

**National Toxicology Program
Board of Scientific Counselors**

February 24, 2009

**National Institute of Environmental Health Sciences
Research Triangle Park, NC**

Summary Minutes

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I. Attendees

Members in Attendance

Tracie Bunton, Eicarte LLC
Edward Carney, The Dow Chemical Company
Russell Cattley, Amgen
David Eastmond, University of California
George Friedman-Jimenez, New York University School of Medicine
William Janzen, The University of North Carolina at Chapel Hill
Mitzi Nagarkatti, University of South Carolina School of Medicine
Raymond Novak, Wayne State University
Michael Pino, Sanofi-Aventis
Kenneth Portier, American Cancer Society (Chair)
Jim Riviere, North Carolina State University
Diane Robins, University of Michigan Medical School
Ruthann Rudel, Silent Spring Institute
James Sherley, Boston Biomedical Research Institute
Gina Solomon, Natural Resources Defense Council

Pending Members

Elaine Faustman, University of Washington
Stephen Looney, Medical College of Georgia
Justin Teegarden, Pacific Northwest National Laboratory

Members not in attendance

David Wegman, University of Massachusetts Lowell

***ad hoc* Members**

Ronald Hines, Medical College of Wisconsin
Dana Loomis, University of Nevada Reno

National Institute of Environmental Health Sciences Staff

Linda Birnbaum	Retha Newbold
Chad Blystone	Christopher Portier
John Bucher	Barbara Shane
Rajendra Chhabra	Michael Shelby
Helen Cunny	Robert Sills
Paul Foster	Diane Spencer
John French	William Stokes
Dori Germolec	Kristina Thayer
Michelle Hooth	Raymond Tice
Gloria Jahnke	Molly Vallant
Grace Kissling	Suramya Waidyanatha
Ruth Lunn	Nigel Walker
Robin Mackar	Lori White
David Malarkey	Kristine Witt

Joyce Martin
Scott Masten

Mary Wolfe
Michael Wyde

Other Federal Agency Staff

Paul Howard, Food and Drug Administration (FDA)

Mark Toraason, National Institute for Occupational Safety and Health (NIOSH)

Public

James Bus, The Dow Chemical Company

Jeffrey Charles, Toxicology Consultant

Barry Clayton, Reichhold, Inc.

Philip Cole, University of Alabama at Birmingham

Angus Crane, North American Insulation Manufacturers Association

George Cruzan, ToxWorks

Charles Elkins, Chuck Elkins & Associates

Sanford Garner, SRA International

Julie Goodman, Gradient Corporation

Arthur Grollman, State University of New York at Stony Brook

John Hadley, Owens Corning

Joe Haseman, J. K. Haseman Consulting

Milton Hetjmancik, Battelle

Marc Jackson, ILS

Bill Jameson, CWJ Consulting, LLC

Edward Leydon, Reichhold, Inc.

Jay Merrell, Industrial Dielectrics, Inc.

John Menkedick, Battelle

Earle Nestmann, Cantox Health Sciences International

J. M. Ratcliffe, SRA International

Lorenz Rhomberg, Gradient Corporation

John Schweitzer, American Composites Manufacturers Association

Jack Snyder, Styrene Information & Research Center

II. Introductions and Welcome

The National Toxicology Program (NTP) Board of Scientific Counselors (BSC) met on February 24, 2009, in the Rodbell Auditorium, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina. Dr. Kenneth Portier served as chair. He welcomed everyone to the meeting and asked the BSC members and attendees to introduce themselves. Dr. Barbara Shane made a few announcements and read the conflict of interest statement. She noted that the *ad hoc* reviewers would not vote and no conflicts of interest were identified.

III. Director's Report to the NTP Board of Scientific Counselors

Dr. Linda Birnbaum, Director of the NIEHS and NTP, welcomed the BSC members and expressed her gratitude to them for their service. She thanked Dr. Samuel Wilson, who had served as Acting Director of the NIEHS and NTP for the past year, for his leadership at the institute.

She discussed her vision for NIEHS and NTP. She said health and the environment are priorities for the Obama administration and identified pollution as a global issue. She pointed out that the best teams of scientists are needed to address complex multifactorial diseases such as diabetes, cancer, and heart disease. It is important for scientists to work together across research disciplines and improve the translation of basic science into public health policy. The NTP is a worldwide leader in toxicology research and has provided scientific information needed by others to make sound decisions to safeguard public health. She is committed to sustain and strengthen that leadership role.

She is pleased that the NTP is exploring new approaches to toxicological research including high throughput screening to target key cellular pathways, understanding molecular events or processes linked to disease or injury, exploring the utility of non-mammalian model systems, and placing a major emphasis on host susceptibility models to understand the genetic basis for sensitivity to toxic substances. She pointed out the importance of training future scientists and noted that the NTP is actively involved in postdoctoral training programs in toxicology, carcinogenesis, pathology, and laboratory animal medicine.

Dr. Birnbaum identified some of her actions since joining NIEHS the previous month and indicated her commitment to restoring close interactions with NIH, extramural communities, and interagency partners. She has decided to open the NIEHS Clinical Research Unit and pointed out that no invasive procedures would be performed and there would be an outside advisory panel. To facilitate more interaction with the Environmental Protection Agency (EPA) located across the lake from NIEHS, she has initiated facility sharing with EPA's premier animal inhalation unit. She will advertise nationally for the top leadership positions at NIEHS namely Deputy Director, Scientific Director, Director of the Division of Extramural Research and Training, and a Clinical Director. Leaders in the ethics office and diversity and education programs also will be appointed.

She briefly discussed the appropriations for the NIEHS during FY08 and FY09. The budget for FY08 was \$733 million (M) including \$77M for Superfund and \$9M as a pass-through from DOE for worker training. The FY09 appropriation is expected to be \$751 M with similar funding for Superfund and from DOE.

She briefly described the American Recovery and Reinvestment Act (ARRA) of 2009 in which NIH will receive \$10.4 billion (B) over 2 years to fund extramural

facility construction and renovation (\$1B), shared instrumentation (\$300M) and research grants (\$8.2B) with \$500M for renovation of buildings and facilities on the NIH campus. Much of the latter will be used to renovate the present clinical center on the NIH campus. A pass-through of \$400M to NIH will support Comparative Effectiveness Research to compare the effectiveness of one treatment/intervention to another treatment/intervention for the same disease. Of the \$7.4B ear-marked for the Institutes/Centers, \$800M will remain in the Office of the Director for challenge grants and other activities. The NIEHS would receive approximately \$187M.

The ARRA research funds are for merit-based grants that benefit the economy, health, and science. The grants will be awarded using as much flexibility as possible within the stimulus guidelines. Much of the funds will support meritorious RO1 proposals that missed the funding cut-off pay line, but funds will only be awarded for grants that can be completed within 2 years. In addition, there will be a call for new RO1s but these monies have to be obligated by September 2009. Supplements to current grants will also be considered.

The Challenge Grant program, which is centrally funded, has an appropriation of ~\$200M and these grants are linked to programs within each institute that address common, defined health and science problems such as nanotechnology.

The Breast Cancer and Environment Research Act, passed in October 2008, authorizes establishment of the Interagency Breast Cancer and Environmental Research Coordinating Committee by April 8, 2009. The committee will consist of 7 federal officials, 6 non-federal scientists or physicians, and 6 advocates. The goal is to coordinate information on existing breast cancer research activities, make recommendations for new research, and develop a comprehensive strategy. The Act authorizes, but does not appropriate \$40M to carry out research to evaluate environmental and genomic factors that may be related to the etiology of breast cancer. The NIEHS currently supports research on breast cancer including the funding of special breast cancer and environmental research centers.

IV. NTP Update

Dr. John Bucher, Associate Director, NTP, said the NTP had spent the time since the November BSC meeting preparing for the BSC and Technical Reports Review Subcommittee meetings on February 24 and 25, respectively. He thanked the BSC members for their preparation for these meetings and for the advice they would provide and welcomed Dr. Birnbaum to her first meeting as the new NTP director.

V. Peer Review of Draft Substance Profiles for the 12th Report on Carcinogens

1. Review of the Process

a. Presentation

Dr. Mary Wolfe, NIEHS, discussed the background to the Report on Carcinogens (RoC) and the review process for its preparation. The RoC is a hazard identification document that identifies agents, substances, mixtures, or exposure circumstances that may pose a carcinogenic hazard to people in the United States. It lists substances as *known* or *reasonably anticipated human carcinogens* and is a congressionally mandated biennial report for which the Secretary, Department of Health and Human Services (DHHS), has responsibility. The first RoC was published in 1980 with 26 listings. The RoC is a cumulative report and the current 11th RoC has 246 listings of which 58 are classified as *known* and 188 as *reasonably anticipated* human carcinogens. It is regarded as an authoritative source for the OSHA Hazard Communication Standard and the California Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65).

The Secretary has delegated preparation of the RoC to the NTP that uses a multi-step process with multiple opportunities for public input. The current process for preparing the 12th Report was released in April 2007. The NTP revised the review process from that used for previous reports to address guidance in the OMB Information Quality Bulletin for Peer Review (2004) and to enhance the scientific rigor of the process and increase opportunities for public input.

Each listing in the RoC is identified by a substance profile that summarizes relevant information that supports the listing based on the substance's carcinogenicity, genotoxicity, mechanism of action in humans and/or animals, and potential for human exposure. It also provides information on the properties of the substance, its use and production, and any current Federal regulations and guidelines that limit exposures. Specific RoC listing criteria, approved by the Secretary, DHHS, in 1996, are used to evaluate the scientific evidence relating to whether a substance should or should not be listed in the report. The RoC listing criteria are based on three categories: *known* or *reasonably anticipated* to be a human carcinogen or *do not list*. Specific standards have been developed that the body of scientific evidence must meet to reach a listing determination for each category. The conclusion is based on scientific judgment with consideration of all relevant information.

The NTP follows a multi-step, formal process for the review of substances selected from among the nominations for review ("candidate substances"). Dr. Wolfe briefly described each of the four parts of the process namely (1) nomination and selection of candidate substances, (2) scientific review of candidate substances, (3) peer review of draft substance profiles, and (4)

preparation of the RoC and its submission to the Secretary. She noted that the NTP invites nominations of substances to review for the RoC from anyone. The RoC review process is posted on the NTP website at <http://ntp.niehs.nih.gov/go/29353>.

In describing the third part, Dr. Wolfe pointed out that the BSC would be carrying out that part today by peer reviewing the draft substance profiles in open forum. This step is new to the RoC review process, as previously substance profiles have not been made public or peer reviewed externally before the RoC was published. Dr. Wolfe said the peer review comments provided by the BSC and the public would be considered by the NTP in finalizing the substance profiles. Once the NTP completes the scientific review process for all candidate substances, the finalized substance profiles and the current 11th RoC would be compiled to prepare the draft 12th RoC. The NTP will submit the draft RoC to the NTP Director who will provide it to the Executive Committee for comment and then transmit the draft RoC to the Secretary, DHHS, for review and approval. Following the Secretary's approval, the RoC is transmitted to the Congress and released to the public. When the 12th RoC is released, the NTP will release three response documents related to the preparation of the report: (1) a response to the expert panel peer review report, (2) a response to the BSC peer review report, and (3) a response to public comments received on the candidate substance since release of the final background document.

Dr. Wolfe stated the charge to the BSC: "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 (i.e., *known to be human carcinogen* or *reasonably anticipated to be human carcinogen*, or *not to list*). She pointed out that the NTP was not asking the BSC to comment on the policy decision.

b. BSC Discussion

Dr. Kenneth Portier asked if a record is kept of substances reviewed and not listed and Dr. Wolfe replied that those substances are listed in an appendix in the back of the RoC.

Dr. James Sherley asked about the remaining four candidate substances of the nine being reviewed for the 12th report since the BSC is only reviewing the profiles for five substances at this meeting. Dr. Wolfe replied that the substances were divided into two groups and the remaining four substances are moving through the process. They will be brought to the BSC next year.

Dr. George Friedman-Jimenez said the RoC provides valuable information and identifies knowledge gaps that have substantial health implications. He asked whether these gaps are compiled, and Dr. Bucher replied that there is no formal document articulating the research needs identified from the RoC reviews other than the minutes and discussions at the meetings. Dr. Birnbaum added that

identified research needs can be funneled into the Division of Extramural Research and Training resulting in a request for applications from grantees.

Dr. Paul Howard asked whether the draft RoC would be prepared after the review of the four remaining substance profiles, and Dr. Wolfe replied that all nine profiles would be incorporated into the 12th RoC at the same time.

2. Peer Review of Draft Substance Profiles

A. *ortho*-Nitrotoluene

a. Presentation

Dr. Gloria Jahnke, NIEHS, presented the information supporting the preliminary NTP listing recommendation for *ortho*-nitrotoluene (*o*-NT) in the 12th RoC as *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity in experimental animals and supporting mechanistic data.

She described the use and exposure of *o*-NT in the United States, cancer studies in humans and experimental animals, and mechanistic evidence supporting the recommendation.

She noted that there is significant U.S. exposure to *o*-NT especially in occupational settings. It is a chemical intermediate used in the synthesis of dyes and numerous other products. Ten to 50 million pounds of *o*-NT are produced per year in the United States (2002). *o*-NT has been detected during chemical production in the air of manufacturing plants and in ground water, surface water, and soil at or near munitions production and military training facilities.

Data from human studies are inadequate to evaluate the relationship between human carcinogenicity and exposure to *o*-NT.

NTP concluded that there is sufficient evidence of carcinogenicity from NTP animal studies based on early onset of mesothelioma of the tunica vaginalis of the testis and cholangiocarcinoma in male rats, as well as clear evidence of carcinogenicity at multiple sites in male and female F344/N rats and B6C3F₁ mice in chronic feeding studies. Thus, in rats, *o*-NT caused subcutaneous skin tumors, hepatocellular adenomas and carcinomas and mammary gland fibroadenomas in both sexes and malignant mesothelioma, cholangiocarcinomas, and lung tumors in males. In mice, it caused hemangiosarcomas in both sexes, cecal carcinomas in males, and hepatocellular adenomas and carcinomas in females. The pattern of mutations (G to A transitions or G to T transversions) in the p53 or *K-ras* genes in *o*-NT induced hemangiosarcomas and colon tumors in mice was consistent with the formation of DNA adducts at guanines and is similar to mutations in colon tumors found in humans.

Dr. Jahnke discussed other mechanistic evidence supporting the carcinogenicity of *o*-NT including the presence of urinary metabolites and proposed bioactivation pathways. Both metabolites, *o*-nitrobenzoic acid and *o*-nitrobenzyl alcohol, were identified in human and rodent urine following exposure to *o*-NT. Intestinal bacteria appear to mediate the bioactivation of *o*-NT to a reactive 2-methylaniline intermediate in intact male rats. The 2-methylaniline intermediate is translocated to the liver where it forms carbonium and nitrenium ions resulting in the formation of DNA adducts and 2-methylaniline hemoglobin adducts in rats. The same hemoglobin adducts have been detected in human workers exposed to *o*-NT suggesting a similar bioactivation pathway in rats and humans.

b. BSC Discussion

Dr. Russell Cattley, the first BSC discussant, said the contents of the profile were correct, clearly stated, and relevant to support the NTP's preliminary policy decision on listing. He thought there should be a better description of human exposure and route of administration as occupationally workers are exposed by inhalation and dermally, while exposure of rodents in carcinogenicity studies is by the oral route. This is important because it appears that metabolism has an effect on the tumor spectrum that differs between mice and rats. He suggested a more detailed description of the differences between biliary excretion in the male and female rat, as it is a key to understanding cancer risk. He thought the profile should contain specific definitions to explain the terms mesothelial-derived neoplasia and hyperplasia.

Dr. Raymond Novak, the second BSC discussant, agreed with Dr. Cattley's comments that the profile was comprehensive. The profile provides detailed and supporting information on exposure, routes of exposure, metabolism, and mechanism(s) of carcinogenicity. He was pleased that the profile acknowledged that the prediction of human effects is difficult because exposure is often to a mixture of compounds. The finding that mutations in certain genes in rodent tumors parallel those reported to occur in human tumors lends additional support to the conclusions. He suggested adding a more detailed discussion of the breakdown products to the metabolism section as the metabolites contribute to the adverse effects of *o*-NT.

Dr. Gina Solomon, the third discussant, agreed with the previous reviewers on the comprehensiveness of the profile. She suggested that the finding that hemoglobin adducts in workers mirrors those found in rodents and should be emphasized.

Dr. David Eastmond added that microbial metabolism in the gut is critical in the mode of action of *o*-NT. The complex metabolic pathway that *o*-NT undergoes may explain why it is negative in some genotoxicity assays. Since 2-methylaniline-hemoglobin adducts are found, this would suggest that *o*-NT had undergone nitroreduction, a reaction usually mediated by gut bacteria.

B. Aristolochic Acids

a. Presentation

Dr. Ruth Lunn, NIEHS, presented the information that supports the preliminary listing recommendation for aristolochic acids in the 12th RoC as *known to be human carcinogens* based on sufficient evidence from studies in humans and supporting mechanistic data.

Aristolochic Acids (AAs) belong to a family of nitrophenanthrene carboxylic acids that occur naturally in *Aristolochia* and *Asarum spp.* AAs are used as traditional folk medicines in China and have been inadvertently used in a weight-loss regimen. Although FDA placed a warning in 2001 on these products, they are still available legally in other countries and in the United States via the Internet.

The evidence in humans supporting a causal association between AA and cancer is the high prevalence and risk estimates for urothelial tumors among AA-exposed patients, consistency of outcomes among different studies, dose/response relationships between AA-consumption and urothelial cancer, identification of AA-DNA adducts in urothelial cancers in patients consuming AA herbal products, and a high percentage of A to T transversions in the p53 gene (a possible fingerprint for exposure to aristolochic acid) in urothelial tumors.

Consumption of herbs containing AA has been associated with nephropathy (“aristolochic acid nephropathy” or AAN), which was first identified in Belgium among individuals ingesting herbal products containing aristolochic acid for weight-loss. Two studies in Belgium reported a high prevalence of urothelial cancer among AAN patients undergoing renal transplantation. AA-DNA adducts were identified in cells from urothelial cancers from these patients. Transplant patients who consumed a higher cumulative dose of AA had a higher risk for developing urothelial cancer compared to transplant patients who did not consume AA. No significant differences were found for other risk factors such as smoking.

A similar type of nephropathy and related urothelial cancers was identified in the Balkans, called Balkan endemic nephropathy (BEN). AA was proposed as the risk factor based on the presence of AA–DNA adducts and A:T to T:A transversions in the p53 gene in urothelial tumors from BEN patients. Exposure is believed to be from contaminated wheat.

In addition to the studies in Belgium and the Balkan states, two clinical studies in China found significantly increased risk for transitional cell cancer among renal transplant or dialysis patients who also consumed herbal remedies containing AA.

Dr. Lunn pointed out that the International Agency for Research on Cancer (IARC) has also determined that plants containing AA and AA (per se) are carcinogenic to humans.

Dr. Lunn reviewed the mechanistic data in humans supporting the listing of AA as *known to be human carcinogens*. AAs are metabolized to aristolactams, which are further metabolized to a cyclic *N*-acylnitrenium ion that forms DNA adducts at purine bases. These adducts are associated with A:T to T:A transversions, which have been detected in mammalian cells and in oncogenes of AA-induced tumors in humans and animals.

Dr. Lunn pointed out that there is also sufficient evidence of carcinogenicity of AA in experimental animals. AA induced tumors (1) at multiple sites (the most important being the forestomach, kidney, and uterus), (2) by multiple routes of exposure, and (3) in multiple species (mice, rats, and rabbits).

Thus, the NTP concluded that there is sufficient evidence in humans based on excess urothelial cancer among AA-exposed patients and mechanistic studies indicating that AAs are the cancer-causing agents in these folk medicines.

b. BSC Discussion

Dr. Jim Riviere, the first BSC discussant, said aristolochic acid I is well characterized and its description accurate, but aristolochic acid II is not identified as well. The known metabolites have been appropriately characterized; however, other naturally occurring AAs found in many herbs, and closely related derivatives (aristolactams, dioxoaporphines) have not been specifically characterized, although they are identified structurally. Also, the nomenclature of traditional Chinese herbs is complex.

He agreed that human exposure is primarily due to consumption of “medicinal” herbs and that occupational exposure is of minimal risk. AA is found in snakeroot extracts used as flavorants in alcoholic beverage, but the potential for exposure and the amounts consumed are not described well in the profile. The actual consumption of AA in herbals is unknown. FDA has banned these herbs; however, they are readily available over the Internet and in traditional sale venues without any warning of their potential toxicity.

The linkage between AA and urinary tract tumors was due primarily to identification of the syndrome in the Belgian weight loss cohort. This signal could have easily been missed in the background of renal disease in the population. The seriousness of exposure to AA was strengthened when the herbal component was implicated in the etiology of Balkan endemic nephropathy where exposure was secondary to flour contamination. The potential for contamination of grains worldwide should be discussed in the profile.

He agreed with the evidence in the profile and strongly supported the listing of AA as a *known human carcinogen* based on the epidemiological evidence, the mechanistic data showing AA is mutagenic, and that AA exposure results in DNA-AAI adducts in tumors with A:T → T:A transversion mutations in the *H-ras* and *p53* genes.

It appears that the major weakness linking AA to cancer in experimental animals is that classic lifetime rodent studies have not been conducted, although administration of AA is associated consistently with tumor formation at multiple sites in rats and mice after exposure for 6-12 months. The finding of the same adducts in human tissues from the Belgian cohort and in animals fed AA provides a strong reinforcement of a causal linkage.

He suggested emphasizing the recent, in-depth review of AA's role in both Chinese herb nephropathy and Balkan endemic nephropathy to demonstrate the continued concern of nephrologists with this syndrome as well as the consensus view that AA is the responsible agent.

Dr. Friedman-Jimenez, the second discussant, said the identity of AA is well described in the draft profile. However, he was concerned about the difficulty of accurately identifying all the herbs, medicinal preparations, and commercial products that contain AA so that the production, sale, and use of these products can be regulated.

The widespread potential for human exposure to AA is well documented in the draft substance profile. The certainty about exposure is addressed by the consistency of studies from different geographic regions of the world, the concentration of AA in commercially available herbal preparations, and the levels of aristolochic acid DNA adducts in samples of kidney, urethra, and bladder tissues of patients with reported renal impairment, renal failure, renal transplantation, and urothelial cancers.

The information presented in the profile strongly suggests that without effective worldwide public health intervention, exposure will continue to occur in many countries. Although control of exposure is not a formal part of the review of the profile, this international challenge to public health is an additional motivation to make the NTP statement on carcinogenicity of aristolochic acids as unequivocal as possible, within the bounds of the available scientific evidence.

Dr. Friedman-Jimenez said the scientific information supports a conclusion of sufficient evidence of carcinogenicity of AA in humans. The human evidence reported in the profile includes case reports, clinical case series, prevalence surveys, and identification of AA-DNA adducts and A:T to T:A transversions in p53 mutations in tumors of selected groups of renal failure patients; however, no population-based case-control or cohort epidemiologic studies that adequately documented AA exposure have been reported. Case reports and prevalence surveys are normally considered to be relatively weak epidemiologic designs for making causal inferences as compared with randomized clinical trials and population-based cohort and control studies; however, Dr. Friedman-Jimenez said several findings from the studies published on AA and urothelial cancers make this body of evidence convincing. These features include:

- Moderate to strong associations between AA exposure and urothelial cancers have been reported or can be inferred from the published studies.
- AA-DNA adduct biomarkers were identified in several of the clinical case reports and case series and in two prevalence studies that established exposure specifically to AA.
- Evidence of a strong association was found between AA exposure and urothelial cancers in one clinical study where a relative risk of 5.85 ($p < 0.0001$) was reported for transitional cell carcinoma among 1,429 renal transplant patients who reported taking AA containing herbs. In this study, the absolute risk of transitional cell carcinoma among renal transplant patients not reporting exposure to AA-containing herbs was 0.97%.
- The strength of association between documented substantial AA ingestion and incidence of urothelial cancers can be inferred from two prevalence studies and several historical comparison populations. In one prevalence study, 7 of 10 renal-failure patients had urothelial cancers within 10 years of taking AA herbals, a risk of 70%. In a second study, 18 of 39 patients who consumed AA had urothelial cancers within 8 years after AA exposure, a risk of 46%. They validated the questionnaire report of exposure by identifying AA-DNA adducts in 100% of subjects reporting AA exposure and none in 8 subjects reporting no AA exposure. A major additional strength of this study was estimation of the total dose of AA by questionnaire with comparison to the prevalence of urothelial cancers. In the group with a history of more than 200 g total exposure to AAs ($10/15 = 67\%$ of patients) compared to the group with a history of less than 200 g total exposure ($8/24 = 33\%$), there was a two-fold elevation in cancer prevalence in the higher dose group compared to the lower dose group. This provides some evidence of an exposure–response gradient between self-reported exposure to AA and incidence of urothelial cancers. Historical comparisons can be made with findings from other studies that suggest that the risk of urothelial cancer among renal failure patients unexposed to AA is in the 0.55 % to 1.76% range, one to two orders of magnitude lower than the risks reported among AA-exposed subjects.

Dr. Friedman-Jimenez said the strength of association between AA dose and incidence of urothelial cancers cannot be quantified directly because none of the published studies that have biomarker-based exposure assessments had a non-AA-exposed comparison group of renal failure patients to use for a direct calculation of relative risk. Dr. Friedman-Jimenez suggested that the profile should contain information about the incidence and prevalence of transitional cell carcinoma in patients that did not take AA but received a renal transplant. Studies not referenced in the profile reported a prevalence of 0.55-1.76% for urothelial cancers among renal transplant patients who have not taken AA. Although these cases are not drawn from the same populations as the cancer cases and cannot be used directly to produce covariate-adjusted estimates of relative risk, these prevalence estimates are consistent with each other and with the estimates in the 1% range from the two clinical studies.

It is unknown whether AAs can cause urothelial cancers without first causing severe nephropathy, or whether severe nephropathy is a required precursor condition to AA-induced urothelial cancers. As long as AAs are known to cause severe nephropathy, AA can be considered the cause of the cancers regardless of whether severe nephropathy always occurs first or not. Thus, the judgment of a causal relationship between AAs and severe nephropathy is directly relevant to the judgment of a causal relationship between AAs and urothelial cancers.

He pointed out that from 3-5% of patients taking the slimming regimen in Belgium developed severe nephropathy, while the rest did not. Potential explanations are possible including variability in AA content of different batches of the herbal preparation, variability in patient compliance with the regimen, and variability in individual susceptibility to AA nephrotoxicity. Available information is inadequate to confidently confirm or refute any of these proposed explanations.

The studies on Chinese Herb Nephropathy and Balkan Endemic Nephropathy published on completely separate and different populations in China, Belgium, and the Balkans, using different study designs and methodology are remarkably consistent in their conclusions that AAs cause severe nephropathy. The associations between AA exposure and nephropathy appear to be moderate to strong, depending on population, other causal factors, and study design.

Dr. Friedman-Jimenez thought the information related to the causal inference process was scattered throughout the profile and suggested that the organization of epidemiologic evidence from human studies could be improved by grouping similar topics in a more logical order that reflects the causal inference process. The strength of association, consistency, exposure-response gradient, biological plausibility, and whether alternative explanations of observed data is due to chance, selection bias, or confounding bias can be excluded with reasonable certainty. Making a more explicit relationship of the causal inference process would help in structuring the profile.

Dr. Friedman-Jimenez opined that, taken together, the human studies in the profile provide sufficient evidence of a causal relationship between AA exposure and urothelial cancers in humans. This meets the single requirement for classification of AA as *known to be a human carcinogen*.

Ms. Ruthann Rudel, the third BSC reviewer, said the document is clear and well presented. She suggested including the chemical structure(s) of the key compounds. She said the issue of nomenclature and identification is necessary for effective intervention.

The document clearly describes the current understanding of exposure to herbals containing AAs. She suggested that the profile describe how and when AA is used intentionally; one reference indicated that it is used to terminate pregnancy, thus, there must be awareness that it is toxic. She asked if the doses in the

Belgium weight-loss cases were much higher than levels typically prescribed or used and suggested that this information to be added to the profile.

She asked for clarification regarding the tissues in which adducts were found and whether AA is a direct acting mutagen, since the profile identifies the metabolites as the active agents.

Dr. Solomon suggested including a discussion in the profile as to whether dermal exposure and transdermal absorption are relevant, as gardeners might be at risk. She asked about the possible use of extracts of AA plants as flavorings and whether the many nitrophenanthrenes in this class have the same effect and are metabolized via the same pathway.

Dr. Sherley suggested a research need would be to examine tissue from kidney tumors from patients who had not taken AA to assess if the same biomarker is found in these tumors.

C. Styrene

a. Presentation

Dr. Lunn said the NTP preliminary listing recommendation for styrene in the 12th RoC is *reasonably anticipated to be a human carcinogen*. Dr. Lunn discussed the evidence from epidemiology studies, cancer studies in experimental animals, and mechanistic studies in humans and animals that supports the recommendation.

Styrene is used worldwide in the production of polymers for rubber reinforced plastics and polystyrene food containers. The 2006 U.S. production was 11.4 billion pounds. Significant occupational exposure occurs in the reinforced plastics industry (RPI), the styrene-butadiene rubber (SBR) industry, and the styrene monomer and polymer industry (SMPI). The general public is exposed to much lower levels than industrial workers through the inhalation of outdoor air contaminated by emissions from industrial processes, tobacco smoke, and via the ingestion of food and water.

NTP concluded that there is limited evidence of carcinogenicity in humans based on studies showing (1) increased mortality or incidence of lymphohematopoietic cancer (LHC) among subgroups of styrene-exposed workers and (2) the finding of styrene-7,8- oxide DNA adducts and increased genetic damage in lymphocytes from styrene-exposed workers.

Dr. Lunn described the strengths and weaknesses of the available studies from the three industries. The strengths of the epidemiology studies from the RPI are the high levels of styrene to which workers were exposed and the absence of other confounding carcinogenic exposures. The limitation to these studies is the large percentage of the workforce that was employed for less than a year with a short follow-up. The strengths of the studies from the SBR are the large number

of long-term workers, the detailed analyses of LHC, and the greater number of cases of lymphoma and leukemia. The limitation of these studies is that the lower levels of exposure to styrene are highly correlated with co-exposure to butadiene.

The characteristics of the studies on styrene that are most informative for this evaluation are the greater number of exposed cases, adequate length of follow-up, and the internal analyses (i.e., use of unexposed members of the cohort as the comparison group) on exposure-response relationships that help to control for confounding. The most informative studies are Kogevinas *et al.* (1994), Kolstad *et al.* (1994), and Delzell *et al.* (2006).

Kogevinas *et al.* (1994) studied a multi-national cohort from the RPI and showed that the risk of LHC and lymphoma increased with increasing time since first exposure and average exposure. For lymphoma there were non-significant increases in relative risks for all exposure levels, but no significant trend.

Kolstad *et al.* (1994) studied an incidence cohort study of Danish workers in the RPI that had some overlap with the workers in the Kogevinas study. Kolstad found 112 cases of LHC, 42 of which were diagnosed as leukemia. An important feature of this study is that the authors measured cancer incidence, which is important for LHC cancers since there is a high survival rate. The authors found a significant increase in the standard incidence ratio (SIR) for leukemia among workers with greater than 10 years since first employment, earlier start dates, or being short-term workers. There was some evidence that short-term workers may have been exposed to high levels of styrene. Dr. Lunn pointed out that earlier start dates may be an indirect measure of exposure levels since exposure decreased by more than 4-fold over the duration of the study; a positive trend was found for earlier start dates in internal analyses.

Delzell *et al.* (2006) studied a multi-plant cohort of U.S. and Canadian workers from SBR facilities that included mostly workers from two earlier cohorts. They found significantly elevated standardized mortality ratio (SMRs) for leukemia, and non-Hodgkin lymphoma (NHL) plus chronic lymphocytic leukemia for workers with a 10-year duration of employment. The relative risk (RR) increased with higher levels of exposure to styrene. Also, workers were exposed to butadiene, a carcinogen, and dimethyldithiocarbamate (DMDTC). Butadiene was not independently associated with the risk of NHL and the exposure-response pattern for styrene was similar in models controlling for DMDTC and butadiene exposure. The internal analyses for leukemia showed a significant RR for the highest levels of exposure to styrene peaks. With cumulative exposure there was an increased risk for styrene, which was attenuated after controlling for butadiene.

In summary, there was an increased risk for LHC, lymphoma, and leukemia with time since first exposure in the RPI and SBR industries. There was an increased

risk for leukemia in SBR workers at peak exposures and in the RPI workers with an earlier start date. With average exposure there was an increased risk for lymphoma and LHC in both industries. With cumulative exposure there was an increased risk for lymphoma in the SBR industry. NTP's conclusion for LHC is consistent with the IARC evaluation (2002) that there is limited evidence in humans for the carcinogenicity of styrene.

Limited evidence is not just based on the LHC findings but also on studies of genetic damage in workers. Multiple studies have identified styrene-7,8-oxide DNA adducts at O⁶-guanine, β-N1-adenine, or N²-guanine in lymphocytes of styrene-exposed workers. In addition, increased single-strand breaks and chromosomal aberrations were found in lymphocytes of styrene-exposed workers. A meta-analysis of 22 studies reported a significant risk (2.18) for chromosomal aberrations among workers when the time-weighted-average exposure was greater than 30 ppm. Chromosomal aberrations have been shown to be a risk factor for cancer.

Dr. Lunn said the NTP concluded that there is sufficient evidence of carcinogenicity in experimental animals based on increased incidences of lung tumors in mice exposed by multiple routes to styrene. Increased incidences of lung tumors in male and female mice were observed in an inhalation study in CD-1 mice performed by Cruzan *et al.* (2001). Ponomarkov and Tomatis (1978) found a significant increase in lung tumors in offspring of female mice exposed *in utero* by gavage and then weekly for 16 weeks after weaning. There was also a significant increase in lung tumors and a significant dose-response relationship in male mice exposed by corn oil gavage for 78 weeks followed by 13 weeks observation (NCI 1978). No tumors were found in the control group. The incidence of lung tumors in the high-dose male mice (21%) in the study exceeded the historical control range (0 to 20%) for 44 studies using untreated and corn-oil gavage controls from two laboratories. Data from studies of styrene in rats were considered inconclusive.

Dr. Lunn discussed the evidence from mechanistic studies by first describing the pathways for styrene's metabolism. In the mouse, Cyp2e1 and Cyp2f2 oxidize styrene to styrene-7,8-oxide, which may be detoxified to styrene glycol, mandelic acid, phenylglyoxylic acid, and finally to phenylglycine and excreted in the urine. A minor pathway is the oxidation of styrene to styrene-3,4-oxide, which is further metabolized to 4-vinyl phenol by Cyp2f2.

Two potential mechanisms for the carcinogenicity of styrene, which are not mutually exclusive, are genotoxicity and cytotoxicity. Evidence supporting a genotoxic mechanism is: (1) the genotoxic styrene-7,8-oxide has been detected in the blood of styrene-exposed workers; (2) styrene-7,8-oxide adducts, single-strand breaks, and sister chromatid exchanges have been found in animals; and (3) styrene-7,8-oxide adducts, single-strand breaks, and chromosomal aberrations have been found in human lymphocytes of styrene-exposed workers.

Evidence supporting a cytotoxic mechanism is derived from studies showing that repeated exposure to styrene causes hyperplasia.

The interspecies difference in tumorigenicity between mice and rats can be explained by the 6-fold higher levels of Cyp2f2 ring-oxidized metabolites, such as the cytotoxic 4-vinylphenol in Clara cells, in the mouse lung than in the rat lung. 4-Vinyl phenol is thought to be more potent than styrene-7,8-oxide in inducing cytotoxicity; however, this hypothesis has not been tested in long-term cancer studies or in Cyp2f2 knockout mice.

In summary, the proposed listing of styrene as *reasonably anticipated to be a human carcinogen* is based on limited evidence in humans due to increased mortality or incidence of LHC in styrene-exposed workers and increased levels of DNA adducts and genetic damage in human lymphocytes. Sufficient evidence in experimental animals is based on lung tumors in mice by two routes of exposure. The supporting mechanistic evidence is the metabolism of styrene to styrene-7,8-oxide, which causes genotoxicity in humans. Styrene-7,8-oxide is listed in the RoC as *reasonably anticipated to be a human carcinogen*.

b. Public Comments

(i) Mr. Jay Merrell, Industrial Dielectrics

Mr. Merrell asked the NTP and the BSC to review all the relevant scientific data regarding the potential for styrene to cause cancer in humans before making a final decision. Industrial Dielectrics uses styrene-polyester resin, glass fibers, and other materials to make highly engineered molding compounds for use in electrical equipment and appliances. People are exposed to styrene both inside and outside the plant. He complies with all the applicable OSHA and EPA regulations to protect the health of his workforce and the surrounding neighborhood. He realizes NTP is conducting a hazard assessment, and that other governmental agencies have the responsibility for full risk assessments and setting exposure limits and control requirements. He has heard that the data on the health effects of styrene are controversial and that industry scientists and independent experts who have reviewed the data regarding the carcinogenicity of styrene have come to different conclusions than the NTP staff.

(ii) Dr. Philip Cole, University of Alabama at Birmingham

Dr. Cole, representing the Styrene Information and Research Center (SIRC), commented on a review paper by Bofetta *et al.* (unpublished) on human epidemiological studies of styrene and cancer. The paper reported that the overall SMRs for LHC (excluding NHL + leukemia), NHL, and leukemia were not increased. He summarized the four major positive studies by Hodgson, Bond, Kolstad, and Delzell and stated that the overall SMRs for the four studies was 1.36 for NHL and 1.18 for leukemia. He summarized the internal analyses of the Kogevinas, Kolstad, and Delzell studies for NHL and leukemia and stated that most of the analyses were negative, four were positive, and two were weakly positive. A positive association was found for leukemia with cumulative exposure

to styrene in the crude analyses, but not in the analyses controlling for butadiene and DMDTC. Based on these findings he could not find a pattern for any strong association of styrene with these cancers.

Dr. Cole also discussed the two NTP categories of classifying carcinogens and the meaning of reasonably anticipated to be a human carcinogen. He maintained that the definition means more than probable. He compared the NTP classification of carcinogenicity to that of IARC and said they are comparable. He did not think that many IARC 2B compounds would be classified as reasonably anticipated to be human carcinogens.

BSC Questions for Dr. Cole

Dr. Elaine Faustman asked about peak exposures. Dr. Cole said the data presented by Delzell in the SBR workers found no association with NHL and a weak relationship with leukemia. He reiterated that there was no relationship of cumulative exposure with leukemia after correction for butadiene and DMDTC exposures. There was some weak support for the peak exposure, but not cumulative exposure.

Dr. Dana Loomis asked whether occupational carcinogens with SMRs of 5-50 refer to exposure in modern facilities or in the past. Dr. Cole replied that these SMRs relate to historical exposures, although present day exposures in industry are higher than for the general population.

Dr. Justin Teeguarden asked if the studies would support a finding of insufficient evidence, sufficient, or limited evidence in humans. Dr. Cole responded that IARC classifies styrene as having limited evidence; he believes it is barely limited. Dr. Cole had no opinion as to whether animal studies are more important if the classification is limited evidence.

(iii) Dr. Julie Goodman, Gradient Corporation

Dr. Goodman, representing the American Composites Manufacturers Association, said the NTP provided an incomplete portrayal of the epidemiological data of styrene by concentrating on the positive findings in the Kogevinas and Delzell studies and failing to discuss the negative epidemiological studies that predominate in the literature. She believed there is no consistent trend of styrene exposure with cancer risk in any cohort. NTP's emphasis on risk is based on one exposure measure, namely, the association between leukemia and peak styrene exposures in the Delzell study. In her opinion, the findings from this study are due to chance and this study alone cannot serve as evidence for an association. Each lymphoproliferative cancer type is a different disease with a different mechanistic pathway for development and should be evaluated separately from the others. No cancer type had an increased risk consistently across exposure measures or studies. The evidence does not support the draft profile's classification of limited evidence of carcinogenicity based on "increased

mortality or incidence of LH cancer" and thus styrene should be characterized as "not classifiable" based on NTP's criteria.

BSC Questions for Dr. Goodman

Dr. Loomis said the large number of null associations in some studies might be due to the number of associations that were assessed. Dr. Goodman agreed. She noted that the best exposure metric is unknown and said the most statistically significant exposure metric is not necessarily the most appropriate. Dr. Solomon asked Dr. Goodman for a justification for separating chronic myelogenous leukemia (CML) and acute myelogenous leukemia (AML) since CML undergoes blast crisis and progresses to AML and it is difficult to differentiate the cell types diagnostically. Dr. Goodman replied that the epidemiological outcomes are not consistent because one study showed an association with leukemia and another with non-Hodgkin's lymphoma. Dr. Solomon said a separation of tumor types makes sense for cancers that are derived from different cell types, but some of the lymphohematopoietic cancers are often derived from common progenitors and develop along the same causal pathway. Dr. Goodman disagreed and said combining of different tumor types is inappropriate. If one finds an association with a particular tumor type in one study and another tumor type in a second study, even though the cohorts were exposed to the same chemical, these findings would not suggest an association. Dr. Loomis said the investigators used different categorizations for the different diseases; Kogevinas classified the tumors as malignant lymphomas and leukemias while Delzell used a large number of categories. Dr. Goodman replied that it is not just consistency across studies that is required, but also consistency within a cohort; different studies with the same cohort looked at different exposure metrics.

(iv) Dr. Jeffrey Charles, Consulting Toxicologist

Dr. Charles, spoke on behalf of the Emulsion Polymers Council Incorporated and addressed issues regarding the animal data. He disagreed that the animal evidence is sufficient to conclude that styrene is an animal carcinogen because tumors were only found in one species, the mouse, in one organ, the lung, and through one route of exposure, inhalation. Also, he said styrene does not cause tumors via a genotoxic mechanism.

There is only suggestive evidence that styrene is a carcinogen in mice in four studies using the oral route. Two of the studies were negative and one exposure regimen caused cytotoxicity that resulted in an increased incidence of lung tumors. In the fourth study, NCI found suggestive evidence of male lung tumors with an incidence of 0, 12, 18% in treated groups of B6C3F1 mice exposed to 0, 150, 300 mg/kg, respectively, for 78 weeks and then observed for an 13 additional weeks. Dr. Charles disagreed with NTP's reevaluation of this study where NTP questioned the 12% incidence of lung tumors in the concurrent historical control animals that received an untreated diet at the testing laboratory compared to a control value of 4% at a second laboratory where the control

animals received corn oil. Dr. Charles added that this study was conducted in an animal room housing a second concurrent study where animals were exposed to known carcinogens. The reviewing pathologists stated that the alveolar/bronchiolar tumors observed in the exposed animals are common in several strains of mice irrespective of treatment and vary in frequency from study to study. In conclusion, he said the increase in lung tumors in mice exposed via inhalation and the suggestive evidence by the oral route do not substantiate the draft substance profile's conclusion of sufficient evidence and thus the draft substance profile is not technically correct.

BSC Questions for Dr. Charles

Dr. Faustman asked Dr. Joe Haseman, a consulting statistician to the RoC, about the appropriate use of historical controls in the NCI study. Dr. Haseman replied that the concurrent control should always be used in analyzing the data. However, in this case the control group received an untreated diet, which was different from the experimental diet of styrene dissolved in corn oil. The second most important control diet would be a corn oil gavage group. The untreated control group had a significantly higher percentage of tumors (12%) than the corn oil controls (0%). The question is how much credence should be given to the untreated control group. Looking at control groups from a second laboratory might be helpful. The incidence of tumors in corn oil control groups from the second laboratory was 2 and 4%, closer to the 0% than the 12% in the untreated controls. A second question is how much weight should be given to the second testing laboratory's control data, which are not consistent with other contemporary data. NCI thought styrene was not carcinogenic based on the incidence of tumors from the concurrent control. In retrospect, by analyzing the data and using more consistent control values, styrene is carcinogenic in mice. It would be up to the BSC to decide if the re-analysis is acceptable.

(v) Dr. Earle Nestmann, Cantox Health Sciences International

Dr. Nestmann commented on the genotoxicity studies with styrene. He said that although the draft substance profile and the background document summarized the genotoxicity studies factually, the discussion is biased toward "positive" results and ignored reviews with alternative interpretations. He indicated areas in the profile that were not adequately addressed, namely, the lack of a meaningful assessment of the genotoxicity data in relation to the metabolism of styrene; the lack of concordance between the results of *in vitro* studies, worker exposure, or carcinogenicity studies; the paucity of discussion on the negative *in vivo* results in assays for chromosome aberrations and micronuclei; and the biological significance of the types of DNA adducts in styrene-exposed workers in relation to DNA repair, as DNA adducts indicate exposure, but not necessarily genetic hazard or risk.

Styrene can be oxidized *in vitro* and *in vivo* by cytochrome P450 enzymes to styrene-7,8-oxide, a genotoxic compound. *In vivo*, styrene-7,8-oxide is rapidly detoxified by epoxide hydrolase and glutathione peroxidase, two enzymes that

are lacking in *in vitro* assays; hence, styrene-7,8-oxide tends to be “positive” in these systems. Pharmacokinetic modeling shows that the concentration of styrene-7,8-oxide in human lung tissue from inhaled styrene is 100-fold and 10-fold lower than in mice and rats, respectively. He concluded that the genotoxicity data do not support the listing of styrene in the 12th RoC as *reasonably anticipated to be a human carcinogen*.

BSC Questions for Dr. Nestmann

Dr. Solomon asked Dr. Nestmann whether he believed that styrene-7,8-oxide is genotoxic. He replied that it is genotoxic, but is rapidly metabolized to non-genotoxic intermediates *in vivo*. He added that styrene-7,8-oxide adducts are found in humans at very low levels.

(vi) Dr. James Bus, Dow Chemical Company

Dr. Bus, representing the Ethylbenzene Industry Panel of the American Chemistry Council, discussed the mode of action of styrene in mouse lung. He agreed that styrene is metabolized to styrene-7,8-oxide in the liver, that low-levels of styrene-7,8-oxide-DNA adducts have been detected in animals and humans, and that styrene-7,8-oxide is genotoxic in *in vitro* mutagenicity assays. However, mouse lung is the only site where tumors are found and oral administration of styrene-7,8-oxide does not cause lung tumors in mice. He posited that lung tumors are not related to the formation of styrene-7,8-oxide in the mouse lung and that styrene-7,8-oxide does not explain the mouse versus rat sensitivity or the negative lung genotoxicity studies. He believed that Cyp2e1 does not mediate the metabolism of styrene to styrene-7,8-oxide in mouse lung because Cyp2e1 knockout mice develop lung tumors. Rather, he proposed that styrene is uniquely metabolized via Cyp2f2 in Clara cells to ring oxidized metabolites including 4-vinylphenol (4-VP) that leads to cytotoxicity, chronic cell proliferation, and tumorigenicity. 4-VP also undergoes further oxidation by Cyp2f2 to ring-oxidized metabolites. Evidence for this hypothesis is the finding that inhibition of Cyp2f2 blocks the toxicity of styrene in mouse lung. He said that rats and humans have very low activities of Cyp2f2 and thus do not develop lung tumors. He concluded that the mouse lung tumor response is not supported by the styrene-7,8-oxide mode of action hypothesis. However, the alternative mode of action hypothesis is consistent with the weight of evidence namely that styrene is metabolized to non-genotoxic, cytotoxic ring-oxidized metabolite(s) by mouse specific Cyp2f2, a cytochrome isozyme of very low activity in rats and humans.

BSC Questions for Dr. Bus

Dr. Faustman asked about the causal agent for the chromosomal aberrations. Dr. Bus replied that the interpretation of those studies were unclear, but he believes that humans do not generate sufficient amounts of toxic metabolites to produce aberrations based on *in vitro* studies using human lung microsomes.

Dr. Teeguarden asked if Cyp2f2 metabolizes styrene to styrene-7,8-oxide. Dr. Bus said it might, but this is not the major pathway since Cyp2e1 knockout mice

do not develop cancer; therefore, the majority of styrene is metabolized to styrene-7,8-oxide through the Cyp2e1 pathway.

Dr. Teeguarden said he believes that the dosimetry of styrene-7,8-oxide in the lung and not the blood compartment is an important measure. Dr. Bus replied that styrene-7,8-oxide levels in the blood are predictive of lung concentrations. He added that if styrene-7,8-oxide is responsible for tumorigenicity in the mouse following oral gavage of styrene, then the concentration of styrene-7,8-oxide should have been high enough to cause tumorigenicity, but it did not. Dr. Teeguarden reiterated that he believes lung metabolism is driving lung dosimetry and Dr. Bus agreed.

Dr. Eastmond asked whether oral administration would produce toxicity in the lung based on his proposed pathway through Cyp2f2 and then presumably progress to cancer. Dr. Bus agreed that toxicity would occur, but thought that the oral administration of styrene did not cause cancer.

(vii) Dr. Lorenz R. Rhomberg, Gradient Corporation

Dr. Rhomberg, representing the National Marine Manufacturers Association, commented on the weight of evidence evaluation. He said there was no consistent evidence for cancer because (1) the human data do not consistently show increased mortality or incidence of cancer, (2) the evidence in experimental animals are species-specific and not applicable to humans, and (3) the mode-of-action in mice is not applicable to humans. He did not believe the human evidence because there was no consistent association between styrene exposure and mortality for any tumor type, there was a lack of exposure-response relationships, and there were confounding factors relating to co-exposure to known carcinogens. The animal data do not meet the standards of "sufficient" evidence because the lung tumors observed in the mice were mostly benign except in one strain, tumors developed late in life in the presence of chronic cytotoxicity, and there were no tumors in rats. He said styrene is weakly genotoxic, but this is not the mode of action for styrene. Rather, Cyp2f2 metabolizes styrene to highly toxic intermediates in the mouse lung resulting in local cytotoxicity and subsequent cell proliferation. Since the formation of the toxic intermediates does not occur in rats and CYP2F2 does not form styrene-7,8-oxide in humans, the mode of action cannot be used as evidence that styrene is a tumorigen in humans. He concluded that a causal interpretation for human cancer is not credible and the standards of "limited evidence" are not met.

BSC Questions for Dr. Rhomberg

Dr. Teeguarden asked what quantitative differences in metabolism between mice and humans would be needed to suggest that there is a real difference in the mechanism of tumorigenesis between mice and humans. Dr. Rhomberg replied that one has to consider functionality rather than fold differences between species. Lung toxicity is required for mouse tumor formation and lung toxicity has not been observed in rats and humans. Although humans metabolize styrene

to styrene-7,8- oxide, humans have high levels of epoxide hydrolase that detoxifies the epoxide. Dr. Teeguarden then added that if the pharmacokinetic data do not indicate that sufficient styrene-7,8- oxide is formed in the human to cause cytotoxicity, this would eliminate a similar mode of action in humans and the mouse.

Dr. Loomis asked Dr. Rhomberg whether he agreed that a possible reason for some of the null associations could be that a number of the epidemiology studies are descriptive and report SMRs for multiple diseases that have been divided into smaller subgroups for analysis. Dr. Rhomberg said this could be the case, but his concern is that the null effects are seen for tumors that have been hypothesized to result from styrene exposure. He would have expected the same type of biological effects in all studies. It is a matter of scientific judgment as to whether the lack of concordance is due to small numbers. Dr. Loomis asked how one might separate the relevant from the non-relevant epidemiology data. Dr. Rhomberg said one could do a meta-analysis or laboriously tabulate all the results to assess which studies were the strongest and which weakest because of methodological reasons. Another concern is the lower proportion of tumors in the RPI workers with higher exposure to styrene compared to the higher proportion of tumors in the SBR cohorts with lower exposure to styrene. Dr. Rhomberg was uncertain how the BSC could make a judgment call with only a limited set of data. He hoped the BSC would consider these discrepancies to be sure their decisions hold up to scientific scrutiny. He said the public commenters have some relevant points that need to be resolved or counter arguments put forth before a decision is made.

(viii) Dr. George Cruzan, ToxWorks

Dr. Cruzan said the public commenters disagree with the NTP's decision to list styrene as *reasonably anticipated to be a human carcinogen*. Only 2 of the 74 analyses in the Kogevinas study showed a significant positive increase in tumors by average exposure and time since first hire, but no increase was noted when cumulative or duration of exposure was considered. NTP has interpreted the epidemiology studies to mean there is limited evidence of carcinogenicity, but Kogevinas did not state that styrene causes cancer. The RPI daily exposure in the 1960s was over 100 ppm.

He said the Delzell study is the only study to report average exposure, which was calculated by dividing the cumulative exposure for each person by the duration of exposure. Peak exposure in the SBR workers was lower than the daily exposure in the RPI.

IARC reviewed the animal studies on styrene and concluded that the evidence of carcinogenicity was limited, not sufficient as the NTP asserts. IARC said the genotoxicity data were inconclusive. One study showed that the levels of styrene-7,8-oxide in the lung are not responsible for lung tumors in mice. Physiologically-based pharmacokinetic (PBPK) modeling estimates that there is

a 10-fold difference in the level of styrene-7,8-oxide between rats and mice, a 10-fold difference between rats and humans, and therefore, a 100-fold difference between mice and humans.

BSC Questions for Dr. Cruzan

Dr. Faustman asked if there are pharmacodynamic differences between rats and mice such as repair rates and how these rates compare to humans. Dr. Cruzan replied that one cannot find cytotoxicity in rat lung up to 1000 ppm of styrene, but it causes toxicity in mouse lung at 20 ppm. He added that other chemicals that are metabolized via the Cyp2f pathway cause mouse lung tumors but not rat lung tumors. Styrene causes cytotoxicity in the mouse and rat nasal olfactory epithelium due to Cyp2f2 metabolism in this tissue, but 5-phenyl-1-pentyne that inactivates Cyp2f2 prevents cytotoxicity.

Dr. Teeguarden asked if the levels of styrene-7,8-oxide in the lung could explain the differences between rats and mice, and since very little styrene-7,8-oxide is produced in human lung, whether this would be evidence that humans cannot be at risk for lung tumors. Dr. Cruzan replied that Dr. Gary Carlson could not identify styrene-7,8-oxide *in vitro* in human lung preparations treated with styrene as it was below the level of detection. Dr. Ronald Hines stated that those findings are inconsistent with *in vitro* studies in human cells showing styrene can be converted to styrene-7,8-oxide more efficiently by CYP2F2 than CYP2E1. Dr. Cruzan stated that Dr. Yost could not duplicate those findings.

c. BSC Discussion

Dr. Pino, the first BSC discussant, said the information on identity, description, use, production, and human exposure in the draft substance profile is clear and accurate. There is abundant documentation for the potential of human exposure.

The conclusion of limited evidence of carcinogenicity based on human data is clearly and adequately described and supported in the draft substance profile. However, it is limited evidence, and it is plausible that any of the results could be due to chance or confounding factors. For completeness and in order to be consistent with a weight of evidence approach he thought the study by Wong *et al.*, (1994) should be included, as this study did not report any evidence of increased risk of LHC in RPI workers.

Based on the data from the two mouse studies using two different routes of exposure, it is not clear that there is sufficient evidence of carcinogenicity in animals. The inhalation study by Cruzan *et al.*, (1998) showed clear evidence of increased lung tumors in the 2-year study with CD-1 mice, but the results of the 2-year oral gavage study in B6C3F1 mice (NCI, 1979) are problematic. It is unclear why the corn oil control mice group had a lower background tumor rate than untreated controls. Dr. Haseman's paper that compared corn oil gavage with untreated controls showed no difference in tumor incidence between these two groups of controls. The Hazleton studies of corn oil and untreated control

groups showed no differences in incidences of background tumors. If styrene is toxic or carcinogenic, non-neoplastic changes in the lungs of the mice would be expected, but such changes were not described in the technical report. Although the study is suggestive of increased lung tumors, it is not conclusive due to the low number of animals in the control group. He indicated that the study in O20 mice by Ponomarev and Tomatis, (1978) was not conclusive based on the severe styrene-related toxicity/mortality. Also, the model and study design were not conventional for assessing carcinogenicity, and he stated that the results should be regarded with caution.

Both the genotoxic and non-genotoxic mechanisms proposed for styrene are plausible and the major route of metabolism is through styrene-7,8-oxide. The RoC lists styrene-7,8-oxide as *reasonably anticipated to be a human carcinogen*.

Dr. Eastmond, the second BSC discussant, thought the draft substance profile was well written, but it did not reflect the range of findings or the controversy around styrene. He thought more of the uncertainties should be brought into the profile, as the listing is a judgment call depending on one's interpretation. The 0.3-1% contamination of styrene with styrene oxide in the exposure studies should be added. The information on occupational exposures appears to be sufficient, but it seems to downplay the maximum levels to which many workers were exposed pre-1989. The exposure of the general population to styrene through food is orders of magnitude lower than those to which workers have been and are exposed.

He agreed that the document supports the conclusion of limited evidence of carcinogenicity in humans; however, it does not reflect the range of the epidemiological results, the variability of the studies, or the controversial nature of the conclusions. Although different types of lymphomas and leukemias are usually considered as different diseases, a strange pattern is seen in the types of lymphohematopoietic tumors found with other epoxides related to styrene, such as ethylene oxide and butadiene that have similar modes of action. Both the suggestive evidence from traditional epidemiology studies and the data from human biomonitoring and molecular epidemiology studies identifying DNA adducts, single-strand breaks, and structural chromosomal aberrations in human lymphocytes from workers exposed to styrene strengthen the conclusions.

The NTP's conclusions for at least two of the epidemiological studies seem to differ from those of the authors. The conclusions stated by the authors of the Delzell and Kogevinas studies were ambiguous or almost negative. He suggested that NTP's evaluation should be more appropriately described as a re-evaluation or re-interpretation of these studies as the NTP selected a broader set of data from these studies for its analysis.

Styrene is carcinogenic by inhalation in the mouse, but the oral route is a judgment call because an equivocal call was made for the original NCI study. In

retrospect, however, when considering the positive results by inhalation with the reevaluation of the control data in the gavage study, there does appear to be a dose-related increase in carcinogenicity. The ability of styrene to induce cell proliferation in the terminal bronchioles of the lung after oral dosing as shown by Green *et al.* (2001) in mice provides additional support for the revised assessment of the NCI study that oral administration of styrene can induce cancer-related changes in the lung. It should be stated in the draft profile whether the rat studies were negative, or if there was inadequate evidence to draw a conclusion.

Dr. Friedman-Jimenez, the third BSC discussant, said the information in the draft profile was presented clearly and the identity of styrene was technically accurate. He focused his remarks primarily on human exposure and human epidemiology studies. There was sufficient evidence presented in the draft substance profile documenting exposure to styrene, despite the lack of documentation of data in older publications. In his opinion, the scientific information on the studies of health effects of styrene in humans in the draft substance profile supports a conclusion of limited evidence of carcinogenicity as defined in the RoC listing criteria.

He commented specifically on the Delzell paper, which presents a Poisson regression analysis of relative risk for leukemia with various combinations of styrene, butadiene, and DMDTC in the models when examining one, two, and three chemicals at a time. The apparent exposure-response trend for styrene and leukemia is attenuated when butadiene is added to the model and disappears when both butadiene and DMDTC are added to the model. The authors interpreted this finding to mean that butadiene is most likely the actual carcinogen. An alternative interpretation is that there is an interaction between butadiene and styrene, between DMDTC and styrene, or among the three chemicals. He was unsure whether it is even appropriate to include DMDTC in the models as a potential confounder, especially if there is an interaction between DMDTC and styrene. The profile should discuss the cross-classification analyses between butadiene and styrene, which suggests an effect modification with butadiene; however, there is not enough power to test for an interaction. The interpretation offered by the authors that butadiene is the only carcinogen causing leukemia in their cohort is a reasonable interpretation of their data, but it is not generalizable to other studies of styrene-butadiene-DMDTC workers and does not completely explain the elevated relative risks reported in other styrene-exposed cohorts. The analyses in this study do not explain the elevated SMRs for leukemia for some of the occupational subgroups, production groups, labor groups, and the longest latency group. He concurred with the NTP profile that styrene is a risk factor for leukemia with the caveat that confounding factors are not excluded as alternative explanations of the observed data.

The reports of non-Hodgkin's lymphomas/chronic lymphocytic leukemia (CLL) and lymphohematopoietic neoplasms provide credible evidence that styrene

causes those neoplasms, but confounding, information bias, and chance might be possible alternative explanations. Negative findings should be interpreted in the context of the statistical power of the study. Very few of the studies that presented relative risks of null findings had sufficient precision to exclude moderate or even strong associations. The profile could benefit from more inclusive and critical discussion of negative and statistically insignificant findings. Inconsistencies of epidemiological findings can often be the result of bias, confounding factors, or chance that vary substantially among the different studies. A discussion of the statistical problem of multiple comparisons relating to the profile should be added. In summary, taken as a whole, the set of studies in the draft profile provides limited evidence of carcinogenicity in humans.

He questioned the definition of “reasonably anticipated to be a human carcinogen” and found it to be self-contradictory, ambiguous, and difficult to implement in a transparent and objective way. He thought the definition relies too heavily on the word “credible” as it introduces a large, subjective element into the decision process. He thought much of the controversy reflects the diversity in understanding the definition of limited evidence. He also thought it might be worthwhile to revisit the definitions to address both the scientific challenge and the public health values of the RoC.

Ms. Rudel, the fourth BSC discussant, said the draft substance profile is clear and represented a synthesis of a large body of information. Styrene is more difficult to assess than related epoxides including butadiene, ethylene oxide, and vinyl chloride, all of which are known human carcinogens with stronger evidence of carcinogenicity in animal studies. The level of styrene-7,8-oxide in the blood of humans that induce DNA damage might be influenced by genetic variation related to detoxification and DNA repair. These differences might explain the variation in responsiveness of cohorts in different parts of the world.

She agreed with the listing of styrene as *reasonably anticipated to be a human carcinogen* based on chromosomal damage, DNA adducts, and styrene-7,8-oxide in exposed workers and the epidemiology database. Also styrene-7,8-oxide is already listed in the RoC. The draft substance profile describes the rationale clearly, but it would be more useful if it conveyed the inconsistencies in the data set. It might be useful to add information on the similarities and differences between the database for styrene and the three epoxide-forming carcinogens that are metabolized through similar metabolic pathways.

The animal data are harder to understand, but there is a credible argument that the mouse lung tumors might have limited relevance to humans. The only other tumors observed were liver and mammary tumors.

Ms. Rudel pointed out four issues that she suggested be addressed:

- The Delzell study was only done in men.
- Add the values of the 95 percentile and maximum blood styrene levels in the general population as measured by the CDC to the profile so they can be put in the context of levels in occupationally exposed workers.
- The limited overall power of the epidemiology database is an important caveat in over interpreting negative findings.
- Clarification and discussion on the outcomes from the rat studies would add to the completeness of the document.

She suggested that a discussion of the incidence of mammary gland tumors in humans and rodents be added to the profile. Only a limited number of women were included in the studies. Styrene and the related epoxide-forming chemicals all cause mammary tumors in rodents but at different rates, with styrene being the weakest of the four. There are no adequate breast cancer studies in humans on these four epoxides even though butadiene, ethylene oxide, and vinyl chloride are all human carcinogens at other sites. The profile should state that breast cancer risk has not been adequately evaluated and it should include the findings from three studies where internal comparisons show an increased risk for breast cancer among subgroups of women exposed to styrene (Kogevinas *et al.*, 1993, Wong *et al.*, 1994, and Cantor *et al.*, 1995).

Dr. Hines, the first *ad hoc* BSC discussant, concurred with the earlier reviewers that the identity and description of styrene are clearly described and technically correct. He had some concerns about the arguments presented in the draft substance profile, although he thought the evidence presented regarding the formation of styrene-7,8-oxide in humans at levels sufficient to form macromolecular adducts after a reasonable exposure supported the listing of styrene as *reasonably anticipated to be a human carcinogen*.

He said it is important to note that occupational exposure levels have decreased over the past several decades from several hundred ppm to levels of less than 100 ppm and causality cannot be established in human studies. As Dr. Eastmond pointed out, one cannot eliminate the confounding exposure to styrene oxide in occupational exposure to styrene. He believed it is inappropriate to use the mouse model to support carcinogenicity in experimental animals because the mouse is a uniquely sensitive species due to differences in certain enzyme levels and pharmacodynamic parameters. Also, lung tumors have not been reported in any other rodent species.

Dr. Hines discussed the metabolic pathways involved in the activation and detoxification of styrene. The draft substance profile failed to describe the lack of the predicted accumulation of styrene in humans based on styrene partition coefficients, which is supportive of relatively efficient styrene detoxification in man. The draft substance profile and the background document are deficient because there was no discussion regarding the presence of CYP2E1 in type II

pneumocytes and Clara cells in the human lung. Although CYP2F1 transcripts have been identified in the human lung, there is no evidence of enzyme activity; hence, it is difficult to predict the quantitative capacity of the human lung to metabolize styrene via the CYP2F1 pathway. He urged that the quantitative data on the formation of styrene-7,8-oxide in pulmonary tissue from mouse, rat, and human tissue be included in the profile because of the limited human carcinogenicity data, as this information would help in interpreting the mechanistic data. Formation of styrene-7,8-oxide is approximately 100-fold less in the human than the rat or mouse, and the formation of styrene glycol, a detoxification product, is equal or better in the human compared to the mouse or rat. These data suggest that enzymes in the human lung poorly activate styrene to styrene-7,8-oxide, a suspected carcinogenic metabolite, in contrast to the robust ability to detoxify styrene-7,8-oxide to the inactive glycol.

Another important omission is the role of genetic polymorphisms in the susceptibility and resistance to carcinogenicity by styrene. No definitive CYP2E1 or microsomal epoxide hydrolase null or hypomorphic variants have been described in humans. In contrast, the *CYP2F1*2* allele, which is present in 20-40% of the human population, contains a single-base insertion in exon 2, resulting in a truncated inactive protein. Thus, a significant number of individuals in a population would have low or no CYP2F1 activity and decreased susceptibility to styrene carcinogenicity. In contrast, the expression of the *CYP2E1*1D* allele is increased in obese humans or those who consume excess alcohol and they would be expected to exhibit increased sensitivity to styrene carcinogenicity.

He thought that the utility and relevance of the document would be increased by a discussion of the differential susceptibility and possible sensitivity of children to styrene as their daily intake is twice that of adults. There is a paucity of information on the ontogeny of cytochrome P450 metabolizing enzymes in the human lung during development, but *in vitro* studies of CYP2E1 expression in the human liver suggest that this cytochrome is present in the fetus by the third trimester at levels 10-20% of an adult. Adult levels of expression are observed by 1-2 years of age. The ontogeny of CYP2F1 has not been studied. In contrast to these activating systems, hepatic epoxide hydrolase expression attains near adult levels during the third trimester. Thus, despite the higher levels of styrene exposure in children, the above data would support children being less sensitive than adults to styrene carcinogenicity.

He thought that the presence of styrene adducts in workers was strong evidence. He identified a pivotal, controlled study by Johanson *et al.*, (2000), not described in the profile, where individuals were exposed to 50 ppm of styrene for 2 hours in an exposure chamber. Styrene was stabilized by hydroquinone to prevent the spontaneous formation of styrene-7,8-oxide. The authors found styrene 7,8-oxide hemoglobin adducts in the volunteers indicating that styrene can be

activated to its mutagenic form in humans and form macromolecular adducts at relatively low exposures.

Since sufficient evidence in any one of the three categories of the RoC listing criteria is adequate to list a compound, he agreed that styrene be listed as *reasonably anticipated to be a human carcinogen* for the purposes of hazard identification based on its metabolism to styrene-7,8-oxide in humans and the fact that styrene-7,8-oxide is listed in the RoC as *reasonably anticipated to be a human carcinogen*. However, an appropriate risk assessment would likely conclude that the risk of styrene carcinogenicity in humans is low.

Dr. Bucher asked Dr. Hines whether he thought that the induction of lymphoproliferative tumors in humans is believable based on the differences in metabolic activation in rodents and humans. Dr. Hines responded that there is strong evidence that CYP2E1 is expressed in human lymphocytes and this might be more relevant in the induction of lymphoproliferative tumors in humans than the metabolism in Clara and type II pneumocytes in the lung.

Dr. Loomis, the second *ad hoc* BSC discussant, said the draft substance profile is clear and accurately written and that styrene production, its use, and exposure are accurately described. He agreed with the NTP that the epidemiologic studies emphasized in the draft substance profile are the key studies. The entire literature suffers from common problems. Most of the studies reported mortality data although incidence data are more informative for lymphohematopoietic cancers because of their relatively low fatality rates. The profile should emphasize that these are rare cancers and even though the cohorts are large, the small numbers of workers in specific exposure groups limit their power. Also, the key studies categorized the different lymphohematopoietic cancers differently, which limits the ability to discern consistent associations between studies.

He addressed the interpretation of the studies in the RPI versus SBR industry. Although the RPI cohorts are reasonably large, the diseases of primary interest are quite rare. The literature is limited by the small numbers of workers in specific exposure and disease groups, the scarcity of quantitative exposure data, and the short follow-up times of several cohorts, particularly during the 1950s and 1960s when exposures were the highest. The studies in the RPI should have been more informative because of the higher exposure levels, but there are difficulties with data collection from workers employed for short periods of time with short follow-up periods. Also, this industry is characterized by large numbers of very small plants, which is a hindrance to the estimation of exposure. Paradoxically, average exposure rates are quite high, but cumulative exposures are not.

The SBR industry presents different challenges, namely low exposure rates and co-exposure to butadiene. He found the interpretation of the adjustment of risk

estimates for styrene in the presence of butadiene and DMDTC problematic, because with the close correlation between exposures it is not clear whether it is possible to identify an independent effect of any compound. It was instructive to look at the evidence for interactions discussed by Delzell, which suggests greater than multiplicative combined effects of styrene and butadiene for leukemia. Interaction with other tumor types was not addressed.

He pointed out that the statement in the draft substance profile that the Kogevinas study adjusted for exposure duration in the cumulative exposure analyses is incorrect, as the analyses adjusted for time since first exposure. Because of the large number of short-term workers, average exposure and cumulative exposure are quite similar, thus, controlling for time since first exposure may be inappropriate. Kogevinas also adjusted for age and calendar time, which are the primary time-related determinants of risk. The risk ratios in addition to the *P* values in the Kogevinas study should be added to the profile. The Wong study also reported statistically significant increases in risk for all lymphohematopoietic tumors with duration of exposure, but not with cumulative exposure, with insignificant increases for NHL and leukemia.

He found the mechanistic data to be important supporting evidence and concluded that the human epidemiology results provide limited evidence that styrene is carcinogenic in humans.

Dr. Teeguarden suggested that the description of genotoxicity be clearer in the profile. Genotoxicity does not necessarily translate into carcinogenicity and a DNA adduct is inconsequential until there is a mutation. He suggested using the terms genotoxic and non-genotoxic rather than genotoxic and epigenetic when describing mode of action. Also, cytotoxicity alone does not result in a tumor, as a mutation must also occur. Since the metabolism of styrene is a key event, it is important to document the quantitative data from all the species and to establish a standard as to what is considered a significant difference between species.

Dr. Hines added that there are qualitative differences in some of the cytochrome P450 enzymes between humans and animals because they arose post-speciation, but CYP2E1 is qualitatively the same in the mouse, rat, and human. CYP2F1 is not the same as Cyp2f2 and CYP2F4. Each protein is assigned a different number because it is regulated differently and has different kinetic constants and different substrate specificities.

Dr. Birnbaum asked whether the CYP2E1 found in human lymphocytes differs from that in other species. Dr. Hines replied that he was not sure whether CYP2E1 is expressed in animal lymphocytes, but qualitatively, CYP2E1 in human lymphocytes mirrors that found in the liver (Raucy *et al*, 1999).

Dr. Faustman said both modes of action and metabolism of styrene are plausible and should be discussed in the document enumerating why both mechanisms

could be believable. She also wondered what proportion of the workforce might be immunosuppressed and hence more susceptible to styrene. She suggested that such information, if available, be added to the profile.

D. Captafol

a. Presentation

Dr. Gloria Jahnke, NIEHS, presented the scientific information supporting the preliminary listing recommendation for captafol in the 12th RoC as *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity in experimental animals and supporting mechanistic data. She described the use of captafol and its exposure in U.S. populations, cancer studies in experimental animals, and mechanistic evidence.

Captafol is a non-systemic fungicide that was used on fruits, vegetables, other plants, and timber products in the United States from 1961 until 1987. Legally, companies were allowed to sell all their existing stocks until 1999 when the U.S. EPA restricted captafol's use and all tolerances were revoked except for onions, potatoes, and tomatoes; the remaining tolerances were revoked in 2006.

Despite its ban in the United States, there is potential for exposure from consumption of imported food products containing residues. Captafol is absorbed following exposure by dermal or oral routes and by inhalation. In the past, there was significant occupational and environmental exposure.

Data from human studies are inadequate to evaluate any relationship between human carcinogenicity and exposure to captafol.

There is sufficient evidence of carcinogenicity in rodents based upon the identification of tumors at multiple sites and in multiple species. Lymphosarcoma, hemangiosarcoma, and Harderian gland adenoma (males) were found in CD-1 mice after chronic feeding of captafol for 110 weeks. Hemangioendothelioma, hemangioma, and tumors of the small intestine and liver were identified in B6C3F₁ male and female mice that survived for 42 weeks in a chronic feeding study. Chronic feeding of captafol for two years to CrI:CD rats resulted in kidney tumors in males and mammary gland fibroadenomas and neoplastic nodules in the liver of females. Similarly, kidney tumors (males) and neoplastic nodules in the liver (males and females) were found in F344 rats following a two-year chronic feeding study.

Mechanistic studies support the experimental animal studies. Captafol is metabolized to tetrahydrophthilmide (THPI), dichloroacetic acid, and inorganic sulfur. During this process an episulfonium ion, which is a potent alkylating agent, is formed. Captafol is genotoxic in bacteria and causes single-strand breaks, sister chromatid exchanges, chromosomal aberrations, and micronuclei in mammalian and human cell lines. It also interferes with spindle formation and transforms cells (i.e., cells show traits characteristic of tumors). *In vivo* captafol

causes mutations in rat germ cells and single-strand breaks and micronuclei in rat kidney cells. Captafol binds to thiols including glutathione and cysteine thus reducing the oxidative capacity of the cell. It also inhibits enzymes involved in DNA replication and DNA and RNA synthesis and induces cytochrome P-450 monooxygenases.

b. BSC Discussion

Dr. Tracie Bunton, the first BSC discussant, said the description of captafol and its use as a fungicide are clearly presented and technically accurate in the draft substance profile. The information regarding the potential for human exposure and possible links to human cancer, including factors confounding the interpretation of these data, is also clearly presented. In addition, the findings from mechanistic and animal studies are clear, and identify captafol as a genotoxic and alkylating agent, an initiator and promoter of carcinogenesis, and a chemical that causes an increase in tumor incidence in mice and rats.

The information presented supports the NTP's preliminary listing decision that captafol is *reasonably anticipated to be a human carcinogen* based on the animal cancer data and the mechanistic information.

Dr. Solomon, the second BSC discussant, said the information on use, production, and exposure is clear and accurate, and the profile describes the historical significance of human exposure to captafol. Current exposure is less clear and appears to be unlikely in the United States. One striking finding is the identification of the metabolite THPI in human serum. She wondered whether this residue is derived from dietary exposure to captan rather than captafol since captafol is now banned in the United States. The data cited for the animal studies clearly indicate that captafol is a multi-site carcinogen in several strains of both rats and mice.

The mechanistic data presented in the profile could be clearer and more focused. Although a number of potential mechanisms are briefly presented, some center on the alteration of the side chain and some on the formation of THPI, and it is unclear which of these mechanisms is the most relevant to captafol's carcinogenicity. If THPI is the common active metabolite of captafol, captan, and other phthilimide fungicides, it might be useful to list these fungicides in a single profile as a class or to discuss why they should not be grouped.

The profile supports a designation of *reasonably anticipated to be a human carcinogen* for all three pesticides, based on the cancer data and the mechanistic information.

Dr. Howard said captafol has not been detected in the United States on locally grown or imported foods since 1998.

Dr. Michael Pino suggested that the definitions of neoplasms and lymphosarcoma be deleted as they have not been included in the other profiles and are not accurate.

E. Riddelliine

a. Presentation

Dr. Gloria Jahnke, NIEHS, presented the scientific information supporting the preliminary listing recommendation for riddelliine in the 12th RoC as *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity in experimental animals and supporting mechanistic data. She described the use and exposure of U.S. populations, cancer studies in experimental animals, and mechanistic evidence.

Riddelliine is a pyrrolizidine alkaloid produced by five *Senecio* genera in the United States. There is potential for significant oral exposure based on its inclusion in herbal products and its contamination of grains, flours, and honey.

No studies were identified that indicate a relationship between human cancer and exposure to riddelliine.

There is sufficient evidence of carcinogenicity of riddelliine, administered by gavage in NTP studies, based upon an increased incidence of tumors in both sexes of rats and mice at multiple sites. In chronic gavage studies, hemangiosarcomas, mononuclear cell leukemia, and hepatocellular adenomas were found in male and female rats and hemangiosarcomas and lung tumors were found in male and female mice, respectively.

Cytochrome P450 enzymes CYP3A and CYP2B6 metabolize riddelliine to dehydroriddelliine, which undergoes hydrolysis to R/S dihydropyrrolizine (R-DHP, S-DHP), and forms various DNA adducts. Both human and rodent microsomes metabolize riddelliine to R-DHP and S-DHP. Dermal application or injection of R-DHP results in malignant skin tumors in female mice and rhabdomyosarcomas in male mice, respectively. Intraperitoneal injection of S-DHP results in tumors at multiple sites in male rats.

Riddelliine, R-DHP, and S-DHP are genotoxic in bacteria and a dose-dependent formation of DHP adducts was identified *in vivo* in the livers of rats exposed to riddelliine. In the liver, a higher, more persistent level of adducts was found in endothelial cells compared to parenchyma cells, and vascular endothelial growth factor (VEGF), an endothelial cell mitogen, increased in these cells. There was a higher frequency of G:C to T:A mutations in exposed endothelial cells. These transversions have been identified in the *K-ras* gene in riddelliine-induced hemangiosarcomas in mice.

b. BSC Discussion

Ms. Rudel asked whether comfrey contains pyrrolizidine alkaloids and their similarity to the alkaloids found in *Senecio spp.* Dr. Bucher replied that comfrey contains pyrrolizidine compounds that are metabolized through a pathway similar to that of riddelliine. He added that comfrey and other plants containing pyrrolizidine alkaloids that are metabolized through this same pathway have been nominated for possible inclusion in a future RoC.

Dr. Howard added that once it was known that riddelliine is metabolized through this pathway, the FDA took action against comfrey and other herbs containing pyrrolizidine alkaloids. He suggested that the scientific and common names of plants that contain riddelliine be listed in the profile.

Dr. Sherley asked if riddelliine and other pyrrolizidine alkaloids cause cancer in the areas where the plants grow and Dr. Jahnke said that information is not available. She added that these compounds are very toxic, thus animals and humans often die from acute exposure.

Dr. Bunton, the first BSC discussant, said the information in the draft substance profile is presented clearly. The profile describes the distribution of riddelliine in the environment, and the potential sources of human exposure via contamination of foodstuffs. *Senecio spp.* are used in herbal medicines and teas, and instances of accidental poisonings of infants in the United States have been reported as well as cases of poisoning in other countries from exposure to grains and flours contaminated by *Senecio* plants. In addition, pyrrolizidine alkaloids have been detected in eggs, honey, and milk indicating they can be transmitted through the food chain.

The evidence for genotoxicity of riddelliine is stated clearly, and IARC concluded that riddelliine is carcinogenic in rats and mice based on findings in NTP bioassays.

She supported the listing of riddelliine as *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity in experimental animals and supporting mechanistic data.

Dr. Raymond Novak, the second BSC discussant, said the profile is clear and appears to be technically accurate. It describes in detail the sources and routes of exposure and absorption of riddelliine, its metabolism to toxic/genotoxic and carcinogenic metabolites, its target organ(s), and the molecular /cellular events that precede organ damage, organ failure, and rodent tumorigenesis.

Sufficient information is provided to document the past exposure and potential current and future human exposure to riddelliine. One area for clarification and reference in the profile would be to provide a table containing all the plant genera and species that contain riddelliine and their common names. The potential for

cumulative effects resulting from low-level, chronic exposure should be emphasized in the profile.

Based on the animal and mechanistic data presented in the draft profile, he agreed that the NTP provides a compelling argument for listing riddelliine as being *reasonably anticipated to be a human carcinogen*.

Dr. Novak suggested that the profile provide some discussion of inflammation and oxidative stress as causes of carcinogenicity although dissecting out the pathways would be difficult. He also asked whether the information on herbals is easily accessible as it would be an important resource for physicians. Dr. Howard replied that there are websites on dietary substances that thoroughly catalogue herbals and their contraindication with other drugs.

Dr. Sherley suggested that the NTP might consider investigating whether humans develop cancer from chronic riddelliine intake in herbal teas.

VI. Conclusion

Dr. Bucher thanked the BSC for their efforts toward a successful peer review of the draft substance profiles. He reiterated that this is the first time the profiles for the RoC were reviewed in public forum and invited feedback from the BSC on the process. Dr. Birnbaum echoed Dr. Bucher's thanks and acknowledged the large amount of material they had to review in preparation for this meeting.

VII. Adjournment

The meeting concluded at 4:45 p.m.