SUMMARY MINUTES

NATIONAL TOXICOLOGY PROGRAM

BOARD OF SCIENTIFIC COUNSELORS'

BIENNIAL REPORT ON CARCINOGENS SUBCOMMITTEE MEETING

November 18-19, 1996

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III. Review of Revisions to the Introduction of the 8th *Biennial Report on Carcinogens* (BRC) and Changes to the Structure of the Report

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The National Toxicology Program (NTP) Board of Scientific Counselors' Biennial Report on Carcinogens Subcommittee (the Subcommittee) held its second meeting on November 18 and 19, 1996, at the National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina. (Attachment 1: *Federal Register* meeting announcement; Attachment 2: Agenda and Roster of Members and Expert Consultants.) Members of the Subcommittee are Drs. Arnold Brown (Chairperson), Eula Bingham, Thomas Goldsworthy, Carol Henry, David Hoel, Robert LeBoeuf, Franklin Mirer, Louise Ryan, Frederick Tyson, and Jerrold Ward. Expert Consultant to the Subcommittee is Dr. Hiroshi Yamasaki. Drs. Bingham, Goldsworthy, Hoel, and Ward were unable to attend; however, Dr. Hoel was able to provide written reviews which were read into the record. Additionally, for this meeting the Subcommittee was supplemented by the participation of three *ad hoc* Expert Consultants: Drs. Stephen Belinsky, Lovelace Biomedical and Environmental Research Institute; Clay Frederick, Rohm and Haas Company; and Kim Hooper, California State Department of Health Services.

I. Introduction and Background: Dr. George Lucier, Director, Environmental Toxicology Program (ETP), noted that the public review of the process for preparation of the *Biennial Report on Carcinogens* (BRC*)* to broaden input to its preparation, broaden the scope of scientific review associated with the report, and provide review of the criteria used for inclusion of substances in the BRC, had commenced with initial review by an *ad hoc* committee of the Board in April 1995 and concluded recently with approval of the revised criteria by the Secretary, DHHS. Dr. Lucier commented that the major change in the process was to establish an external review group, the BRC Subcommittee. With regard to the criteria review, he said there had been the opportunity for multiple public and interagency inputs. There was a consensus that: (1) the current criteria should be revised; (2) all relevant information should be used, especially mechanistic information, (3) the categories (*known to be a human carcinogen* and *reasonably anticipated to be a human carcinogen* should remain the same as described in the original legislation; and (4) there should be a formal mechanism which allows the removal of substances from the BRC.

Dr. Bill Jameson, NIEHS, gave background for the actions required of the Subcommittee and consultants at this meeting. The first action was a review of the 15 chemicals under consideration for listing or upgrading in the 8th BRC. The substances had originally been reviewed under the former process with the former criteria for inclusion in the 8th and 9th *Annual Reports on Carcinogens*. Final action on these nominations were put on hold during the review of the BRC criteria, and then reevaluated using the approved revised criteria by the NIEHS BRC Review Group (RG1) and subsequently by the NTP Executive Committeem Working Group for the BRC (RG2). Dr. Jameson briefly described the actions of the two review groups noting the initial development of a draft summary document and draft BRC citation for each chemical which was revised for each chemical based on the recommendations made by RG1 and RG2. This resulted in a single summary statement for 14 of the 15 chemicals. For one chemical, Disperse Blue 1, there was a divergence of opinion between the two groups so for this chemical, the Subcommittee was provided two summary citations. Dr. Jameson said that the supporting documents and references for each nominated chemical were available in the room during the meeting and that representatives of both RG1 and RG2 were available to answer questions.

The Chairman then outlined how the reviews would be conducted during the meeting. Dr. Brown stated that the reviews would be carried out in the following manner. The discussion of each chemical would be led by the primary reviewer who is asked to summarize his/her evaluation of the nomination and what recommendation they would make concerning the chemical under consideration. In most cases, this should be no more than a 10 minute discussion. The secondary reviewer would then be asked to comment on the nomination, emphasizing areas of agreement and contrast with the primary reviewer. This would be followed by any public comment concerning the nominated chemical. The Subcommittee would then discuss the nomination and a vote would be taken on the Subcommittee's recommendation to be forwarded to the NTP.

II. Peer Review of Substances Nominated for Listing in the 8th *Biennial Report on Carcinogens*:

Cyclosporin A -- Cyclosporin A was proposed to be listed as *known to be a human carcinogen* based on studies in humans that indicate a causal relationship between exposure to cyclosporin A and human cancer.

Dr. Hoel, the primary reviewer, was unable to attend the meeting but had submitted his review, which Dr. Hart, NIEHS, read into the record. Dr. Hoel agreed with the recommendation for listing cyclosporin A (CsA) as *known to be a human carcinogen*, based on the position of the International Agency for Research on Cancer (IARC) (Monograph # 50) and on his interpretation of a cohort study by Starzl and coworkers showing substantial increases in lymphomas in organ transplant patients receiving CsA as an immuno-suppressant. Otherwise, he stated that the many other cohort studies cited in the data summary reporting use of immune suppression drugs and subsequent cancers were not sorted or evaluated as to whether they were very relevant to the issue of CsA carcinogenicity.

Dr. Yamasaki, the secondary reviewer, agreed with the proposed listing. He commented that the conclusion regarding possible carcinogenic mechanisms may be supported even more strongly. The conclusion that CsA may act through non-genotoxic mechanisms and probably by immune suppression could be supported from human studies: (I) lymphomas only after a short time following initiation of treatment, as short as one month (cohort studies) or three months (case reports), and (2) tumors regress after discontinuation of treatment. Dr. Yamasaki suggested that additional information from human studies should be added to the front section (BRC-1) of the summary document, and as well information on carcinogenicity data in monkeys. Reference to a rat study in which no control data were available should be deleted.

In other discussion, Dr. Ryan opined that the use of other drugs beside CsA in many of the cohort studies were, to her, confounding factors suggesting limited evidence of carcinogenicity in humans. Dr. Yamasaki emphasized that there were several studies with CsA alone noting, as did Dr. Hoel, that it was not made clear in the summary document which studies used CsA alone and which included other drugs He cited a study by Vilardell which included both groups treated with CsA alone and CsA along with other drugs. Drs. Ryan and Hooper agreed that a summary table displaying epidemiological data would be helpful in the support document. Dr. Mirer commented that in the BRC reviews, one could not expect a depth of review especially with regard to primary journal articles that the IARC monograph meeting format allows.

Dr. Henry moved that the nomination of cyclosporin A for listing in the BRC as *known to be a human carcinogen* be accepted. Dr. LeBoeuf seconded the motion, which was accepted unanimously with five votes.

Thiotepa -- Thiotepa was proposed to be listed as *known to be a human carcinogen* based on studies in humans which indicate a causal relationship between exposure to thiotepa and human cancer.

Dr. Hoel, the primary reviewer, was unable to attend the meeting but had submitted his review, which Dr. Hart, NIEHS, read into the record. Dr. Hoel did not agree with the recommended listing contending that IARC Monograph # 50, which provides the basis for the recommendation, probably overstates the human data. He stated that case-reports as cited may be suggestive but are not useful in causality arguments, leaving one case-control study by Kaldor et. al. Dr. Hoel said that clearly, a dose response was lacking for these data, and therefore, causality was not established in spite of the IARC conclusions. Thus, he would support *reasonably anticipated to be a human carcinogen.*

Dr. Yamasaki, the secondary reviewer, agreed with the proposed listing as a *known to be a human carcinogen*. He said that there were many more case-reports than were cited in the summary document. Further, mammalian carcinogenicity data were supportive in that neoplasia occurred in multiple sites in both sexes of rats and mice. Also, thiotepa was shown to be a potent mutagen *in vivo* and *in vitro*.

In other discussion, Dr. Ryan questioned the statistical significance of the case-reports which would be needed to establish causality and said she would like to see more detailed information for the one case-control study. Dr. Hooper asked whether there were unusual tumors cited in the case-reports. Dr. Jameson said that acute lymphocytic leukemia was the only neoplasm reported. Dr. Mirer again noted that the IARC conclusions were based on a more in-depth review with access to primary literature sources. Dr. Brown stated that the problem is when case reports like this come out, a case control study is very difficult to do because people stop using the drug. Dr. Hooper urged that in future summary documents there be more shaping of some of the data, e.g., mutagenicity data, human blood data. Dr. LeBoeuf said it was his understanding that IARC might upgrade a chemical where there was limited evidence of carcinogenicity if there were strong animal data and mechanistic information.

Dr. Mirer moved that the nomination of thiotepa for listing in the BRC as *known to be a human carcinogen* be accepted. The motion was tabled for lack of a second. Dr. Ryan moved that thiotepa be accepted for listing in the BRC as *reasonably anticipated to be a human carcinogen*. Dr. Henry seconded Dr. Ryan's motion. In discussion, Dr. Henry opined that this uncertainty leading to the alternative motion reflected on the summary data received as well as the lack of time by members to delve into primary references. Dr. Hooper noted that there was a third option given under Recommendation on the review form, and that was to defer listing. Dr. Mirer said that would leave thiotepa unlisted and thought that to be undesirable. Dr. Brown reminded the Subcommittee that their recommendations to the Director, NTP, were only advisory. The Director would make the decision on listing. The motion that thiotepa be listed as *reasonably anticipated to be a human carcinogen* was accepted by four yes votes to one no vote (Mirer). Dr. Mirer said he voted no because he believed it should be listed as *known to be a human carcinogen*.

Reconsideration: Later in the meeting, a motion was made by Dr. Henry and seconded by Dr. LeBoeuf to bring thiotepa back for reconsideration. The motion was accepted unanimously with five votes. Dr. Henry then moved that reconsideration be given to determining whether the listing for the BRC of thiotepa should be changed from *reasonably anticipated to be a human carcinogen* to *known to be a human carcinogen*. Dr. LeBoeuf seconded the motion, which was accepted by three yes votes to one no vote (Ryan) with one abstention (Tyson). Dr. Ryan said she considered the previously agreed on listing to be correct.

In discussion, Dr. LeBoeuf asked whether there were additional case reports that had not been discussed during the previous conversations. Dr. Bucher replied that there were eight additional case reports beyond the seven cited in the document but each of these tended to be weaker, so the one case-control study cited should be given the most weight. He also commented on Dr. Hoel's assertion that lack of dose-response in the case-control study detracted from the causality argument He said it was not unusual in studies of chemotherapeutic agents for there to be atypical dose-response relationships in secondary tumors. Dr. Ryan said she remained unconvinced by the available epidemiological data. Dr. Yamasaki said that over and above the available evidence each reviewer's expertise must be factored in. Dr. Mirer commented that IARC in its analysis of all the data including the case reports undoubtedly examined the data in the case reports much more intensively in making their determination.

Dr. Henry moved that the listing for thiotepa be changed to *known to be a human carcinogen*. Dr. Mirer seconded the motion, which was accepted with four yes votes to one no vote (Ryan). Dr. Ryan said that the change in listing was not decided on the scientific evidence available to the Subcommittee.

Azacitidine -- Azacitidine (5-AzaC) was proposed to be listed as *reasonably anticipated to be a human carcinogen* based on evidenced of malignant tumor formation at multiple tissue sites in multiple species of experimental animals.

Dr. Hoel, the primary reviewer, was unable to attend the meeting but had submitted his review, which Dr. Hart, NIEHS, read into the record. Dr. Hoel agreed with the proposed listing. He observed that it was interesting that a

cytostatic drug used for leukemia treatment in humans was found to accelerate the development of leukemias in mice.

Dr. Belinsky, the secondary reviewer, agreed with the proposed listing. He said that the carcinogenicity studies cited do provide evidence that 5-AzaC is carcinogenic; however, it is difficult to assess potency and by what mechanism this compound is acting. Dr. Belinsky commented that it is clear from the genotoxicity studies that 5-AzaC damages DNA either through direct or indirect mechanisms. However, the mechanistic information provided and emphasized in the summary statement implies that the major mechanism involved in carcinogenesis is via inhibition of DNA methylation rather than leading to genotoxicity. Dr. Belinsky stated that this section needs to be modified to reflect alternative mechanisms, at least providing equal weight for hypomethylation causing effects on chromatin structure. Further, the strong promoting characteristics of 5-AzaC and potential importance of this property in carcinogenicity observed in the animal model systems should be emphasized.

Dr. LeBoeuf moved that the nomination of azacitidine for listing in the BRC as *reasonably anticipated to be a human carcinogen* be accepted. The motion was seconded and accepted unanimously with five votes.

***p*-Chloro-*o*-toluidine and its hydrochloride salt** -- *p*-Chloro-*o*-toluidine and its hydrochloride salt (PCOT) were proposed to be listed as *reasonably anticipated to be human carcinogens* based on limited evidence of carcinogenicity from studies in humans and evidence of malignant tumor formation in experimental animals.

Dr. Mirer, the primary reviewer, believed that the human and animal studies reported supported a listing of *known to be a human carcinogen*. He cited two industrial studies finding an excess of bladder cancers among workers exposed to PCOT. Using the Levels of Evidence employed in interpreting NTP bioassays, he opined that both studies provided "clear evidence of carcinogenic activity". Dr. Mirer stated that three studies in mice provided clear evidence for carcinogenicity in mice although the studies were not up to current NTP standards, while the two rat studies reported were probably inadequate. He said that references to mechanisms for species differences should be modified or deleted from the summary.

Dr. Tyson, the secondary reviewer, agreed with the proposed listing of *reasonably anticipated to be a human carcinogen*. He agreed that the excess bladder cancers found in the exposed workers suggested PCOT might be a human carcinogen. He noted there was evidence of genotoxicity but mechanistic data was lacking. In particular, Dr. Tyson believed that an analysis of oncogene mutational spectra or aberrant expression patterns would have been useful to determine if PCOT induction of solid tumors had an oncogene component.

Dr. Mirer moved that the proposed listing be changed to *known to be a human carcinogen*. There being no second, the motion was tabled. Dr. Tyson then moved that the proposed listing *reasonably anticipated to be a human carcinogen* be accepted. Dr. LeBoeuf seconded the motion. Dr. Ryan asked Dr. Mirer to further discuss the human studies. Dr. Mirer cited the excess bladder cancers in workers at American Hoechst Corporation and a cohort of workers exposed in a German chemical plant. Dr. LeBoeuf thought the exposure of workers to other chemicals in these studies was a confounding factor. Dr. Mirer added that carcinogenic amines were more often associated with mouse liver tumors and much less frequently with urinary tract tumors in rodents. Dr. Tyson's motion supporting the proposed listing was accepted by four yes votes to one no vote (Mirer). Dr. Mirer voted no because he believed the epidemiological data supported a listing of *known to be a human carcinogen*.

Chlorozotocin -- Chlorozotocin is proposed to be listed as *reasonably anticipated to be a human carcinogen* based on evidence from studies in experimental animals, and because it is a member of a well defined, structurally-related class of substances listed in a previous *Annual Report on Carcinogens* as either *known to be human carcinogens* , or *reasonably anticipated to be human carcinogens.*

Dr. Ryan, the primary reviewer, agreed with the proposed listing. She said that although the evidence of carcinogenicity in animals is reasonably convincing, the conclusion is based on a relatively small number of studies. Given that the animal data were suggestive but not particularly strong, she stated that this made the structure-function information on other nitrosoureas more important to the proposed listing, and thought that a statement to that effect might be added in the summary statement. More detail on findings with these structurally-related alkylating agents would be helpful.

Dr. Henry, the secondary reviewer, agreed with the proposed listing. Her two areas of potential concern for scientific criticism of the database were that (1) only one species (rats) had been evaluated for long-term exposure, and (2) no apparent evaluation had been made of any dose-response. Further, the most important animal study had been by the intraperitoneal route while human studies as an investigational cytostatic agent were by the intravenous route. She

said these issues were of lesser importance than the observation that chlorozotocin was a member of a well-defined structurally-related class of agents listed in previous Annual Reports.

Dr. Ryan moved that the nomination of chlorozotocin for listing in the BRC as *reasonably anticipated to be a human carcinogen* be accepted. Dr. Henry seconded the motion. In discussion, Dr. LeBoeuf commented on the consistency of the genotoxicity data across species and test systems. The motion was accepted unanimously with five votes.

Furan -- Furan is proposed to be listed as *reasonably anticipated to be a human carcinogen* based on evidence of malignant tumor formation at multiple tissue sites in multiple species of experimental animals.

Dr. Ryan, the primary reviewer, agreed with the proposed listing. She thought the document presented information on animal studies, including those by the NTP, that clearly made the case for the carcinogenicity of furan in animals. Dr. Ryan stated that the section on modes of action to be particularly important under the new listing/delisting criteria. However, she would also like to see a subsection that clearly discussed the implications for human carcinogenicity of mechanistic findings in animals.

Dr. Tyson, the secondary reviewer, agreed with the proposed listing. He found the animal data compelling but thought that use of a strain of mice other than $B6C3F_1$ that does not have such a high incidence of spontaneous H-*ras* activated liver tumors might have more clearly addressed the potential for furan to induce H-*ras* activated liver tumors.

In discussion, Dr. Mirer said there was inconsistency in the document with regard to the weak and variable genotoxicity results cited and the considerable importance given to genotoxicity. Dr. Henry expressed concerns about the section on human exposure in the lack of information on production volumes, lack of data on human exposure for such an important commercial chemical. Dr. LeBoeuf commented that furan was consistently clastogenic including *in vivo* in bone marrow and there should be more discussion of mechanisms including the role of cell proliferation.

Dr. Ryan moved that the proposed listing of furan as *reasonably anticipated to be a human carcinogen* be accepted. Dr. Tyson seconded the motion, which was accepted unanimously with five votes.

NITROARENES

Five of the chemicals nominated for listing in the BRC as *reasonably anticipated to be a human carcinogen* were nitroarenes, which comprise a large class of structurally-related chemicals. The nitroarenes are 1-nitropyrene(1-NP), 4-nitropyrene (4-NP), 1,6-dinitropyrene (1,6-DNP), 1,8-dinitropyrene (1,8-DNP), and 6-nitrochrysene (6-NC). Dr. LeBoeuf, as primary reviewer for all five, chose to address these chemicals as a class before then reviewing each individual nomination.

Dr. LeBoeuf discussed the cited toxicology data moving from *in vitro* assays such as *Salmonella* across assays with increasing relevance to humans. He reported that all were mutagenic in *Salmonella,* with 1-NP considerably weaker than the others, most were positive in cell transformation assays including human cells, formed DNA-adducts, and all formed mutagenic metabolites via a nitronium ion. With regard to neoplasia, all showed evidence of inducing neoplasia both at the site of administration (usually by injection) and at distant sites. However, the major route of human exposure is inhalation, and there are limited animal studies by this route with some confounded by poor survival and other factors. That adducts have been detected in rodent lung following intratracheal instillation of 1-NP supports there being *in vivo* genotoxic activity in a likely target organ in humans. Dr. LeBoeuf stated that the NTP might more carefully evaluate the findings from the intratracheal/inhalation studies. He thought that the potential for neoplastic effects in humans could not be ruled out. Dr. Brown said that discussion of an NTP summary statement for the five nitroarenes would be held following review of the individual nitroarenes.

1-Nitropyrene -- 1-Nitropyrene (1-NP) is proposed to be listed as *reasonably anticipated to be a human carcinogen* based on evidence of malignant tumor formation in multiple species of experimental animals, at multiple sites and by multiple routes of exposure.

Dr. LeBoeuf, the primary reviewer, agreed with the proposed listing.

Dr. Belinsky, the secondary reviewer, deferred his recommendation regarding the proposed listing until after the discussion. He expressed concern about the purity of the test material particularly in the short-term and *in vitro* studies as contaminants such as other of the nitroarenes could affect results obtained. Dr. Belinsky reviewed the major carcinogenicity studies in animals, concluded that 1-NP is a weak carcinogen, and thought that the studies did not

provide compelling evidence that 1-NP would cause tumors in man. He also noted that the metabolic activation of 1-NP leading to DNA adducts occurs largely via nitroreduction, and clearly there is very little nitroreductase activity in the lung. Thus, the critical question that arises is whether the anaerobic nitroreductase activity will be active in man at levels needed to produce DNA adducts.

In discussion by the Subcommittee, Dr. Mirer stated that using the revised criteria, we would have to list 1-NP because it is a genotoxic chemical which is positive in multiple species by multiple routes. Dr. Frederick commented that the route of administration becomes less of an issue when the chemical induces tumors at many sites other than the administration site. Dr. Yamasaki said that it is very difficult to transform human cells in culture, thus positive effects with 1-NP would be strong evidence of a potential for being a human carcinogen.

Dr. LeBoeuf moved that the proposed listing of 1-nitropyrene as *reasonably anticipated to be a human carcinogen* be accepted. Dr. Mirer seconded the motion, which was accepted unanimously with five votes.

4-Nitropyrene -- 4-Nitropyrene (4-NP) is proposed to be listed as *reasonably anticipated to be a human carcinogen* based on evidence of malignant tumor formation at multiple tissue sites in multiple species of experimental animals.

Dr. LeBoeuf, the primary reviewer, agreed with the proposed listing. He said the only mutagenicity data were in *Salmonella* and there were no studies by the relevant route of human exposure, i.e., inhalation. However, the structural relation to other nitroarenes and the multiple sites of tumors in rats and mice after subcutaneous or intraperitoneal injections provided sufficient evidence for the proposed listing.

Dr. Frederick, the secondary reviewer, agreed with the proposed listing, and thought the summary statement highlighted the evidence supporting the listing.

Dr. LeBoeuf moved that the proposed listing of 4-nitropyrene as *reasonably anticipated to be a human carcinogen* be accepted. Dr. Mirer seconded the motion, which was accepted unanimously with five votes.

1,6-Dinitropyrene -- 1,6-Dinitropyrene is proposed to be listed as *reasonably anticipated to be a human carcinogen* based on evidence of malignant tumor formation in multiple species of experimental animals, at multiple sites and by multiple routes of exposure.

Dr. LeBoeuf, the primary reviewer, agreed with the proposed listing. He said that in terms of carcinogenicity by the most relevant route, the evidence was the strongest among the five nitroarenes proposed for listing. In particular, he said that a study by Takayama *et. al.,* was important as it represented a relevant route of human exposure and the incidences of myeloid leukemia and lung adenocarcinomas were significantly increased in male and female hamsters. The analytical purity was 99.6 %.

Dr. Mirer, the secondary reviewer, agreed with the proposed listing. He noted that the data presented did not include a standardized bioassay at a Maximum Tolerated Dose (MTD). Thus, the full range of tumor types and organ locations has not been explored, nor has low-dose toxicity been evaluated.

Dr. LeBoeuf moved that the proposed listing of 1,6-dinitropyrene as *reasonably anticipated to be a human carcinogen* be accepted. Dr. Mirer seconded the motion, which was accepted unanimously with five votes.

1,8-Dinitropyrene -- 1,6-Dinitropyrene is proposed for listing as *reasonably anticipated to be a human carcinogen* based on evidence of malignant tumor formation in multiple species of experimental animals, at multiple sites and by multiple routes of exposure.

Dr. LeBoeuf, the primary reviewer, agreed with the proposed listing. He said the genotoxicity profile in a wide variety of assays was very similar to that for 1,6-dinitropyrene, and there was evidence of cell transformation activity. Although carcinogenic activity by a route of exposure relevant to humans was not demonstrated, the structural similarities to 1,6-dinitropyrene, consistent evidence of genotoxicity, cell transforming abilities, and similar metabolic pathways make it likely that carcinogenic activity via the inhalation route would be observed.

Dr. Ryan, the secondary reviewer, agreed with the proposed listing. She said she would like to see a subsection in the summary statement that clearly discusses the implications for human carcinogenicity of mechanistic findings in animals.

Dr. LeBoeuf moved that the proposed listing of 1,8-dinitropyrene as *reasonably anticipated to be a human carcinogen* be accepted. Dr. Ryan seconded the motion, which was accepted unanimously with five votes.

6-Nitrochrysene -- 6-Nitrochrysene is proposed for listing as *reasonably anticipated to be a human carcinogen* based on evidence of malignant tumor formation at multiple tissue sites in multiple species of experimental animals.

Dr. LeBoeuf, the primary reviewer, agreed with the proposed listing. He commented that although there are no data indicating carcinogenicity via the likely route of human exposure, the lung is a target organ via the intraperitoneal route of exposure. Dr. LeBoeuf said that evidence for DNA adducts in tumor target tissue supports further the possibility that tumors induced by this chemical are at least in part a result of chemical-induced DNA damage.

Dr. Tyson, the secondary reviewer, agreed with the proposed listing.

Dr. Tyson moved that the proposed listing of 6-nitrochrysene as *reasonably anticipated to be a human carcinogen* be accepted. Dr. LeBoeuf seconded the motion, which was accepted unanimously with five votes.

Proposed Summary Statement for the Five Nitroarenes in the 8th BRC

Dr. Jameson read the proposed summary statement:

The nitroarenes comprise a large class of structurally-related chemicals and encompasses several carcinogens including 1-nitropyrene, 4-nitropyrene, 1,6-dinitropyrene, 1,8-dinitropyrene, and 6-nitrochrysene. These meet the criteria established for listing as *reasonably anticipated to be a human carcinogen* primarily on the basis of carcinogenicity results with experimental animals. Although these chemicals have limited intentional uses, they are normally found in particulate emissions from many combustion sources, most notably, diesel exhausts. The mutagenicity and carcinogenicity of nitroarenes vary, but those that are positive appear to be active at relatively low concentrations when compared to other classes of chemicals also positive in experimental systems.

Few of this large class of chemicals have been rigorously evaluated in "state of the art" rodent cancer studies. Typically, the chemicals were administered by injection, over short periods of time, and with less than optimal time allowed for tumors to fully develop. Despite these factors, the experimental carcinogenesis results are generally similar and demonstrate tumor formation at sites away from as well as at the site of injection. The chemicals also show genotoxic activity in a variety of *in vitro* and *in vivo* assays, and metabolic pathways for the creation of reaction products with the ability to cause gene mutations or changes in the structure of DNA have been described in tissues from animals as well as humans.

There are no adequate studies on which to judge the human carcinogenicity of these chemicals. However, diesel engine exhausts have been evaluated for carcinogenic potential by the IARC (1989). The IARC Working Group of Experts concluded that there was "limited evidence for the carcinogenicity of diesel exhaust to humans" and "sufficient evidence for carcinogenicity to experimental animals". There is also "sufficient evidence of carcinogenicity" to experimental animals of extracts of diesel exhaust which contain the nitroarene fraction. Whether the nitroarenes are responsible for, or contribute to the human carcinogenicity of diesel exhaust has not been determined.

In discussion about the summary statement, Dr. LeBoeuf proposed an addition to line 5 of the first paragraph as follows (addition bold faced): "These meet the criteria established for listing as *reasonably anticipated to be a human carcinogen* primarily on the basis of carcinogenicity results with experimental animals **but also on the basis that similar plausible mechanisms of nitroarene-induced cancer in experimental animals would also occur in humans.**" Dr. LeBoeuf proposed an addition to the first sentence of the second paragraph as follows: "Few of this large class of chemicals **and none of the nitroarenes reviewed for inclusion in the BRC to date** have been rigorously evaluated in 'state of the art' rodent cancer studies. Dr. Brown agreed to these changes. Next, Dr. LeBoeuf proposed that a statement be added after the second sentence of the second paragraph: "**Furthermore, the bioassay results generated for the nitroarenes reviewed for inclusion in the BRC are not adequate for assessing quantitatively the likely carcinogenic risk to humans from exposure to these chemicals.**" Dr. Brown stated that this sentence fell into the area of risk assessment and therefore, he thought it inappropriate for the BRC. Dr. Lucier agreed but said instead that a statement to the effect that the available data do not provide for establishing dose-response relationships. Dr. LeBoeuf was agreeable to this. He suggested that the last sentence in paragraph two pertaining to genotoxicity be strengthened, as follows: "The chemicals also show genotoxic activity in a variety of *in vitro* and *in vivo* assays; **they have shown the ability to transform both finite life span and immortalized cell lines (including human cells) *in vitro***

to preneoplastic or neoplastic phenotypes and have the ability to react with DNA both *in vitro* and *in vivo*. Evidence for chemical-DNA adduction has been observed in some target tissues for tumor induction with the nitroarenes." This addition was agreed to. Finally, Dr. LeBoeuf proposed that a qualifying word be added to the last sentence of paragraph three: "Whether the nitroarenes are responsible for, or contribute to the **probable** human carcinogenicity of diesel exhaust has not been determined." There was discussion suggesting that the appropriate qualifier be left to the NTP, and some other minor changes were suggested.

Dr. Henry moved that the proposed summary statement for nitroarenes be accepted with agreed to changes and additions recommended by Dr. LeBoeuf and other reviewers. Dr. Mirer seconded the motion, which was accepted unanimously with five votes.

Danthron -- Danthron (1,8-dihydroxyanthraquinone) is proposed for listing as *reasonably anticipated to be a human carcinogen* based on evidence in experimental animals. When administered in the diet to male rats, danthron induced adenomas and adenocarcinomas of the colon and adenomas of the cecum. When administered in the diet to male mice, danthron caused an increase in the incidence of hepatocellular carcinomas.

Dr. Henry, the primary reviewer, agreed with the proposed listing. She commented that the animal data were somewhat limited by there being only one dose and sex. The human exposure information seemed weak, and although the chemical has been banned by the FDA, she wondered if there were concerns about previously exposed individuals. Dr. Henry said the single case report was so extreme and unusual and does not convey the concern for use of the chemical with pregnant women and the issue of transplacental transfer which may be far more significant.

Dr. Hooper, the secondary reviewer, agreed with the proposed listing. He said that if a comparison of initiation/promotion studies done with those cited for 7,12-dimethylbenz[a]anthracene or dimethylhydrazine then route of exposure needs to be included. Dr. Hooper said the highly mutagenic activity in the *Salmonella* assay should be mentioned.

Dr. Henry moved that the proposed listing of danthron as *reasonably anticipated to be a human carcinogen* be accepted. Dr. Mirer seconded the motion, which was accepted with four yes votes and one abstention (LeBoeuf). Dr. LeBoeuf abstained based on his company's commercial interest in laxative products.

Disperse Blue 1 -- Disperse Blue 1 (1,4,5,8-tetraaminoanthraquinone) was evaluated by the NIEHS BRC Review Committee (RG1) with the majority opinion being that Disperse Blue 1 should not be listed in the BRC as *reasonably anticipated to be a human carcinogen,* because (1) the carcinogenic effect was limited to a single species and site, (2) carcinogenicity occurred only at the higher doses, where it was often associated with the formation of urinary bladder stones, and (3) there was uncertainty regarding whether the mechanism of carcinogenesis is relevant to humans.

Subsequently, the NTP Executive Committee BRC Working Group (RG2) proposed that Disperse Blue 1 be listed in the BRC as *reasonably anticipated to be a human carcinogen* based on evidence of malignant tumor formation in experimental animals to an unusual degree with regard to incidence, site, and type of tumor, and because it is a member of a well defined, structurally related class of substances listed in a previous *Annual Report on Carcinogens* as either *known to be human carcinogens,* or *reasonably anticipated to be human carcinogens.*

Dr. Henry, the primary reviewer, noted the disagreement between the two previous review groups (RG1 and RG2) and the reasons for their recommendations. She listed the three types of bladder tumors observed in rats (transitional cell neoplasms, leiomyomas or leiomyosarcomas, and squamous cell neoplasms), but only at high doses, and commented on the strong association between the formation of calculi and the formation of tumors in rats. She said that Disperse Blue 1 had not been tested extensively for genotoxicity, but was directly mutagenic in *Salmonella* and mouse lymphoma cells, while inducing SCE and chromosomal aberrations in Chinese hamster ovary cells. Dr. Henry said that if consideration must be given to significant numbers of people exposed, then more information on exposure and genotoxicity or other information about mode of action would be important. Dr. Henry stated that she agreed with the RG1 recommendation not to list Disperse Blue 1 in the BRC.

Dr. Hooper, the secondary reviewer, said Disperse Blue 1 (DB1) causes urinary bladder tumors and calculi, so the issue is whether there is a causal relationship between the calculi and the tumors. He also reviewed the genotoxicity data and the neoplasia data in considerable detail. He noted that urinary bladder epithelial (squamous cell and transitional cell) tumors in male and female rats are rare, and urinary bladder mesenchymal (leiomyoma/leiomyosarcoma) tumors are very rare (almost never seen). Dr. Hooper pointed out that while DB1 causes dose-related increases in calculi in rats

and mice, it does not cause bladder tumors in mice. He compared the data with that from four other structurally-related aminoanthraquinones tested by NTP in rodent bioassays. Of these, two produced urinary bladder neoplasms in rats but none produced calculi. Dr. Hooper discussed the lack of concordance between tumors and calculi, i.e., there were a significant number of tumor-bearing animals that did not have calculi, and suggested modifying some of the statements about the association between tumors and calculi. He acknowledged that some calculi might be lost in the pathology work-up but said there was no evidence for this. Dr. Hooper concluded that barring more convincing data that calculi cause urinary bladder tumors, his preference would be to list Disperse Blue 1 as a chemical that can be *reasonably anticipated to be a human carcinogen.*

Public Comment: Dr. W. E. Dressler, Director of Toxicology and Regulatory Affairs, Clairol, stated that in the NTP bioassay a number of the rats that had bladder tumors but no evidence of calculi, upon further examination demonstrated calculi in the renal pelvis. He commented that unlike Disperse Blue 1, the other two anthraquinones producing bladder tumors in NTP bioassays were multi-sex/species and multi-site carcinogens.

Dr. Frederick reported on a workshop sponsored by International Life Sciences Institute (ILSI) on bladder cancer in which he had been a participant that showed calculi can be excreted in the urine, and urged more diagnostic efficiency in detecting calculi. There ensued a lengthy discussion on bladder tumors. Dr. R. Maronpot, NIEHS, reported that in the NTP bioassay of Disperse Blue 1 there were dose-related hyperplastic and inflammatory changes in the bladder of rats at the end of 90-day studies. Dr. Maronpot pointed out that the difference in prosecters is enough to lose some calculi, but that ~90% of the calculi would be found sincee the prosectors were specifically directed to look for them. Dr. Mirer asked whether there were chemicals that cause stones but not tumors in rats. Dr. Brown observed that current thinking is that stones cause tumors in rats. Dr. Frederick commented that the NTP Technical Report on Disperse Blue 1 reports the presence of pigment granules in the submucosa. It was noted that the granules were likely the dye but positive identification has not been made to confirm this. Dr. J. Swenberg, University of North Carolina, said there was clearly a threshold in the rat, a dose orders of magnitude higher than any realistic human exposure and therefore carcinogenicity of the chemical was not relevant to humans. Dr. Yamasaki said that *p*-cresidine was associated with leiomyosarcomas but not calculi in the rat.

Dr. Henry said she would like additional information to help her decide on a motion; if a request for additional information was included in a motion, what would be the NTP's response. Dr. Bucher responded that a request for more information should be very specific. Dr. Maronpot suggested that the NTP could go back with in-depth retrospective analysis of both 90-day and two-year materials including sectioning and PCNA staining. This review could define if submucosal inflammation is present with cell proliferation. The down side of doing additional work is that the ecology of the bladder at the end of a 2-year study, with tumors, is not the normal situation because one is looking at the end of a process and associations at that stage are difficult to make. Dr. LeBoeuf said this might serve to increase the association among cell proliferation, calculi and tumors. Dr. Lucier said that the NTP could go back and do additional studies; however, this would have to be considered along with other priorities. Dr. Hooper proposed that Disperse Blue 1 be accepted for listing and then depending on development of further information, the chemical could be brought back and proposed for delisting.

Dr. Henry moved that Disperse Blue 1 be accepted for listing as *reasonably anticipated to be a human carcinogen.* Dr. Mirer seconded the motion. Dr. Frederick stated that he could not see it being carcinogenic to humans under any feasible human exposure scenario. Dr. LeBoeuf appealed again that the vote be deferred to allow development of more mechanistic data. The motion was accepted with three yes votes to one no vote (LeBoeuf) and one abstention (Ryan). Dr. Ryan said she still did not have enough information to make a decision one way or the other. Dr. LeBoeuf said that he voted against the motion because he thought the decision on whether to list or not list should have been deferred to provide time to collect additional information/data related to the possible association between calculi/crystals and the induction of leiomas and leiomyosarcomas.

***o*-Nitroanisole** -- *o*-Nitroanisole is proposed to be listed as *reasonably anticipated to be a human carcinogen* based on evidence of malignant tumor formation at multiple sites in multiple species of experimental animals.

Dr. Mirer, the primary reviewer, agreed with the proposed listing. His discussion focused on information given about the NTP chronic bioassay in rats and mice. He said that for mice, there was no effect on survival in either males or females, and , thus, the Maximum Tolerated Dose (MTD) was not exceeded at the highest dose. Tumors were increased at the lowest dose tested in male rats and mice, so a no effect level was not observed at 666 ppm (mice) or 222 ppm (rats) in diet. For rats, increased incidences of leukemias were seen in both sexes.

Dr. Frederick, the secondary reviewer, agreed with the proposed listing. He had examined the NTP Technical Report and thought the leukemias in rats and increased incidences of hepatocellular neoplasms in mid and low dose mice supported listing. Dr. Frederick commented that there was decreased incidence of hepatic tumors in high dose mice suggesting an exceeding of the MTD and decreased survival and body weight gain in rats from stop studies and suggested these data not be part of the discussion. Male and female rats in the stop studies (dosed feed for 27 weeks, followed by control diet for 77 weeks) had shown increased incidences of tumors of the urinary bladder, large intestine, and kidney. There was some discussion by reviewers about altered physiological states such as in the high dose mice where decreased food intake and/or decreased body weight would be confounders for detecting tumor effects.

Dr. Mirer moved that the proposed listing of *o*-nitroanisole as *reasonably anticipated to be a human carcinogen* be accepted. Dr. Ryan seconded the motion, which was accepted unanimously with five votes.

1,2,3-Trichloropropane -- 1,2,3-Trichloropropane is proposed for listing as *reasonably anticipated to be a human carcinogen* based on evidence of malignant tumor formation at multiple sites in multiple species of experimental animals.

Dr. Mirer, the primary reviewer, agreed with the proposed listing. He stated that the animal studies cited were very strong with tumors found in both sexes and species at several sites and even at the low dose. Dr. Mirer found the genotoxicity information difficult to absorb and the strength of the data appeared to vary depending on how they were displayed, in narrative or tabular form. He thought that perhaps some standard genotoxicity conclusory language would be helpful.

Dr. Yamasaki, the secondary reviewer, agreed with the proposed listing. He noted that 1,2,3-trichloropropane was positive in various tests for mutagenicity and related effects, and enhanced Syrian hamster embryo cell transformation. Further, he said that the chemical induced cell proliferation in various organs, many of which were tumor sites, in mice (lung and forestomach), and in rats (kidneys, nasal turbinates, bile ducts, forestomach, pancreas, lungs, and liver.

Dr. J. Swenberg, University of North Carolina, reported that in studies in his laboratory with 1,2,3-trichloropropane, there were striking differences in cell proliferation depending on the route of administration. Specifically, if given in drinking water, there was no increase in cell proliferation vs. a seven-fold increase in animals dosed by corn oil gavage, the route used in NTP studies. He thought that with a weaker carcinogen such route differences would need to be considered.

Dr. Mirer moved that the proposed listing of 1,2,3-trichloropropane as *reasonably anticipated to be a human carcinogen* be accepted. Dr. Tyson seconded the motion, which was accepted unanimously with five votes.

III. Review of Revisions to the Introduction of the 8th *Biennial Report on Carcinogens* (BRC) and Changes to the Structure of the Report: Dr. Jameson said he wanted to walk the Subcommittee through the draft Introduction and point out changes that had been made and ask for advice on some appendices under consideration for inclusion in the 8th BRC. The Introduction was updated to reflect the new formal review procedures for listing or delisting substances and the revised criteria. He noted changes on p. 2 of the draft that (1) update frequency of publication from annual to biennial to reflect change in legislation, (2) emphasize that the Report is a hazard identification and not a risk assessment document, and (3) points out the need for risk/benefit analysis, e.g., with cancer chemotherapeutic agents. Dr. Jameson referred to a section on p.4 - 'Identifying Carcinogens' - which was rewritten to identify sources of information used in considering listing a chemical. On pp. 4-7 under a section titled 'Human and Animal Studies', the former criteria were retained since the chemicals previously listed were listed using these criteria, there was a description of the recent review of the BRC process and criteria, and the revised criteria to be used with the 8th and subsequent BRCs were highlighted. He said the point-by-point discussion of the relationship of the ARC criteria to the IARC criteria which was contained in the 7th ARC Introduction was deleted. On pp. 7-9, under a section titled 'Inclusion of Substances", 15 manufacturing processes, occupations and exposure circumstances classified by IARC as known to be carcinogenic to human were identified. Dr. Jameson said all were based on IARC evaluation and classified as Group 1 - Known Human Carcinogen. He described how such were handled differently in previous reports, sometimes dividing them between an index and the Introduction, and gave a chronology of when each was included in the ARC, and now BRC. On pp. 9-11. the section on 'Preparation of Reports on Carcinogens' was updated to include procedures and the various levels of peer review. On p. 13, 'Estimating Risk Reduction', a paragraph describing regulation of asbestos was removed. Finally, on pp. 15-16, the references were updated. Dr. Jameson said an overall aim was to make the document more user friendly with header and footer identification which he illustrated with a draft write-up for one of the chemicals under review.

Dr. Jameson said the Program sought input from the Subcommittee on proposals to add several new appendices to the Report, these being: (1) chemicals evaluated but not listed; (2) chemicals nominated but not yet evaluated for listing or delisting; (3) chemicals delisted from the BRC; and (4) "Manufacturing Processes, Occupations and Exposure Circumstances classified by IARC as sources which are known to be carcinogenic to humans because of the associated increased incidences of cancer in workers in these settings." This last group, as previously noted, is currently in the Introduction. Dr. Brown said the Subcommittee was not being asked to make motions and vote on these proposals but rather to provide guidance. Written comments had been received concerning appropriateness of inclusion of boot and shoe manufacturing and repair and nickel refining in the BRC under "Manufacturing Processes, Occupation and Exposure Circumstances...", and a request to make a formal public statement concerning inclusion of boot and shoe manufacturing and repair.

Public Comments -- Ms. LeAnn Johnson, attorney, and Dr. Ralph Mosely, expert consultant, represented the Footwear Industries of America, Inc. (FIA). Ms. Johnson said she intended to address the appropriateness or legality of listing manufacturing processes in the 8th BRC. She stated that FIA opposes the NTP proposal to reference manufacturing processes anywhere in the 8th BRC because: (1) NTP is expressly exceeding its statutory authority under the Public Health Service Act to list chemical substances by including references to manufacturing processes in any part of the Report; and (2) industries whose manufacturing processes are referenced in the Report are denied the procedural safeguards applicable to "listed" chemical substances which ensure that a listing is based on valid, current and reliable data. Moreover, she said while there are procedures available to challenge the listing of substances in the Report, there is no comparable process for protesting references to manufacturing processes, i.e., delisting procedures only apply to "an agent, substance, or mixture." Dr. Mosely commented that (1) there is not a shred of evidence associating cancer with footwear manufacture under modern conditions in the U.S.; (2) the only comprehensive and recent epidemiological study of the modern domestic footwear industry found virtually no significant incidences of cancer; (3) the IARC references cited were accepted by NTP at face value without independent review to determine whether those findings were applicable to industrial conditions in the U.S.; and (4) only substances and not industries or industrial processes were mandated by Congress for inclusion. Dr. Mosely then reviewed the IARC studies referenced regarding boot and shoe manufacturing and repair and their alleged relationship with various types of cancers and why they did not apply to modern U.S. footwear makers.

Discussion: Dr. Brown stated that the legal issues fell outside the expertise and charge of the Subcommittee. Dr. Lucier said that ultimately the Department and their legal counsel had the authority and expertise to deal with the legal issues. Dr. Frederick wondered whether the future scope of the BRC might extend to physical and biological agents. Dr. Lucier said he could envision that physical agents could be considered in the future but that biologics would be difficult to come to grips with. Dr. Hooper stated that coming from a state public health agency, he was sensitive to the need to keep the public informed of potential hazards and saw this as a function of the BRC. He suggested putting the 15 manufacturing processes, occupations and exposure circumstances into the Appendix along with a caveat that "certain of these occupations or processes might not apply to the U.S. " Dr. Bucher said to note the language on p. 8 of the draft Introduction which would stay with the 15 either here or in an appendix. Dr. LeBoeuf said it was essential that there be relevant disclaimers, and said the second sentence on p. 8 -- "The NTP has not reviewed the data supporting the listing of these occupational situations as posing a carcinogenic threat to humans, and recognizes that certain aspects of occupational exposures may differ in different parts of the world" -- pretty well covers what is needed. Dr. Frederick suggested that something such as "may have changed over time" should be added. Dr. Brown said he would like to gain a sense of the Subcommittee as to where they should be referenced in the Report or whether they should be referenced at all. Dr. D. Sharpnack, NIOSH, said that moving them into the Appendix would clarify their ownership to IARC. Dr. Belinsky said there should be information in the disclaimer noting likely changes in the process since many of the IARC references are not very recent. Dr. Ryan thought the general issues here should be in the Introduction while putting the specifics in the Appendix. There seemed to be a consensus opinion of the Subcommittee that in the interest of public health the BRC should contain references somewhere in the Report, and further, if included, there should be a carefully worded disclaimer which indicates that these "Manufacturing Processes, Occupations and Exposure Circumstances" have not formally been reviewed by the NTP and are provided for information only in the interest of public health. The disclaimer should refer the reader to the original IARC references and emphasize that manufacturing processes and occupations are different throughout the world and that manufacturing processes and occupations reviewed by IARC in their determinations may differ greatly from what has been or is currently used in the United States.

Dr. Brown said the next topic for consideration was the proposal by the NTP to add a table to the Appendix listing chemicals nominated and evaluated for listing but not accepted for listing in the ARC or BRC. Dr. W. Allaben, NCTR/FDA, said from the FDA view there was considerable potential for misuse or misunderstanding of such a

table. Dr. LeBoeuf commented that only chemicals known or anticipated to be human carcinogens should be in the document. Dr. Bucher said we do plan to put such a listing in the *Federal Register* also but this reaches a limited readership. The thought was that putting a table in the BRC might stimulate receipt of data which even could lead to reevaluation of a chemical. Dr. Henry suggested the World Wide Web or other mediums for communication of information might be a better idea. Dr. Brown asked for comment on the proposal to have a table in the appendix of chemicals nominated but not yet evaluated for listing or delisting. Similar thoughts were expressed by the reviewers as for the proposed table for chemicals evaluated but not listed. Thus, the sense of the Subcommittee was that appendix tables for (1) chemicals evaluated but not listed, and (2) chemicals nominated but not yet evaluated for listing or delisting, may lead to confusion by the reader. The Subcommittee and Expert Consultants suggested that this may be better handled by referring the reader to another source, such as the NTP web site, to get this information. The Subcommittee thought an appendix table for chemicals delisted from the BRC was needed.