Discussion

Dr. Toraason commended the expansion of CERHR, and asked Dr. Bucher if CERHR's name would be changed as a result of its evolving mission. Dr. Bucher said no decision had been made on a new name. In terms of CERHR's mission, the focus on exposures during development would continue, but assessment of consequences of those exposures would expand to broader areas, including consideration of adult exposures as appropriate.

Dr. Howard asked where the review by the Interagency Committee for Chemical Evaluation and Coordination would take place under the proposed new nomination process. Dr. Bucher said with a particular nomination, the individual agency points of contact would be polled to assess the overall need to convene the larger group. Dr. Birnbaum added that it should be clear that although a single agency may nominate a chemical for testing, the questions involved may go beyond those posed by the nominating agency.

XII. NTP Testing Program: Nominations and Proposed Research Projects

A. Overview

Dr. Bucher briefed the committee on the NTP's Testing Program and on how nominations and proposed research concepts are developed. He briefly reviewed the areas of emphasis in NTP's Testing Program and described the NTP study nomination review process. The BSC's charge is: *To review and comment on draft research concepts and determine whether the proposed*

research projects are an appropriate use of NTP testing program resources. A research concept is a brief document outlining the nomination or study rationale, and the significance, study approach, and expected outcome of a proposed research program.

B. Hydroxyurea

Dr. Barry McIntyre, Toxicology Branch, presented the proposed research concept on hydroxyurea. Hydroxyurea is an off-patent pharmaceutical that was originally developed for cancer treatment, but is now labeled to treat sickle cell anemia in adults. In 2007, it was reviewed by an expert panel convened by CERHR, which recommended animal studies to assess the long-term effects of prenatal and postnatal exposures on postnatal development, including developmental neurotoxicity, reproductive function, and carcinogenicity. It was nominated to the NTP for toxicological testing by a private citizen in 2006, and subsequently by the NIEHS based on the CERHR report. The drug is in wide clinical use in the treatment of rare diseases. It has demonstrated mutagenicity and clastogenicity, and in the absence of alternative treatments, is seeing increasing use in infants and children, and during pregnancy.

An initial nomination was considered by the BSC in 2008, but the NTP recommended, and the BSC concurred, that no additional work be done at that time. At the same time, an NIH Consensus Development Conference on Sickle Cell Disease called for additional animal studies to characterize adverse developmental and reproductive effects and carcinogenic risks of hydroxyurea. The original patent holder, Bristol Myers Squibb, has declined to conduct pediatric studies, but clinical trials in infants and children in the United States and Europe are reportedly showing efficacy. Thus, he said, conduct of additional animal studies is supported, and has been endorsed by the NTP Interagency Committee for Chemical Evaluation and Coordination and the NICHD.

He described sickle cell disease as a group of genetic disorders that strikes 1 in 5,000 Americans, approximately 72,000 patients, primarily individuals of African descent. The most severe form is sickle cell anemia characterized by a loss of red blood cell flexibility and "sickling," which causes subsequent obstruction of capillary blood flow and presents clinically as a vaso-occlusive crises. Hydroxyurea is the only approved disease-modifying therapy for sickle cell anemia, in that it decreases the incidence and severity of vaso-occlusive crises. In clinical use, both branded and generic hydroxyurea products are available. It was approved to treat sickle cell in 1998, after having originally being approved as a cancer therapy in 1967. It is used off-label to treat sickle cell disease in children, as well as several other illnesses. Given the lack of any effective alternative treatments, there are clinical trials in progress evaluating the drug as sickle cell therapy in children, including infants. There is currently a black box warning that hydroxyurea is not recommended for use during pregnancy, although it is known to be used to mitigate the severity of vaso-occlusive crises in pregnant women. Part of the consideration is that the drug would be seen as long-term, lifetime therapy.

Dr. McIntyre described the carcinogenic potential of hydroxyurea. It is a clear genotoxicant, with case reports in the literature of acute leukemia and skin cancers. There have been reports of increased incidence of acute leukemia myelodysplastic syndrome in small cohort studies, as well as increased incidence of mammary tumors in female rats, although a 12-month mouse study exhibited no evidence of carcinogenesis. In 2000 the IARC concluded that the drug was "not classifiable as to its carcinogenicity in humans" (Group 3).

Dr. McIntyre pointed out that although International Conference on Harmonisation (ICH) guidelines do not require carcinogenicity studies in drugs characterized as unequivocal genotoxicants, nonetheless a chronic toxicity study up to one year in length may be necessary to detect early tumorigenic effects if the drug is intended to be administered chronically to humans. Thus, with the longest-duration previous study being three months, a question arises as to the adequacy of the available carcinogenicity studies, particularly given the potential for lifelong exposures.

Briefly summarizing the 2007 CERHR report on hydroxyurea, Dr. McIntyre went over the limited human and animal data. Most importantly, he said, the animal data show that there is "no doubt" the drug is a teratogen. Also, the potential for reversibility of potential effects on fertility are unclear, an important consideration given lifelong dosing. There is also limited information in animals on the potential effects of hydroxyurea on neurological and immune functions.

He depicted the ICH Segmented Design for drug studies, along with the FDA/European Medicines Agency Pediatric Design, which fills a gap in the sequence, allowing for pediatric studies. Looking at the available data on hydroxyurea, there are clear gaps in the fertility and peri/postnatal studies. The proposal is for NTP to fill in those data gaps regarding potential effects on fertility, developmental outcomes, and carcinogenicity, particularly for chronic exposure beginning early in life. The data generated in rodent studies, he added, would provide important information to clinicians in counseling patients about the risks and benefits of the therapy. The proposed study design would begin by dosing pregnant dams prior to parturition (mimicking the clinical paradigm), and then dosing pups through their lifespan, including mating. Specific aims would be:

- Generate toxicity information in rodents by dosing pregnant animals late in gestation, and directly dosing their offspring
- The offspring would be assessed for the endpoints (1) fertility (including reversibility/recovery); (2) neurobehavioral assessment/neurotoxicity; (3) immune function; (4) malformations, litter size, etc.; (5) carcinogenicity; and (6) exposure/absorption, distribution, metabolism, and elimination (ADME)

The Preliminary Study Plan, reported Dr. McIntyre, involves two tiers. Tier 1 would be an initial dose range-finding study followed by a full developmental toxicity modified one-generational design study and Tier 2 would be carcinogenicity assessments in rats and mice. The proposal

hypothesizes that toxic effects will be observed following exposure to hydroxyurea, with prenatal and neonatal periods being particularly sensitive to the adverse effects of the drug, and that the immune, nervous, and reproductive systems will show developmental abnormalities and long-term adverse effects. Expected data, he said, will provide critical information on long-term outcomes to aid risk/benefit decision-making by regulators and clinicians, also perhaps spurring research to develop a better therapy for sickle cell disease.

C. BSC Questions and Discussion

Dr. Miller asked for clarification regarding whether the original patent holder conducted a carcinogenicity study. Dr. McIntyre replied that it appears from the data in the public domain that the original patent holder relied on data not produced by the drug company in combination with its mutagenic activity to define the potential risk in the target population. Dr. Eastmond asked whether the proprietary data submitted to FDA on the drug might be accessible through a Freedom of Information Act (FOIA) petition. Dr. McIntyre said that particular information was not available even through FOIA, which Dr. Howard confirmed. However, Dr. McIntyre said the FDA's interpretation of the sponsor's data was in fact available on the FDA's website, with a FOIA request no longer necessary to access it.

Dr. Zelikoff asked whether Dr. McIntyre was aware that hydroxyurea has been used as a topical treatment for pre-neoplastic skin lesions. Dr. McIntyre said the NTP is aware of that literature. Dr. Nagarkatti asked whether the proposed research would explore reproductive effects, or simply examine the issue of reversibility. Dr. McIntyre confirmed that looking at postnatal reproductive performance would be one aspect of the study, along with the ability to reverse that potential effect, mimicking the potential life experience of a human patient. Dr. Sherley asked about the available clinical data on longest treatment and secondary cancers. Dr. McIntyre replied that there are reports of secondary cancers in patients who had been treated with hydroxyurea for an initial cancer, and that the development of a secondary cancer later in life is not uncommon with this type of agent. He said the dosing paradigm for cancer is typically short, but for sickle cell, there may be patients who have been receiving the drug for as long as 20 years. Dr. Miller asked if the results of the proposed study would be captured in the proposed CERHR study regarding chemotherapy during pregnancy. Dr. McIntyre was unsure, but said he assumed that they would be included.

Dr. Faustman, first lead reviewer, found the proposal laudable and extremely important, and was pleased to see the inclusion of kinetics. She found the additional details provided in Dr. McIntyre's presentation, compared to the written proposal, helpful, but felt that still more information was needed. To help the clinician, she said, more data on dose, kinetics, and dosimetry are necessary. She urged the idea be linked with CERHR and whatever epidemiological and clinical data they might have available to assess the doses and timing of dosing of hydroxyurea during pregnancy when used as a chemotherapy agent or a sickle cell

agent. She recommended using the more than 20 years of clinical use data to help design kinetics studies, with the goal of modifying the clinical dosing scheme.

Dr. Eastmond, second lead reviewer, found the rationale for the proposed studies to be clear and valid, and given the current and anticipated uses for the drug, considered the proposed studies important. Regarding the merit of the proposed program, he felt that it supports two of the NTP's main goals: to provide valuable information on a potentially hazardous substance, and to strengthen the science base in toxicology. He felt that it would be valuable, especially by providing information to clinicians to aid dosing decisions. He deemed it a high priority research program.

Dr. Solomon, third lead reviewer, was by her own description more negative than the other reviewers. Given the rough calculation that just 100-150 children per year would receive the drug, she questioned whether the project would rise to the level of public health importance justifying a high priority score. She also wondered how much a rodent bioassay would help clinicians with their risk/benefit analysis, given that there are no alternatives to the drug. She felt the data from the ongoing clinical trials and post-marketing surveillance would be important to have, and might be more useful than the rodent bioassay. The proposed program seemed to be a reasonable approach to filling the identified gaps in knowledge, but she wondered whether there might be shorter-term bioassays that might provide some of the significant information being sought. Overall, she suggested that the proposal be given a low-to-moderate priority.

Dr. Bunton, fourth lead reviewer, agreed with Dr. Solomon's comments. She said there was a great deal of clinical information available, and that the proposed animal studies would provide limited new information of value. Much was already known about the drug, and new studies using a large number of animals and many resources, would not add significantly to knowledge. She referred to older fertility studies in animals, which potentially examined many of the same endpoints as the proposed program. She recommended careful examination of the pre-existing literature, including data on human exposures, before going back to animal studies because many of the effects in animals are well known, and would be of limited value to clinicians. She gave the program a low priority in terms of carcinogenicity studies, with only a slightly higher value for studies of reproductive toxicity.

Dr. McIntyre said some of the reviewers' comments mirrored discussions his group had had internally as to why to do any more research work on a drug that already carries a black box warning. He said without further study, the children who will be administered the drug will be the test subjects, with the outcomes only becoming clear 10-20 years later. In that context, he said, running controlled animal studies might shed new light on some of the questions involved, and allow better-informed clinical decision-making to improve those outcomes. He said stakeholders had indicated a desire for the data.

Dr. Howard described the situation faced by a clinician trying to manage risk associated with prescribing hydroxyurea in a child in sickle cell crisis. He said the clinician currently does not have enough information about the reproductive toxicity of the drug to make a well-informed choice, particularly with a peri-pubescent male. He said that is the crux of the problem right now, and waiting for human epidemiologic studies could take 10 more years. He said the FDA and NICHD were particularly anxious to have this analysis to aid in decision-making related to the "crisis" in risk management with the drug. He noted that although this use of the drug is off-label, it is the truly the "treatment of option."

Dr. Novak said a consortia of physicians, some of whom are at Children's Hospital of Michigan, are participating in hydroxyurea clinical trials, and that several clinical trials are identified in *ClinicalTrials.gov*. He recommended the data in a recent paper on long-term use of the drug in sickle cell anemia be reviewed, as well as a fertility paper. He suggested that NTP contact the many clinicians using the drug in children currently and gather information on their experiences, particularly in terms of serious adverse effects. He urged that information be combined with data from the literature to form a basis for design of the proposed experiments.

XIII. Adjournment

Dr. Birnbaum thanked the BSC members for their thoughtful comments and considerable work associated with the meeting. Dr. Novak adjourned the meeting.