NATIONAL TOXICOLOGY PROGRAM
BOARD OF SCIENTIFIC COUNSELORS

Summary Minutes

Peer Review of Draft Technical Reports of
Toxicology and Carcinogenesis Studies
by the Technical Reports Review Subcommittee

October 18, 2001

Contents Page Numbers

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BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE
Summary Minutes – October 18, 2001

The meeting began at 8:30 a.m. on October 18, 2001 in the Rodbell Conference Center of the David P. Rall Building, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. Members of the subcommittee were Drs. Steven Hecht (chairperson), Norman Drinkwater, James Klaunig, David Malarkey, Michele Medinsky, Walter Piegorsch, Mary Anna Thrall, Linda Chatman, Harold Davis, and Yvonne Dragan. Drs. Klaunig, Chatman, Davis, and Dragan were not present. For further information, contact Dr. Mary S. Wolfe, Executive Secretary, at 919-541-3971 or wolfe@niehs.nih.gov.

Riddelliine Dr. Po Chan, NIEHS, introduced the toxicology and carcinogenesis studies of riddelliine by describing the structure and occurrence of the chemical, the results of the previously published 13-week studies, and the design of the present 2-year studies. He discussed the body weights, survival, and the variety of neoplasms observed in the exposed animals. The proposed conclusions for the report were:

> Under the conditions of these studies there was *clear evidence of carcinogenic activity* of riddelliine in male and female F344/N rats based primarily on increased incidences of hemangiosarcoma in the liver. The increased incidences of hepatocellular adenoma and mononuclear cell leukemia in male and female rats were also considered to be treatment related. There was *clear evidence of carcinogenic activity* of riddelliine in male $B6C3F_1$ mice based on increased incidences of hemangiosarcoma in the liver. There was *clear evidence of carcinogenic activity* in female $B6C3F_1$ mice based on increased incidences of alveolar/bronchiolar neoplasms.
>
> Administration of riddelliine by gavage resulted in nonneoplastic lesions in the liver and kidney of male and female rats; the liver and kidney of male and female mice; and the lung and arteries (multiple tissues) of female mice.
>
> Decreased incidences of hepatocellular neoplasms in male and female mice were related to riddelliine administration.

Dr. Ming Chou, NCTR, described companion studies to characterize the metabolism of riddelline by rat and human liver microsomes and the DNA adducts formed in both systems.

Dr. Drinkwater, a principal reviewer, expressed reservations about the unbalanced study design, with male rats and female mice having just one dose group. He also questioned whether the hepatocellular neoplasms and leukemia in the male and female rats could definitely be attributed to chemical exposure.

Dr. Piegorsch, the second principal reviewer, also questioned the unbalanced design but did not feel that any neoplastic effects went undetected in this instance. He noted the potential benefits for risk assessment from the multiple-dose studies in female rats and male mice.

Dr. Medinsky, the third principal reviewer, agreed with the proposed conclusions and appreciated the importance of the DNA adduct studies in characterizing the genotoxic mechanism for this chemical.

Dr. John Bucher, NIEHS, explained the rationale for use of the unbalanced dosing scheme. In preliminary 13-week studies riddelliine caused liver neoplasms in female mice. Because the amount of test material was limited and the carcinogenicity of the chemical was already established, the NTP decided to use several, lower, dose levels in one sex for each rodent species to try to determine dose-response relations or no-observed-effect levels.

Dr. Joseph Haseman, NIEHS, explained that the liver neoplasms and leukemia in female rats were not part of the "clear evidence" conclusion but were still considered attributed to chemical administration and by themselves would have constituted "some evidence" of carcinogenic activity. The numerical increase in leukemia rates was deemed more significant after survival-adjusted analyses, reflecting the early deaths of many animals. Dr. J.R. Hailey, NIEHS, added that mononuclear cell leukemia normally occurs after 18 months in Fischer rats, while most of the exposed rats in the present studies had already died by that time.

Dr. Medinsky moved that the conclusions be accepted as written, and Dr. Drinkwater seconded the motion, which was accepted unanimously with five votes.

Vanadium Pentoxide Dr. Nancy Ress, NIEHS, introduced the toxicology and carcinogenesis studies of vanadium pentoxide by describing the uses and occurrence of the chemical, the design of the short- and long-term inhalation studies, and the body weight, survival, and respiratory effects of chemical exposure. The proposed conclusions were:

> Under the conditions of these 2-year inhalation studies there was *some evidence of carcinogenic activity* of vanadium pentoxide in male F344/N rats *and equivocal evidence of carcinogenic activity* of vanadium pentoxide in female F344/N rats based on the occurrence of alveolar/bronchiolar neoplasms. There was *clear evidence of carcinogenic activity* of vanadium pentoxide in male and female $B6C3F_1$ mice based on increased incidences of alveolar/bronchiolar neoplasms.
>
> Exposure to vanadium pentoxide caused a spectrum of nonneoplastic lesions in the respiratory tract (nose, larynx, and lung) including alveolar and bronchiolar epithelium hyperplasia, inflammation, fibrosis, and alveolar histiocytosis of the lung in male and female rats and mice and an unusual squamous metaplasia of the lung in male and female rats.

Dr. Theodora Devereux, NIEHS, described studies to characterize the pattern of K-*ras* mutations in lung carcinomas taken from mice exposed to vanadium pentoxide. Nearly three-fourths of these carcinomas exhibited K-*ras* mutations, compared with a rate of about 30% in spontaneously occurring alveolar/bronchiolar carcinomas from untreated mice. In addition, many of the tumors with K-*ras* mutations also exhibited a loss of heterozygosity at chromosome

6 in the region of the K-*ras* suppressor gene. Samples with both the K-*ras* mutation and loss of heterozygosity also had the highest activity of MAP kinase. Taken together, these findings were suggestive of a mechanism for the carcinogenicity of vanadium pentoxide.

Dr. Medinsky, a principal reviewer, agreed with the proposed conclusions and commented on the discussion of the toxicokinetic modeling. She suggested that the area under the lung burden versus time curve might be a better metric for expressing exposure than deposition rates, particularly for use in comparisons of exposures between different rodent species and humans.

Dr. Malarkey, the second principal reviewer, also agreed with the conclusions and commended the inclusion of several supplemental studies, including immunotoxicology, reproductive toxicology, and pulmonary lavage. He inquired whether decreases in heart rate and blood pressure might have been secondary effects of the pulmonary disease and also if uterine stromal polyps should be included in the list of chemical-related effects.

Dr. Thrall, the third principal reviewer, also agreed with the conclusions. She suggested that the observed erythrocytosis may have been due to hypoxemia secondary to the lung disease.

Dr. Ress clarified that the lung burden measures were based on weighing entire lungs rather than extrapolation from measurements from samples. Regardless of the metric, mice were seen to be more sensitive than rats to vanadium pentoxide. Dr. Medinsky added that one benefit of the measures that factor lung clearance and solubility is that evaluation of dose-setting and comparisons with human studies become more realistic.

Dr. James R. Hailey, NIEHS, explained that uterine stromal polyps occur spontaneously fairly often and the incidence in the high dose group was not statistically significant, so these tumors were not considered related to chemical administration.

Martha Marrapese, representing the Vanadium Committee of the Ferroalloys Association, questioned whether the conclusion for the male rat study might more appropriately be "equivocal evidence" given the lack of statistical significance of the lung tumors. Dr. Malarkey noted that the incidence of tumors in the control group was unusually high compared with the historical control incidence, which reduced the statistical significance of the increase in tumors in the exposed groups. Dr. Ress presented a slide comparing the incidences of lung adenomas in the present studies with several other NTP male rat studies for which conclusions of "some evidence" or "equivocal evidence" were based on lung tumors. The incidences in the vanadium pentoxide study were even higher than in studies of cobalt sulfate and nickel oxide, which were judged "some evidence," and markedly higher than in other studies with calls of "equivocal evidence." Dr. Hailey added that the occurrence of carcinomas in six vanadium pentoxide exposed animals, compared with only two in over 600 historical control males, provided further support of a chemical-related effect.

Ms. Marrapese also asked that the panel consider deferring the review if they felt they needed more time for public comment to arrive at a conclusion. No motion to defer was offered.

Dr. Medinsky moved, and Dr. Malarkey seconded, that the conclusions to the report be accepted as written. The motion was approved unanimously with five votes.

2,-Hexadienal Dr. Po Chan, NIEHS, introduced the toxicology and carcinogenesis studies of 2,4-hexadienal by describing the uses and natural occurrence of the chemical, the design of the short- and long-term gavage studies, and the effects of exposure on body weight, survival, and forestomach lesions in rats and mice. The proposed conclusions were:

> Under the conditions of these 2-year gavage studies there was clear evidence of carcinogenic activity of 2,4-hexadienal in male and female F344/N rats and male and female B6C3F_1 mice based on increased incidences of squamous cell neoplasms of the forestomach. The occurrence of squamous cell carcinoma of the oral cavity (tongue) in male B6C3F_1 mice may have been related to the administration of 2,4-hexadienal.
>
> Hyperplasia of the forestomach in male and female rats and mice was associated with administration of 2,4-hexadienal.

Dr. Klaunig, a principal reviewer, was unable to attend the meeting, so Dr. Mary Wolfe, NIEHS, read his comments for the record. Dr. Klaunig agreed with the conclusions regarding forestomach neoplasms but did not feel the oral cavity carcinomas in two male mice constituted evidence of carcinogenic activity. He also noted that mutagenicity findings from different laboratories were inconsistent.

Dr. Drinkwater, the second principal reviewer, felt the statement that oral cavity carcinomas may have been treatment related could be included in the conclusions. He asked for clarification of the description of the isomeric mixture at the start of the report.

Dr. Malarkey, the third principal reviewer, agreed with the conclusions and felt the oxidative stress and DNA adduct studies were worthwhile additions.

Dr. Chan explained that different concentrations of S9 metabolic activation enzymes were used in different mutagenicity assays and that a lack of response in tests at one laboratory does not negate positive responses at another. He noted that carcinomas of the tongue were rare in NTP studies and the intent was to note their presence without implying statistical significance. Dr. John Bucher, NIEHS, explained that the term "may have been related" to chemical exposure proposed for the oral cavity neoplasms was meant to distinguish these lesions from those constituting the "clear evidence" of carcinogenic activity in the same sex/species group. By themselves, the tongue neoplasms would be considered only an equivocal finding.

Dr. Drinkwater moved, and Dr. Thrall seconded, that the second sentence of the conclusion statement regarding oral cavity carcinomas be deleted. The motion was defeated by a vote of

three to two. Dr. Malarkey then moved, and Dr. Drinkwater seconded, that the conclusions be accepted as originally written. The motion was carried unanimously with five votes.

Diazoaminobenzene Dr. Nancy Ress, NIEHS, introduced the report on the metabolism, toxicity, and predicted carcinogenicity of diazoaminobenzene by describing the study design process and the results of metabolism and disposition studies and 16-day dermal toxicity studies. She also described results of a mouse bone marrow micronucleus study (not presented in the draft report) that showed that diazoaminobenzene, like benzene, is a potent inducer of micronuclei. The proposed conclusions to the report were:

> Diazoaminobenzene is metabolized to the known carcinogens benzene and aniline. Some toxic effects associated with aniline (Heinz body anemia, methemoglobinemia) and benzene (atrophy of the lymphoid tissue, hematopoietic cell proliferation) were identified. Based on these results, it is predicted that diazoaminobenzene is a carcinogen.

Dr. Thrall, a principal reviewer, agreed with the prediction of carcinogenicity based on the metabolism of diazoaminobenzene to benzene and aniline but questioned whether Heinz body anemia was truly an effect, as only one of 20 treated groups had a statistically significant increase in Heinz body formation. She asked for clarification if there was oral exposure during the dermal study and suggested rearranging the conclusion statement to make clear that the prediction of carcinogenicity was based on metabolism. Dr. Ress replied that some oral exposure occurs in dermal studies as a result of the animals grooming themselves. In this study the animals were housed individually to minimize such exposure.

Dr. Klaunig, the second principal reviewer, was unable to attend the meeting, so Dr. Mary Wolfe, NIEHS, read his comments for the record. Dr. Klaunig agreed that the study results supported the premise that diazoaminobenzene may be carcinogenic.

Dr. Piegorsch, the third principal reviewer, agreed with the conclusions and inquired if the results on the micronucleus studies would be included in the final version of the report. Dr. John Bucher, NIEHS, indicated that the micronucleus data could be added to the report with a notation that these data were not used by the review panel in formulating the conclusion statement.

Dr. Hecht asked if phenyl hydrazine would also have been an expected metabolite of the compound and also if any consideration had been given to possible interactive effects between the metabolites benzene and aniline. Dr. Ress replied that while it was possible that phenyl hydrazine could be a metabolite, it was not observed in this study. The possibility of interactive effects between the metabolites was being examined in further micronucleus tests.

Dr. Thrall moved that the conclusions be modified to eliminate mention of Heinz body anemia and hematopoietic cell proliferation. The revised conclusion was:

Diazoaminobenzene is metabolized to the known carcinogens benzene and aniline. Further evidence of this metabolism is that some toxic effects associated with aniline (methemoglobinemia) and benzene (atrophy of the lymphoid tissue) were identified. Based on these results, it is predicted that diazoaminobenzene is a carcinogen.

Dr. Piegorsch seconded the motion, which was approved unanimously with five votes.