SUMMARY MINUTES
NTP Board of Scientific Counselors
Report on Carcinogens Subcommittee Meeting
January 20-21, 2000

The National Toxicology Program (NTP) Board of Scientific Counselors' Report on Carcinogens (RoC) Subcommittee (the Subcommittee) held its fifth meeting on January 20 and 21, 2000, at the Crystal City Marriott, 1999 Jefferson Davis Highway, Arlington, Virginia. (Attachment 1: *Federal Register* meeting announcement; Attachment 2: Agenda and Roster of Members.) Members of the Subcommittee are Drs. Clay Frederick (Chairperson), John Bailer, John Froines, Stephen Hecht, Kim Hooper, Karl Kelsey, Michele Medinsky, Rafael Moure-Eraso, Jill Pelling, Jose Russo, Allan Smith, and Shelia Zahm. Expert Consultants to the Subcommittee were David Phillips, Eula Bingham, and Hiroshi Yamasaki. Drs .Hecht and Russo were not present. Drs. Bailer and Kelsey also were not present but had provided written reviews that were read into the record by the Executive Secretary.

I. Introduction and Background: Dr. George Lucier, Director, Environmental Toxicology Program (ETP), NIEHS, welcomed Subcommittee members and members of the public who had made it to the meeting despite an overnight snow storm in the Washington, DC area. Dr. Lucier reported on a public meeting on the *Report on Carcinogens* held on October 21-22, 1999, in Rockville, Maryland, where 41 persons provided comments on the RoC process and any issues they thought of importance for the RoC. He said that one of the recommendations was to move the RoC Subcommittee meeting from Research Triangle Park to the Washington area to increase accessibility for people who might not be able to travel to North Carolina. Thus, the present meeting was being held in Crystal City in response to that recommendation. He said that another recommendation had to do with allowing more time for public review of background documents prior to a meeting. This seemed to be reasonable and he noted that the documents were made available on the NTP web site in early November. Another comment had to do with the length of time allowed for formal presentation of public comments with the five minutes allocated thought to be too short. Dr. Lucier reported that the time had been increased to seven minutes, and when possible, up to 10 minutes would be allowed starting with today's meeting. Other comments pertained to providing as early as possible information on issues that might be critical to deliberations on whether or not something should be listed in the RoC. He suggested that making the background documents available as early as possible should allow interested persons adequate time for review and response to a proposed listing or delisting. Dr. Lucier concluded by noting that the nominations being reviewed today and tomorrow were the first group of nominations to be considered for the 10th *Report on Carcinogens* and a second group would be considered a year from now. He reported that the 9th *Report* was awaiting approval by the Secretary of the Department of Health and Human Services (DHHS).

Dr. Bill Jameson, NIEHS, went over the review format to be used with each nomination. Each nomination will be presented by an NTP scientist who will discuss the nomination, including data relating to human cancer, animal cancer, and mechanistic information, and summaries of the arguments for or against listing and will provide the recommendations, including the votes, of the two previous Federal scientific review groups, the NIEHS Review Committee for the Report on Carcinogens (RG1) and the NTP Executive Committee Interagency Working Group for the Report on Carcinogens (RG2). Dr Jameson reported that the presenters for the first two nominations were unable to be present due to the weather, but would be substituted for by other NTP staff members who were involved in the deliberations on those nominations. Following the staff presentation, Subcommittee members will be allowed to ask clarifying questions. Then members of the public will be invited to make their comments, following

which Subcommittee members will be able to ask further clarifying questions. Next, members of the Subcommittee who have primary review responsibilities for the nomination will present their views. This will be followed by further discussion among the Subcommittee members, concluding with motions and votes by Subcommittee members on recommendations to be forwarded to the NTP.

II. Peer Review of Substances Nominated for Listing in the 10th *Report on Carcinogens*:

2, 3-Dibromo-1-Propanol -- Dr. Jameson, substituting for Dr. Raymond Tennant, NIEHS, presented the nomination and said that 2,3-dibromo-1-propanol (DBP) was nominated for listing as *reasonably anticipated to be a human carcinogen* based on the results of a 1993 NTP bioassay in which DBP was found to provide *clear evidence of carcinogenic activity* in both rats and mice. DBP is an intermediate in the synthesis of flame retardants, insecticides, and pharmaceuticals, although its use is now much less than when it was used as a starting ingredient for the flame retardant, TRIS, that was used in children's sleepware during the 1960s and 1970s. Dr. Jameson said that DBP is not found naturally in the atmosphere, is not included in the Toxic Release Inventory, and there is no information on occupational exposure in the most recent Hazardous Substance Database. No studies of potential carcinogenicity in humans were identified. With regard to genetic toxicity, DBP is positive for mutagenicity in *Salmonella, E. coli, Drosophila*, and mouse lymphoma cells. He said that the mutagenicity of DBP suggests a mechanism of carcinogenicity shared by many known human carcinogens, and mutagenic human carcinogens also demonstrate multi-species and multi-site carcinogenic potential. Dr. Jameson reported that RG1 and RG2 both voted unanimously with nine votes to recommend that 2,3-dibromo-1-propanol be listed in the 10th *Report* as *reasonably anticipated to be a human carcinogen*.

Dr. Hooper, a primary reviewer, agreed with the proposed listing based on the findings of the NTP rodent studies. He noted that the mutagenicity of DBP is enhanced by metabolic activation presumably to the reactive mutagen. Since DBP is a metabolite of TRIS, he suggested adding a paragraph to the Summary Statement summarizing the animal bioassay findings for TRIS, comparing the TRIS results with DBP, as well as comparing the mutagenicity results for both. Further, although the mechanism of action is not fully understood, a sentence should be added that DBP is thought to be metabolized to a number of DNA reactive species, including three epoxides.

Dr. Zahm, a second primary reviewer, also agreed with the proposed listing. She noted that the chemical is still present in U.S. industry, although at much lower levels than in the past, and that there are no meaningful epidemiologic data.

Dr. Smith observed that there were 10 million pounds of DBP produced in 1976, and thought it strange that there were no epidemiological data. He asked whether the NTP asks manufacturers of a high volume production chemical if they have exposue or epidemiologic data on their workers. Dr. Jameson said that the NTP publishes its intent to review a nomination in the open literature including the *Federal Register* and trys to solicit as much input as possible but does not specifically ask producers or manufacturers if they have epidemiologic or human exposure data. Dr. Frederick surmised that there are inadequate resources being

deployed in this area. In response to a question from Dr. Moure-Eraso, Dr. Lucier responded that the NTP does not have legal authority to compel provision of such data. Dr. Bingham commented that there are close relationships between NTP and NIOSH and OSHA that could be exploited to obtain such data.

Dr. Hooper moved that the nomination of 2,3-dibromo-1-propanol for listing in the 10th *Report* as *reasonably anticipated to be a human carcinogen* be accepted. Dr. Zahm seconded the motion, which was accepted unanimously with seven votes.

2,2-Bis-(bromomethyl)-1,3-Propanediol -- Dr. John Bucher, NIEHS, substituting for Dr. June Dunnick, NIEHS, presented the nomination and said that 2,2-bis-(bromomethyl)-1,3-propanediol (BBMP) was nominated by RG1 for listing in the *Report* as *reasonably anticipated to be a human carcinogen* based on *clear evidence of carcinogenic activity* in both rats and mice in an NTP bioassay. The material studied was the technical grade, which is the material used as a flame retardant, with the major component being BBMP (about 80 %). The other major components are the monobromo- and tribromopentyl alcohols. Dr. Bucher said there were no case reports or epidemiological studies of the occurrence of human cancer and exposure to BBMP available. With regard to genetic toxicity, BBMP is mutagenic in two strains of *Salmonella*, produces chromosomal aberrations (CAs) in Chinese hamster ovary (CHO) cells, and causes micronucleated erythrocytes *in vivo* in mice. No sister chromatid exchanges were seen in CHO cells. Dr. Bucher reported that there had been a previous bioassay by the Dow Chemical Company in rats at lower doses than used by the NTP that was reported as a negative study. He went over the salient tumor sites in the NTP bioassay to provide an idea of the magnitude of the responses and extensive evidence for malignant tumors, especially in male rats. A stop-exposure study, which was run concurrently in male rats with BBMP given in the feed for three months followed by maintenance on a control diet for up to two years, resulted in neoplasms at the same sites as in the two-year bioassay and for many tumor sites at greater incidences. Dr. Bucher reported that RG1 voted unanimously with nine votes, and RG2 unanimously with eight votes to recommend listing BBMP in the 10th *Report* as *reasonably anticipated to be a human carcinogen*.

Dr. Bailer, a primary reviewer, was unable to be present so Dr. Mary Wolfe, NIEHS, read his comments into the record. Dr. Bailer agreed with the proposed listing. His comments primarily pertained to the presentation of findings in the Summary Document for the NTP bioassay. He thought the findings for the two-year study in male rats needed to be better differentiated from those for the stop study. Dr. June Dunnick, NIEHS, said the stop study results could be put into a different section to give better differentiation. Dr. Bailer asked why only pairwise test results were reported, noting that presentation of trend test results would make the presentation of results consistent between carcinogenicity and genotoxicity sections. Dr. Dunnick agreed that the trend test results could be added to the document.

Dr. Moure-Eraso, also a primary reviewer, agreed with the proposed listing. He found the section on human exposure to be superficial and sometimes ambiguous; for example, he noted the statement that "BBMP was not identified as being released by industry into the environment through the Toxic Release Inventory (TRI 1996)". He said that all this means is that the threshold of 25,000 pounds for reporting might not have been met, and emissions to air or water

might still be occurring. He urged more formal engagement by the NTP of agencies with access to production and exposure data. Dr. Lucier acknowledged that exposure assessment is often a very weak part of any overall health assessment and stated that the NTP is working actively with EPA, NIOSH, OSHA, and the Centers for Disease Control to coordinate and enhance activity in this area. Dr. Frederick reminded the group that the Subcommittee deliberations are primarily a hazard identification exercise.

Dr. Moure-Eraso moved that the nomination of 2,2-bis-(bromomethyl)-1,3-propanediol for listing in the 10th *Report* as *reasonably anticipated to be a human carcinogen* be accepted. Dr. Hooper seconded the motion, which was accepted unanimously with seven votes.

Beryllium and Beryllium Compounds -- Dr. Freya Kamel, NIEHS, presented the nomination and said that beryllium and beryllium compounds are currently listed in the 8th *Report on Carcinogens* as *reasonably anticipated to be human carcinogens*, and were nominated by RG1 for upgrade to *known to be human carcinogens* based on the 1993 monograph by IARC which listed beryllium and beryllium componds as Group 1, i.e., carcinogenic to humans. Dr. Kamel reported that beryllium (Be) and beryllium compounds are found in ores and as alloys, oxides, and salts with annual consumption in the U.S. about 220 metric tons. They are found in soil, air, and water with the major anthropogenic source being coal combustion. Occupationally, about 800,000 workers have been exposed to either Be or Be compounds. In 1971, OSHA set a permissible exposure limit of 2 $\mu g/m^3$ as an eight-hour time weighted average. Subsequently, NIOSH set a limit of 0.5 $\mu g/m^3$ and the American Conference of Industrial Hygienists set a limit of 0.2 $\mu g/m^3$. However, civilian employees are still regulated by the OSHA standard. Dr. Kamel said evidence for carcinogenicity comes from both human and animal studies, and discussed in detail the two primary human studies. One is a retrospective cohort study of lung cancer mortality by Ward *et al* on over 9,000 male workers in seven Be processing plants. The excess relative risk in the cohort was only 1.26, but quite precise, while in certain subgroups receiving the highest exposures, elevation in risk was as high as 3-fold. Dr. Kamel discussed the findings in two older plants in Lorain, Ohio and Reading, Pennsylvania, characterized by higher exposures and longer latency. She discussed two potential confounders, cigarette smoking and exposure to sulfuric acid mist (specifically at Lorain), and concluded that they cannot entirely account for the excess of the cohor. Dr. Kamel then reported on the retrospective cohort mortality study of lung cancer by Steenland and Ward based in the Beryllium Cancer Registry. The overall risk, measured by the standardized mortality ratio (SMR), was 2.0, and elevated risk was seen in both men and women. The primary strength of the two cohort studies was the precision of the risk estimate, and the primary limitation was the lack of individual exposure measurements. Dr. Kamel briefly described the animal data in which multiple studies in rats and mice have shown that either inhalation or intratracheal instillation of Be and Be compounds can produce lung tumors, and by either single or prolonged exposure, which is consistent with human experience. With regard to carcinogenic mechanism, she noted that a direct genotoxic mechanism was not involved. Dr. Kamel said that RG1 voted unanimously (8/0) and RG2 voted five yes to four no votes to recommend that Be and Be compounds be listed in the *Report* as *known to be human carcinogen*s. The votes against by RG2 members related to concerns about the small effect size overall in the cohorts, potential confounding by smoking and sulfuric acid mists, and the lack of a genotoxic mechanism.

Public Comments: Dr. H. Daniel Roth, Roth & Associates, Inc., on behalf of Brush Wellman, Inc., stated that many of the critical findings in the NTP draft document are not supported by the data, especially the claim that "an association with lung cancer has been consistently observed in several populations, with an excess risk of 1.2 to 1.6…" In fact, he said that using adjustments for smoking as reported by Ward, of the six work sites, only the Lorain plant data indicated a statistically significant elevation of SMR, and further, of the six plants, three had relative risks below one, indicating no consistent association between beryllium and lung cancer. Dr. Roth maintained that even Ward recognized that exposed workers smoked more heavily than the referent population. Further, he said more consideration should have been given to the fact that a preponderance of the plant workers, especially in Lorain and Reading, lived in the city not the county, the city having higher levels of air pollution. Finally, Dr. Roth stated that significant excess lung cancers were observed only at the Lorain plant, the only plant using the sulfuric acid process, and the excess disappears when adjustments are made for city referant populations. Dr. Kamel noted that there were actually eight categories of plants in the original Ward article, one being multiple plants and the other being unknown plants, and for both plants relative risks were elevated. Also, she urged looking at not just overall rates but at longer latency subgroups where statistically significant elevations were seen in more than just the Lorain and Reading plants.

Dr. Dimitrios Trichopoulos, Harvard School of Public Health, said that every study reported had been done using the same study base from the six plants, except that every study was really overriding the previous one because there was a longer time of followup. He thought the Ward study was an excellent study, which found when adjustments were made for smoking, that there was a relative risk for cancer of 1.12, which was not significant. Dr. Trichopoulos displayed results from a doctoral thesis on the confounding effects of fluorides on lung cancer incidence at the Reading plant where a hydrofluoric acid process was used. There was considerable discussion and questioning by Subcommittee members of Dr. Trichopoulos on data he presented. Dr. Elizabeth Ward, NIOSH (present by speaker phone), urged caution in considering selected data from a doctoral thesis when the Subcommittee has not had access to the full document.

Primary Reviews. Dr. Zahm, a primary reviewer, agreed with the proposed listing. She commented that beryllium causes cancer in multiple species of animals and by a variety of routes of exposure, and is associated with lung cancer in humans in studies with patterns of risk that support a causal relationship and where the excesses cannot be explained by confounding by smoking or other occupational exposures. Dr. Zahm noted the berylliosis registry study, reported on by Steenland and Ward, and believed to have most of the recognized cases in the U.S., and in which, lung cancer was elevated two-fold overall and 2.3-fold among those with acute disease, the group thought to have highest exposure to Be. She commented that although sulfuric acid mists were postulated as potential confounders in the Lorain plant, there was no evidence of an excess of laryngeal cancer, which is more strongly associated with sulfuric acid mists than lung cancer. There was some discussion among members and staff concerning the role of animal data in evaluation of a nomination as a *known to be human carcinogen*. There was general agreement that human data was preeminent, but that animal data was important for establishing consistency of effect and evaluating biological plausibility. Further, the criteria emphasize use of scientific judgement.

Dr. Kelsey, also a primary reviewer, was unable to be present so Dr. Wolfe read his comments into the record. Dr. Kelsey agreed with the proposed listing. He stated that the proposed listing is consistent with results of animal bioassays, data on the genotoxicity and mutagenicity of Be and certain Be compounds, as well as with the expanding epidemiologic literature. Dr. Kelsey said that more recent epidemiologic findings have not generated any evidence that tobacco or sulfuric acid mists confound the observed elevated risks for lung cancer.

Dr. Froines, also a primary reviewer, agreed with the proposed listing. He said he would like to see more discussion on the issue of smoking adjustment. Dr. Froines argued that sulfuric acid mist would not be a confounder because aerosols of sulfuric acid alone or combined with beryllium would be coarse, over 10 microns, and would not reach the lung. Rather, one would expect to see nasal, esophageal, and laryngeal cancers.

In further discussion, Dr. Bingham noted that while Be is primarily an occupational hazard, there are consumer products made out of Be, and discussion of consumer exposures should be expanded. Dr. Hooper thought the two major epidemiologic studies used as the basis for the proposed listing should be given more detail in the Report as should the consideration of potential confounding by smoking and sulfuric acid. Dr. Smith commented that the issue of latency or time to tumor needed to be addressed in the human cancer data. He discussed the difficulty of assessing a confounding effect of smoking on lung cancer in a population where a large proportion of workers and the nonworker population are or have been smokers at one time.

Dr. Zahm moved that the nomination of beryllium and beryllium compounds for upgrading in the *Report* to *known to be human carcinogens* be accepted. Dr. Froines seconded the motion, which was accepted unanimously with seven votes.

Dyes Metabolized to 3,3'-Dimethoxybenzidine -- Dr. Scott Masten, NIEHS, presented the nomination and said that dyes metabolized to 3,3'-dimethoxybenzidine (DMOB) were nominated by RG1 for listing as *reasonably anticipated to be human carcinogens* based on the fact that DMOB-based dyes, which contain an azo linkage, are enzymatically cleaved to form free DMOB. DMOB is currently listed in the *Report* as *reasonably anticipated to be a human carcinogen*. Further, he noted that dyes metabolized to the parent compound, benzidine, were recommended for listing in the 9th *Report* as *known to be human carcinogens*. Dr. Masten summarized the NTP findings for carcinogenicity of DMOB dihydrochloride in F344/N rats. DMOB was clearly carcinogenic based on a number of tumor sites, including skin, Zymbal gland, preputial gland, liver, oral cavity, and small and large intestines in males; and skin, Zymbal gland, clitoral gland, large intestine, mammary gland, and uterus in female rats. He reported that the dyes are synthesized by reacting DMOB with a variety of aromatic amines to form azo linkages and have been used in coloring textiles, plastics, rubber, and leather goods, with most recent usage primarily in textiles. Dr. Masten summarized findings for carcinogenicity with one of the DMOB-based dyes, C.I. Direct Blue 15, in an NTP bioassay in rats, wherein the tumor sites were strikingly similar to those for DMOB. With regard to human exposure, he said highest exposures occur occupationally in dye manufacturing and processing facilities while some consumer exposure may occur through contact with products containing

the dyes. Exposure can be assessed by biomonitoring urinary DMOB. He said there is often occupational exposure to other carcinogenic aromatic amines, e.g., benzidine, 2-napthylamine, in the same facility. Several DMOB-based dyes are metabolized *in vivo* in rodents and *in vitro* by intestinal bacteria to free DMOB. Dr. Masten reported that DMOB and DMOB-based dyes are mutagenic in bacteria when tested with metabolic activation and an azo-reductive preincubation protocol. He said that RG1 voted unanimously (9/0) while RG2 voted eight to zero with one abstention to recommend that dyes metabolized to 3,3'-DMOB be listed in the *Report* as *reasonably anticipated to be human carcinogens*.

There ensued a discussion about studies on DMOB and DMOB-based dyes in other species besides the rat. Dr. Medinsky said she was aware of studies in mice and hamsters where the dyes were only minimally or not carcinogenic, and she wondered if these were adequate studies or perhaps these other species were inherently less sensitive. Dr. William Allaben, NCTR, reported that mouse bioasssays were performed on DMOB at NCTR, and it was felt that the doses were not high enough to drive an expected tumor response, although the studies were adequate otherwise.

Dr. Pelling, a primary reviewer, agreed with the proposed listing. She noted the metabolism studies published in the early 1980s, which demonstrated that these dyes as a class were metabolized to DMOB in the dog and rat. She pointed out that there were positive findings for carcinogenicity of DMOB in Syrian golden hamsters, which supported the criteria of sufficient evidence of carcinogenicity in animals.

Dr. Medinsky, also a primary reviewer, agreed with the proposed listing. She thought that there needed to be more information in the document concerning public and worker exposure being much less at present than in the past. Dr. Bingham inquired as to whether we really know this to be true. Dr. Pelling commented that the U.S. leather and tanning industry has stated that they had ceased using DMOB-based dyes, no later than 1997. Dr. Medinsky commented that in general, rats appear to be more sensitive to carcinogenic effects of DMOB than mice or hamsters. Although this could be explained by a more robust experimental design in the more recent rat studies, she said it could also be the result of inherent differences in sensitivity among species, leaving the question of the relative sensitivity of humans toward carcinogenic effects of these dyes.

Dr. Pelling moved that the nomination of dyes metabolized to 3,3'-dimethoxybenzidine for listing in the 10th *Report* as *reasonably anticipated to be human carcinogens* be accepted. Dr. Medinsky seconded the motion, which was accepted unanimously with seven votes.

Dyes Metabolized to 3,3'-Dimethylbenzidine -- Dr. Scott Masten presented the nomination and said that dyes metabolized to 3,3'-dimethylbenzidine (DMB) were nominated by RG1 for listing as *reasonably anticipated to be human carcinogens* based on the fact that DMB-based dyes, which contain an azo linkage, are enzymatically cleaved to form free DMB. Like DMOB, DMB is currently listed in the *Report* as *reasonably anticipated to be a human carcinogen*, and dyes metabolized to benzidine were recommended for listing in the 9th *Report* as *known to be human carcinogens*. Dr. Masten summarized the findings for carcinogenicity of DMB dihydrochloride in F344/N rats. The dye in drinking water was clearly carcinogenic in dose-dependent fashion

in male and female rats with similar tumor sites to DMOB, including skin, Zymbal gland, preputial gland (males), clitoral gland (females), liver, oral cavity, small and large intestines,, lung (males), and mammary gland (females). Unlike DMOB, where there were no increases in tumors in mice, there were significant increases in lung alveolar adenomas and adenocarcinomas combined in male BALB/c mice. Dr. Masten reported that the dyes are synthesized by reaction of DMB with a variety of aromatic amines to form azo linkages with at least 95 dyes reported and a smaller, but unknown number, presently in commercial use. DMB-based dyes have been used primarily in printing textiles, with some minor uses as biological stains and in color photography. He summarized the carcinogenicity for one of the derived dyes, C.I. Acid Red 114, in NTP studies in rats. Like DMB, C.I. Acid Red 114 was clearly carcinogenic in male and female F344/N rats with a similar pattern of tumor sites as for the parent compound, DMB. With regard to human exposure, highest exposure occurs occupationally to workers in dye manufacturing and processing facilities, while some consumer exposure may occur through contact with products containing DMB-based dyes. Like DMOB, there were increased cancer incidences found for workers employed at some facilities producing DMB-based dyes, while there are no adequate studies to assess cancer incidence in relation to exposure to DMB-based dyes alone since other aromatic amines are produced in the same facility. Several DMB-based dyes are metabolized *in vivo* in rodents and *in vitro* by intestinal bacteria to free DMB. Dr. Masten reported that DMB and DMB-based dyes are mutagenic in bacteria when tested with metabolic activation and an azo-reductive preincubation protocol, and DMB is genotoxic in mammalian *in vitro* and *in vivo* test systems. He said that RG1 voted with five votes for and one against with one abstention, while RG2 voted unanimously (9/0) to recommend listing dyes metabolized to DMB in the 10th *Report* as *reasonably anticipated to be human carcinogens*.

In discussion, Dr. Frederick asked why there was disagreement among RG1 members. Dr. Masten responded that it had to do with a perceived inadequacy of the earlier draft of the background document's rationale in support of the nomination. There was some discussion about the DNA reactivity of benzidine, DMOB, and DMB, with agreement that all formed DNA adducts. Dr. Frederick noted that benzidine itself is not directly DNA reactive but has to be metabolized. Dr. Masten said that more discussion on carcinogenic mechanisms for both DMOB and DMB-based dyes could be added to the respective background documents.

Dr. Medinsky, a primary reviewer, agreed with the proposed listing. She said that her concerns were similar to those that she had expressed in her review of dyes metabolized to 3,3'-dimethoxybenzidine (DMOB). She stressed that the NTP needed to carefully consider public comments received, especially regarding current human exposures to the dyes. Secondly, she asked for more consistency and similarity of format between the background document with that for the document for dyes metabolized to DMOB with respect to useful tables and figures that were found in one document but not the other.

Dr. Pelling, also a primary reviewer, agreed with the proposed listing and said that all of her comments had been covered by Dr. Medinsky.

In other discussion, Dr. Moure-Eraso asked that there should be inclusive lists in the documents of the dyes that are known to be metabolized to DMOB and DMB. Dr. Frederick cautioned

against being too prescriptive since there may be other dyes of this class that may qualify for listing and since the information is proprietary, it would be not known to the NTP. Dr. Hooper suggested that all of the supporting literature on metabolism of these dyes be inserted in the document as an appendix.

Dr. Medinsky moved that the nomination of dyes metabolized to 3,3'-dimethylbenzidine for listing in the 10th *Report* as *reasonably anticipated to be human carcinogens* be accepted. Dr. Pelling seconded the motion, which was accepted unanimously with seven votes.

2-Amino-3-methylimidazo[4,5-*f*]quinoline (IQ) -- Dr. Bucher presented the nomination and said that IQ was nominated for listing in the 10th *Report* as *reasonably anticipated to be a human carcinogen* based on sufficient evidence of benign and malignant tumor formation at multiple tissue sites in multiple species of experimental animals. IQ is one of a series of heterocyclic amines that are formed in cooked meats, eggs, and found in cigarette smoke, and formed from heating certain amino acids, reducing sugars and fats. Dr. Bucher summarized the extensive genetic toxicology of IQ. IQ is mutagenic in *Salmonella* with metabolic activation, induces sex-linked recessive lethal and somatic mutations in *Drosophila*, induces *hprt* locus mutations in Chinese hamster ovary cells with activation, and induces *hprt* and thymidine kinase locus mutations in human lymphoblastoid cells *in vitro* with activation. There have been a number of studies showing induction of abnormal chromosomal effects of IQ, including chromosomal aberrations , sister chromatid exchanges, DNA strand breaks, and unscheduled DNA synthesis, some in human tissues. Dr. Bucher listed the consistently positive findings in *in vivo* genetic toxicity assays. He reviewed IQ carcinogenesis studies in experimental animals. In CDF1 mice, there were increases in liver, lung, and forestomach benign and malignant tumors. In a one-year study in female Sprague Dawley rats, there were increases in mammary gland and liver tumors, including some rare hemangioendotheliomas and Zymbal gland carcinomas. In F344 rats, there were marked increases in liver, large intestine, and Zymbal gland tumors in both sexes, small intestine and skin tumors in males, and clitoral gland tumors in females. In monkeys, dosed for 60 weeks with IQ, there were high incidences of hepatocellular tumors with none in controls. Finally, in newborn $B6C3F_1$ mice dosed with IQ,, there were increased incidences of hepatocellular adenomas at 8 and 12 months. Dr. Bucher said that in terms of human studies, there is a large literature associating red meat consumption and methods of food preparation with several cancers, especially breast and colorectal, but the findings are at best suggestive. He briefly discussed a proposed mechanism of carcinogenesis involving N-hydroxylation of IQ, followed by acetylation of the N-hydroxy moiety, leading to an unstable nitrenium ion, which has been shown to cause a number of DNA adducts. Dr. Bucher reported that RG1 voted unanimously (7/0) and RG2 voted unanimously (8/0) to recommend that IQ be listed in the 10th *Report* as *reasonably anticipated to be a human carcinogen*.

Dr. Kelsey, a primary reviewer, was unable to be present, so Dr. Wolfe read his comments into the record. Dr. Kelsey agreed with the proposed listing noting that it is consistent with the results of animal bioassays, data on the metabolism, genotoxicity and mutagenicity of IQ, as well as with the limited epidemiologic literature. Dr. Kelsey noted that exposure to IQ is very difficult to quantify, as cooked meats contain multiple carcinogens and most studies are subject to recall and other bias.

Dr. Phillips, also a primary reviewer, agreed with the proposed listing. He pointed out that although IQ is a 'super mutagen', it is not a 'super carcinogen', i.e., other heterocyclic amines formed from cooking of proteins are equi or more potent carcinogens than IQ. Further, IQ is only a minor contributor to the total heterocyclic amine burden. Dr. Phillips disagreed that N-acetyl-IQ was the primary reactive metabolite, but rather he contended that the literature supported the major route of activation being N-hydroxylation followed by O-esterification. He agreed that the ultimate reactive species was probably the nitrenium ion.

Dr. Smith, also a primary reviewer, agreed with the proposed listing. However, he reported that he had received a statement from Dr. Rashmi Sinha which stated that IQ has not been detected in meat samples in the United States. Dr. Zahm commented that Dr. Sinha is in the Nutrition Epidemiology Branch at the NCI and had provided peer reviewed papers, primarily 1995 or more recent publications, to her of work done with the USDA where meat and fish were prepared in the usual mode of preparation and when analyzed for heterocyclic amines, IQ was not detected. Dr. Smith noted that the criteria for listing in the *Report* state that there be significant human exposure in the United States to the agent in question. Dr. Zahm said that earlier studies outside the U.S. had reported detecting IQ in incinerated meat or fish. Dr. Phillips commented that IQ was found in smoked fish in Japan Dr. Pelling pointed out that IQ also was reported to be present in cigarette smoke condensates.

Dr. Smith moved that the nomination of 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) for listing in the10th *Report* as *reasonably anticipated to be a human carcinogen* be accepted. Dr. Froines seconded the motion, which was accepted unanimously with seven votes.

Styrene-7,8-oxide -- Dr. James Huff, NIEHS, presented styrene-7,8-oxide (styrene oxide) as being nominated for listing as *reasonably anticipated to be a human carcinogen* based on the RoC criteria of experimental (long-term carcinogenesis bioassays and genotoxicity) and mechanistic findings and human genotoxicology data. There were no adequate data available to evaluate the carcinogenicity of exposures to styrene oxide in humans. Styrene oxide is produced by cyclization of styrene chlorohydrin and is used mainly in preparation of fragrances and as a reactive diluent in epoxy resin formulations. It is the major metabolite of styrene and has been detected in association with styrene, but at lower levels in industries where unsaturated polyester resins are used. With regard to animal carcinogenesis studies, styrene oxide caused high incidences (44 to 97 %) of uncommon benign and malignant cancers of the forestomach via oral exposure at all exposure concentrations in both sexes of three strains of rats, and $B6C3F_1$ mice, and exposure responses were dose related. Dr. Huff noted that of 500 chemicals studied by the NCI/NTP only 31 caused tumors of the forestomach, and of these 25 were *Salmonella* positive, while only six caused only forestomach tumors. Further, he pointed out that invariably other epoxides studied as well as styrene oxide induce "application site" tumors, suggesting they are direct acting mutagens and carcinogens. Dr. Huff summarized the data supporting the proposed listing: styrene oxide (1) induces gene mutations *in vivo* and *in vitro*; (2) induces chromosomal aberrations (CAs), micronuclei, and sister chromatid exchanges (SCEs) in human cells *in vitro*; (3) induces CAs and SCEs in mice *in vivo*; (4) forms covalent adducts with DNA in humans, rats, and mice; (5) is carcinogenic in both sexes of several strains of rats and one strain of mice; (6) styrene is likewise carcinogenic to experimental animals; and (7) mechanistically, no epidemiological or experimental data are available that suggest that

mechanisms thought to account for genotoxicity and tumor induction by styrene oxide would not also operate in humans. Dr. Huff reported that RG1 voted seven to one and RG2 voted six to three to recommend that styrene-7,8-oxide be listed in the10th *Report* as *reasonably anticipated to be a human carcinogen.* Dr. Frederick asked for the rationale for the dissenting votes. Dr. Huff responded that the dissenting votes were apparently because humans do not have a forestomach, yet the cell type of the human esophagus and rodent forestomach is the same. There ensued a discussion about the metabolism of styrene and styrene oxide. Dr. Froines inquired that since styrene causes tumors of the lung, is the compound metabolized to styrene oxide in lung cells. Dr. Lucier replied that human and rodent lung cells carry out this conversion.

Public Comment. Dr. Chris Bevin, Styrene Information and Research Center (SIRC), said that SIRC believes the forestomach tumors observed at high oral doses in rats and mice are the result of tissue damage at the site of contact. With regard to the only other tumors reported, liver tumors at the low dose but not the high dose in male mice, the lack of liver tumors in the high dose could not be accounted for by reduced survival. Dr. Bevin noted that while genotoxicity of styrene oxide *in vitro* has been well documented, *in vivo* tests have been largely negative. Further, evidence for DNA adduct formation in the forestomach was marginal. Dr.Bevin concluded that SIRC requests that the NTP give careful consideration to information given and make a recommendation not to list styrene oxide in the 10th *Report.* Dr. Hooper commented that some of the *in vivo* genotoxicity tests are not very sensitive and, further, because of styrene oxide's reactivity, the chemical may not be reaching germline tissues. There was some discussion about considering data being cited from an inhalation bioassay of styrene in rats and mice by SIRC that had not been published in the open literature. Dr. Frederick suggested that public presenters be asked to make supporting documentation fully available to the Subcommittee prior to the meeting. Dr. Lucier noted that the 10th *Report* will not be submitted to the Secretary, DHHS, until 2002, so new information published subsequent to the meeting will be evaluated in terms of the review. Dr. Bevin reported that the styrene rat studies have been published and the mouse studies submitted for publication.

Dr. Hooper, a primary reviewer, agreed with the proposed listing. He said that despite the assertion by SIRC to the contrary, there is a considerable body of evidence that supports a genotoxic mechanism of action for styrene oxide, and noted that some of the more recent studies in animals and workplace exposure studies in humans supporting this were not cited by SIRC. Dr Hooper discussed in detail findings from these recent human studies, especially those by Rappaport and coworkers and Hemminki and coworkers with regard to chromosomal effects, HPRT mutations, and protein and DNA adducts. Dr. Hooper conceded that cell proliferation in the forestomach from a bolus dose may also play a role in tumor response. He suggested that because styrene oxide is so reactive in the acid environment of the stomach very little is taken up by the systemic circulation, and therefore, it would be of interest to know the tissue distribution of styrene and the oxide when administered by gavage and also by inhalation. Dr. Hooper commented that styrene given by inhalation is metabolized primarily to styrene oxide and causes tumors of the lung and liver in mice.

Dr. Smith, also a primary reviewer, agreed with the proposed listing. However,he did not think that based on the evidence presented that there is sufficient evidence of carcinogenicity of

styrene oxide at multiple sites in experimental animals. That is the liver tumors in the low dose female mice were not convincing. However, he didn't consider this a problem with respect to the proposed listing. Regarding human studies, Dr. Smith suggested a main area of confusion, especially with reference to Rappaport's studies, was the supposition that when workers are exposed to styrene in air, it is the styrene oxide contaminant and not the styrene oxide resulting from metabolism of styrene to styrene oxide in the body that is important in molecular and biochemical effects seen. Dr. Smith proposed that based on the epidemiological studies he would have preferred presentation of styrene and styrene oxide in a single document.

Dr. Froines, also a primary reviewer, agreed with the proposed listing. He thought the epidemiology section of the document needed some reworking, and noted that some of the studies with styrene-butadiene were more about butadiene. Dr. Frederick agreed, and said he was not convinced of the relevance of the styrene-butadiene epidemiology review, because of the confounding concerns with butadiene, already a known human carcinogen.

Dr. Hooper suggested that there should be a section devoted to human studies on genotoxicity. Dr. Phillips commented that compared with many well studied genotoxic carcinogens, looking at styrene or styrene oxide studies in animals, the level of adducts is much lower, i.e., there don't seem to be many adducts for the tumor response seen, making it not look like a predominantly genotoxic mechanism. However, based on the criteria he did think styrene oxide should be listed in the *Report*.

Dr. Hooper moved that nomination of styrene-7,8-oxide for listing in the 10th *Report* as *reasonably anticipated to be a human carcinogen* be accepted. Dr. Smith seconded the motion, which was accepted with six yes votes and one abstention (Medinsky). Dr. Medinsky said that she abstained since she is consulting for the Harvard School of Public Health on a risk assessment of styrene being supported by SIRC.

Vinyl Bromide and Vinyl Fluoride -- Dr. Ronald Melnick, NIEHS, presented the nominations together because these two chemicals have very similar chemical and biological behaviors. Vinyl bromide (VB) and vinyl fluoride (VF) were nominated by RG1 for listing as *reasonably anticipated to be human carcinogens* (1) because inhalation exposures of rats and/or mice induced tumors at multiple organ sites, including hepatic hemangiosarcomas; (2) because VB and VF are close structural analogues of vinyl chloride (VC), a chemical *known to be a human carcinogen*; and (3) because VB and VF are listed by IARC as probably carcinogenic to humans (Group 2A). Both chemicals are colorless gases at room temperature and used in the production of polymers. Dr. Melnick said that neither occur naturally with industrial release primarily accounting for their environmental presence. The primary route of occupational exposure is inhalation. No studies of potential carcinogenicity of VB or VF in humans have been identified. However, the related congener, VC, is identified as a known human carcinogen by IARC and the *Report on Carcinogens* and targets the liver (primarily hemangiosarcomas), brain, lung, and lymphohematopoietic system. With regard to experimental carcinogenicity data, Dr. Melnick reported there was one two-year study of VB by inhalation exposure in Sprague Dawley rats resulting in hemangiosarcomas of the liver, hepatocellular neoplasms, and Zymbal's gland carcinomas in both sexes. For VF, a two-year inhalation study in Sprague Dawley rats resulted in the same pattern of tumors. An 18-month inhalation exposure study of VF in CD-1 mice

resulted in hemangiosarcomas of the liver, lung neoplasms, and Harderian gland adenomas in both sexes, and mammary gland adenocarcinomas in females. The most common and predominant effect of VB and VF, as well as VC, is the induction of the rather uncommon tumor, hemangiosarcomas of the liver. Dr. Melnick noted that epidemiologic studies have established a causal association between VC exposue and liver hemangiosarcomas. He said that all three vinyl halide congeners are genotoxic in *in vivo* and *in vitro* systems, and they are metabolized to similar DNA reactive intermediates, i.e., haloethylene oxides and haloacetaldehydes. Dr. Melnick said the DNA adducts formed from VB and VF are the same as those formed from VC and he discussed these in detail. Dr. Melnick reported that RG1 voted unanimously with 10 votes, and RG2 voted unanimously with nine votes to recommend that VB be listed in the 10th *Report* as *reasonably anticipated to be a human carcinogen,* while RG1 voted seven yes to two no votes and RG2 voted unanimously with nine votes to recommend that VF be listed in the 10th *Report* as *reasonably anticipated to be a human carcinogen.* Dr. Melnick commented that the two no votes by RG1 on VF pertained to feeling that VF should be a *known human carcinogen* despite the lack of human data because the tumor profiles, the genotoxicity, metabolism, and adducts formed were directly analogous to VC.

Dr. Bailer, a primary reviewer for VB, was unable to be present , so Dr. Wolfe read his comments into the record. Dr. Bailer agreed with the proposed listing. Since the listing of industries with potential VB exposure was based on a 1978 reference, he thought this might be updated. Dr. Bailer suggested that trend test results be added in addition to pairwise comparisons.

Dr. Yamasaki, also a primary reviewer for VB, agreed with the proposed listing. However, he wondered if VB and VF, for which there is no human data, could be combined with VC, for which there is human cancer data, as "vinyl halides" and listed as *known to be human carcinogens*.

Dr. Phillips, also a primary reviewer for VB, agreed with the proposed listing. He agreed with Dr. Yamasaki that based on the fact VC is a known human carcinogen and the similarity of mechanisms of activation between VC and VB, it could be strongly suggested that VB exposure would present a similar carcinogenic hazard to humans.

Dr. Yamasaki, a primary reviewer for VF, agreed with the proposed listing. His comments regarding considering listing the three "vinyl halides" as *known to be human carcinogens* were the same as he had made in the review of VB.

Dr.Moure-Eraso, also a primary reviewer for VF, did not agree with the proposed listing. He stated that on the basis of the previous discussion concerning the similarities with VC in mechanisms of chemical activation, chemical structure, genotoxicity, DNA reactivity, and site concordance for carcinogenic effects among these three chemicals, it seemed to him that the definition of *known to be a human carcinogen* given in the criteria allowed such a recommendation for VF, and VB for that matter. Dr.Moure-Eraso quoted from the criteria as follows: "Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information."

Dr. Frederick asked for clarifying comments by NTP staff on the issues raised by Drs. Yamasaki and Moure-Eraso and reminded the group that they are only advisory. Dr. Lucier said that to call these agents human carcinogens, we need evidence from molecular epidemiology or other human studies that the same mechanisms are operating for all three of the halides. Dr. Pelling thought it too much of a 'jump' without any human data. Dr. Yamasaki reminded the Subcommittee that his proposal was to consider the halides as a group, lacking human data for VB andVF. Dr. Bingham expressed frustration about "the rules", and doubted that epidemiology data would ever be developed for these chemicals. Dr. Medinsky agreed with Dr. Pelling but sympathized with Dr. Bingham's statement. Dr. Smith noted that the difficulty derives from the phrase in the criteria: "There is sufficient evidence of carcinogenicity in humans…" Dr.Lucier said that we tried not to be too prescriptive in development of the criteria. Dr. Phillips cautioned that we would be setting a precedent for listing agents as human carcinogens with no human data. Dr. Yamasaki commented that VC mutates the *ras* oncogene in humans, and this type of mutation occurs in animals exposed to VB and VF. Dr. Moure-Eraso stated that this information would fall under "all relevant information" and could be entered into the realm of "scientific judgment". Dr. Hooper agreed with a listing of VB and VF as *known to be human carcinogens.* Dr. Frederick proposed a motion on these materials as *known to be human carcinogens,* and then a motion for *reasonably anticipated to be human carcinogens*. Dr. Froines suggested a third motion on vinyl halides. Dr. Lucier said that would be inappropriate as the public would not have the opportunity to be informed or offer comments. Dr. Allaben said there was nothing to preclude nomination of the three vinyl halides as a class to be returned for future re-review.

Dr. Yamasaki moved that the nomination of vinyl bromide for listing in the 10th *Report* as *reasonably anticipated to be a human carcinogen* be accepted. Dr. Medinsky seconded the motion. After some discussion, Dr. Yamasaki withdrew his motion. Dr. Moure-Eraso moved that the nomination of vinyl bromide for listing as *known to be a human carcinogen* be accepted. Dr. Hooper seconded the motion, which was accepted by four yes votes (Froines, Hooper, Moure-Eraso, Smith) to three no votes (Medinsky, Pelling, Zahm). Then, Dr. Pelling moved that the listing of vinyl fluoride as *reasonably anticipated to be a human carcinogen* be accepted. Dr. Medinsky seconded the motion. Dr. Pelling withdrew the motion as this left the reason for her no vote on vinyl bromide unstated, and moved that the listing of vinyl bromide as *reasonably anticipated to be a human carcinogen* be accepted. Dr. Medinsky seconded this motion. After further discussion, Dr. Bucher and others suggested that members voting no on Dr. Moure-Eraso's motion express their reasons for the record, Dr. Frederick agreed. Dr. Medinsky and Dr. Pelling both stated that they voted no on the motion for listing vinyl bromide as *known to be a human carcinogen* because the lack of human data made it more appropriate for the chemical to be listed as *reasonably anticipated to be a human carcinogen*. Dr. Zahm said she voted no because according to her reading of the criteria, vinyl bromide should be *reasonably anticipated to be a human carcinogen.* Dr. Pelling withdrew her motion on vinyl bromide. Dr.Yamasaki opined that those voting yes on the original motion should substantiate their reason for deviating from the formal criteria for *known to be a human carcinogen*. Dr. Froines said he voted yes because he considered that the human data on vinyl chloride was supportive given all the chemical, biochemical, and genetic similarities associated with the three congeners. Dr. Smith said he voted yes because the findings with vinyl chloride were sufficient to support *known* with respect to the other two congeners. Dr. Moure-Eraso said he voted yes because he thought the

human data on vinyl chloride were relevant to both vinyl bromide and vinyl fluoride. Dr. Hooper said he voted yes because the sufficient evidence from carcinogenicity studies in humans with vinyl chloride indicates a causal relationship between exposure to vinyl bromide and vinyl fluoride and human cancer. This is supported by the fact that the *ras* mutations in vinyl chloride exposed humans would be expected to be the same for VB and VF based on the similar adducts formed by these three vinyl halides. Dr. Hooper moved that the nomination of vinyl fluoride for listing in the 10th *Report* as *known to be a human carcinogen* be accepted. Dr. Moure-Eraso seconded the motion. In discussion, Dr. Phillips stated that this vote was being taken without even the existence of a biomarker study and could have the effect of squelching molecular epidemiology studies on these chemicals. Dr. Froines opined that this vote will actually stimulate more research than diminish it. Dr. Smith said he was a strong supporter of molecular epidemiology but thought limited resources would be better spent on other topics. Dr. Moure-Eraso returned to the issue of using all relevant information in this case. The motion was accepted by four yes votes (Froines, Hooper, Moure-Eraso, Smith) to three no votes (Medinsky, Pelling, Zahm). The members indicated that the justifications for their votes were the same as stated for the votes on vinyl bromide.

The meeting of the RoC Subcommittee was adjourned.