Board of Scientific Counselors
National Toxicology Program

Summary Minutes
from
Peer Review of Draft Technical Reports of Long-Term
Toxicology and Carcinogenesis Studies and Short-Term Toxicity Study
by the Technical Reports Review Subcommittee

on

November 29, 1994

Research Triangle Park, N.C.

The meeting began at 8:30 a.m. on November 29 in the Conference Center, Building 101, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. Members of the Subcommittee are: Drs. Arnold Brown (Chairperson), Thomas Goldsworthy, Meryl Karol, Curtis Klaassen, Claudia Miller, Janardan Reddy, Irma Russo, Louise Ryan, Robert Taylor, Mary Jo Vodicnik, and Jerrold Ward. Drs. Karol, Reddy, and Vodicnik were not present, although written reviews were provided by Drs. Karol and Reddy and read into the record by the Executive Secretary. These minutes have been reviewed and approved by all members of the Subcommittee who participated. They were written by Dr. Larry G. Hart, Executive Secretary.

When available, a limited number of final NTP Technical Reports for the studies may be obtained free of charge from the NTP Central Data Management, MD A0-01, P.O. Box 12233, Research Triangle Park, N.C., 27709. Telephone: 919/541-3419. Subsequently, they may be purchased from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, Va., 22161, 703/487-4650.

The next NTP technical reports peer review meeting will be held June 20 and 21, 1995, in Research Triangle Park, North Carolina. For information, contact Dr. Hart, 919/541-3971.

Contents

2,2-Bis(bromomethyl)-1,3-Propanediol. Dr. J. K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of 2,2-bis(bromomethyl)-1,3-propanediol (BMP) by discussing the rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic lesions in rats and mice, and possible compound-related non-neoplastic lesions in rats and female mice. The conclusions for the studies were that:

> Under the conditions of these two-year feed studies, there was **clear evidence of carcinogenic activity** of 2,2-bis(bromomethyl)-1,3-propanediol (BMP) in male F344/N rats based on increased incidences of neoplasms of the skin, subcutaneous tissue, mammary gland, Zymbal's gland, oral cavity, esophagus, forestomach, small and large intestines, mesothelium, urinary bladder, lung, thyroid gland, seminal vesicles, and the increased incidence of mononuclear cell leukemia.
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> There was **clear evidence of carcinogenic activity** of BMP in female F344/N rats based on increased incidences of neoplasms of the oral cavity, esophagus, mammary gland, and thyroid gland.
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> There was **clear evidence of carcinogenic activity** of BMP in male B6C3F$_1$ mice based on increased incidences of neoplasms of the harderian gland, lung, and kidney.
>
> There was **clear evidence of carcinogenic activity** of BMP in female B6C3F$_1$ mice based on increased incidences of neoplasms of the harderian gland, lung, and subcutaneous tissue.
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> Slight increases in the incidences of neoplasms of the pancreas and kidney in male rats; forestomach in male mice; and forestomach, mammary gland, and circulatory system in female mice may have also been related to treatment.
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> Exposure of male and female rats to BMP was associated with alveolar/bronchiolar hyperplasia in the lung (males only); focal atrophy, papillary degeneration, transitional epithelial hyperplasia (pelvis), and papillary epithelial hyperplasia in the kidney; follicular cell hyperplasia in the thyroid gland (males only); hyperplasia in the seminal vesicle; mucosal hyperplasia in the forestomach (males only); and urinary bladder hyperplasia (males only). Exposure of mice to BMP was associated with hyperplasia of the alveolar epithelium in females.

Dr. Russo, a principal reviewer, agreed with the conclusions. She said there should be discussion on possible effects of the impurities detected in the compound tested on mutagenesis and carcinogenesis, and additional data on the metabolism of BMP and contaminants should be added.

Dr. Ryan, the second principal reviewer, agreed with the conclusions. She wondered as to the rationale for feed exposure since the text suggested dermal and inhalation exposures

were most likely for humans. Dr. Dunnick said the oral route was chosen to provide maximum exposure to the tissues. Dr. Ryan remarked on the large differences between the overall and adjusted incidence rates for several tumors and asked for discussion as to why. Dr. J. Haseman, NIEHS, said the adjusted rate provides an estimate of overall tumor incidence if all animals survive to the end of the study. In many cases this adjusted rate is reasonable, but it is less meaningful when there are only a few survivors as in the high dose groups of rats in the BMP study.

Dr. Miller, the third principal reviewer, agreed with the conclusions. She inquired as to how the rodent doses would compare with likely human exposures and suggested that information be added as to the sources, routes and degrees of human exposure. Dr. Dunnick responded that the one company that produces BMP had not published information on worker exposure but noted that the EPA has requested such information. Dr. J. Haartz, NIOSH, added that no information had turned up in the National Occupational Exposure Survey on BMP so there was no estimate of potentially exposed workers. Dr. Miller asked whether there should be concerns with vapor or pyrolysis products in the event of a fire. Dr. Dunnick said the chemical volatilizes at temperatures greater than 200 degrees Centigrade and at high temperatures would form hydrogen bromide.

Dr. Miller moved that the Technical Report on 2,2-bis(bromomethyl)-1,3-propanediol be accepted with the revisions discussed and with the conclusions as written for male and female rats and mice, **clear evidence of carcinogenic activity**. Dr. Ryan seconded the motion, which was accepted unanimously with seven votes.

Isobutyl Nitrite. Dr. K. M. Abdo, NIEHS, introduced the toxicology and carcinogenesis studies of isobutyl nitrite by discussing the uses of the chemical and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and non-neoplastic lesions in male and female rats and mice. The conclusions for the studies were that:

> Under the conditions of these two-year inhalation studies, there was **clear evidence of carcinogenic activity** of isobutyl nitrite in male and female F344/N rats based on the increased incidences of alveolar/bronchiolar adenomas and alveolar/bronchiolar adenomas or carcinomas (combined). There was **some evidence of carcinogenic activity** of isobutyl nitrite in male and female B6C3F$_1$ mice based on the increased incidences of alveolar/bronchiolar adenomas or carcinomas (combined) in males and females. The increased incidence of thyroid gland follicular cell adenomas in male mice may have been related to isobutyl nitrite exposure.
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> Exposure of rats and mice to isobutyl nitrite by inhalation for two years resulted in increased incidences of alveolar epithelial hyperplasia (male and female rats and mice), thyroid gland follicular cell hyperplasia and splenic hemosiderin pigmentation (male mice), and serous exudate of the olfactory epithelium of the nose (female mice).
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> Exposure of rats to isobutyl nitrite by inhalation for two years resulted in decreased incidences of mononuclear cell leukemia in males and females.

Dr. Taylor, a principal reviewer, agreed with the conclusions. He commented that a statement in the Introduction saying "there is little information ... on the carcinogenicity of isobutyl nitrite in humans" should be amended to indicate there is no information in the literature in that human data were equivocal and that data were obtained from an immunocompromised population. Dr. Abdo agreed.

Dr. Karol, the second principal reviewer, was unable to attend the meeting but had submitted her review which Dr. L. Hart, NIEHS, read into the record. Dr. Karol agreed with the conclusions. She stated that in view of a rationale for studying isobutyl nitrite being its possible contribution to the high incidence of Kaposi's sarcoma among male homosexual AIDS patients, a discussion was needed of the relevance of the findings to development of the lesions in AIDS patients. Dr. Abdo acknowledged that the primary neoplastic lesions were in the lungs while Kaposi's sarcoma is a skin lesion but noted that it is not unusual for a chemical to have different target sites in different species. Dr. R. Sills, NIEHS, commented that in laboratory rodents there are no spontaneously occuring lesions morphologically similar to Kaposi's sarcoma in humans.

Dr. Goldsworthy, the third principal reviewer, agreed with the conclusions although he thought that if further certainty could be obtained associating chemical exposure with increases in thyroid follicular cell adenomas in male mice, the conclusion in male mice could be **clear evidence**. Dr. Sills responded that these neoplasms were placed in the category of uncertain findings for several reasons, such as there were no significant increases in carcinomas, there was no dose-response for the follicular cell adenomas, and there was not a carcinogenic effect in female mice. Dr. Goldsworthy commented on the

four different lots with differing purities of isobutyl nitrite used and wondered if this could have potentially affected the results observed. Dr. Abdo reported that the lots used for the 2-year studies were 97-99% pure and it was thought that the results were not affected by the level of contaminant present.

Dr. Miller asked whether there was information on the short-term concentrations that humans would experience in using this chemical, presumably from aerosol cans. Dr. Abdo said the labels of the cans did not give concentrations but the NTP could try to obtain information from the manufacturer.

Dr. Taylor moved that the Technical Report on isobutyl nitrite be accepted with the revisions discussed and with the conclusions as written for male and female rats, **clear evidence of carcinogenic activity**, and for male and female mice, **some evidence of carcinogenic activity**. Dr. Russo seconded the motion, which was accepted unanimously with six votes (Dr. Ward was not present).

Nickel Oxide. Dr. J. K. Dunnick, NIEHS, gave a general introduction for the three nickel compounds for which draft Technical Reports were to be reviewed at this meeting. Dr. Dunnick introduced the toxicology and carcinogenesis studies of nickel oxide by describing the experimental design, reporting on survival and body weight effects, and commenting on possible compound-related neoplastic lesions in male and female rats and female mice and compound-related non-neoplastic lesions in rats and mice of both sexes. The conclusions for the studies were that:

> Under the conditions of these two-year inhalation studies, there was **some evidence of carcinogenic activity** of nickel oxide in male F344/N rats based on increased incidences of alveolar/bronchiolar adenomas or carcinomas (combined) and increased incidences of benign or malignant pheochromocytomas (combined) of the adrenal medulla. There was **some evidence of carcinogenic activity** of nickel oxide in female F344/N rats based on increased incidences of alveolar/bronchiolar adenomas or carcinomas (combined) and increased incidences of benign pheochromocytomas of the adrenal medulla. There was **no evidence of carcinogenic activity** of nickel oxide in male $B6C3F_1$ mice exposed to 1.25, 2.5, or 5 mg/m^3. There was **equivocal evidence of carcinogenic activity** of nickel oxide in female $B6C3F_1$ mice based on marginally increased incidences of alveolar/bronchiolar adenomas in 2.5 mg/m^3 females and of alveolar/bronchiolar adenomas or carcinomas (combined) in 1.25 mg/m^3 females.
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> Exposure of rats to nickel oxide by inhalation for two years resulted in inflammation and pigmentation in the lung and lymphoid hyperplasia and pigmentation in the bronchial lymph nodes. Exposure of mice to nickel oxide by inhalation for two years resulted in inflammation and pigmentation in the lung and lymphoid hyperplasia and pigmentation in the bronchial lymph nodes.

Dr. Klaassen, a principal reviewer, agreed with the conclusions. He asked why the two interim evaluations were done at seven and 15 months. Dr. Dunnick said these time points were chosen for looking at progression of lesions and lung nickel levels and are ones where we have a large data base. Dr. Klaassen inquired as to why Shirley's test was used for statistical evaluation of lung burdens of nickel. Dr. Haseman explained that since lung burden data were not normally distributed, a test like Shirley's using rank based methods was considered to be most appropriate.

Dr. Ward, the second principal reviewer, agreed with the conclusions for male and female rats and male mice but considered a level of **no evidence of carcinogenic activity** to be appropriate for female mice. He based this on lack of dose-response, no increase in multiplicity of tumors, and statistical significance only for combined adenomas and carcinomas in low-dose females. Further, the nickel burden in lung was dose-related but tumor incidence was not. Dr. Haseman commented that the lack of a dose-response in females at the top dose may be due to the significantly increased lung weights in these animals, and the significant correlation between increased lung weight and reduced lung tumor incidence, an association consistently observed in mice in the NTP nickel studies. Dr. Ward noted that chronic inflammation in lung is found in high incidence in control rats but not mice and wondered whether these spontaneous lesions were more specifically alveolar macrophages, focal, rather than chronic inflammatory lesions resulting from persistent toxins. Dr. M. Elwell, NIEHS, said the background inflammatory lesions in

controls are morphologically different from those in treated animals and are primarily increases in macrophages.

Dr. Reddy, the third principal reviewer, was unable to attend the meeting but had submitted his review which Dr. Hart read into the record. Dr. Reddy agreed in principle with the conclusions although he thought the data in female mice supported **some evidence** rather than the stated **equivocal evidence**. Further, he expressed concern that the high dose of nickel oxide, 2.5 mg/m^3 used in rats was too low, and therefore, the conclusion of **some evidence** was a conservative one.

Public Comment: Ms. D. J. Sivulka, Executive Director, Nickel Producers Environmental Research Association, Inc. (NiPERA), stated that NiPERA was a not-for-profit research organization whose main role is to support nickel related health and environmental research as well as to comment on documents pertaining to nickel produced by regulatory and advisory bodies outside of the industry. She commended the NTP for their well conducted and well reported studies and noted that NiPERA' s purpose was to offer suggestions for improvements in the Technical Reports in two main areas: (1) in their discussion of the human evidence for nickel toxicity and carcinogenesis; and (2) in their presentation of the significance of findings relative to existing TLVs. She said that many of their concerns related to conclusions in the reports being expressed in terms of existing TLVs, implying that current TLVs were inadequately protective of nickel workers. Ms. Sivulka described the cohorts of workers that have been examined and maintained that the plethora of information obtained gives no evidence of nickel-related excess non-neoplastic lesions at low doses. She concluded by stating that NiPERA's primary recommendations to the NTP were: (1) to discuss the absence of respiratory disease in humans more completely, and (2) to either omit references to TLVs in conjunction with the results of these animal studies or discuss them in the broader context of the human studies and the uncertainties of extrapolating rodent results to humans.

Discussion; Dr. Miller noted that TLVs are based to some degree on animal data. Dr. Russo asked whether any of the human studies had corrected for confounding factors such as alcohol or tobacco exposure. Ms. Sivulka said they had not but said that the incidence of neoplasia in nickel workers could not be attributed solely to factors such as cigarette smoking. Dr. Goldsworthy asked whether the practice wasn't to measure nickel without identifying the forms or species. Ms. Sivulka agreed while noting that NiPERA is supporting exposure measurements and speciation analyses. Dr. Miller stated that there should be information in the Abstract interpreting the animal studies in terms of likely human exposures. Dr. R. Griesemer, NIEHS, said the report deals with a specific set of experiments and does not review other people's animal or human studies and as such is a step in the sequence of events trying to characterize toxicity. Dr. W. Allaben, NCTR, commented that the Discussion section is the appropriate place to speculate on such issues. He said it was the regulatory agencies role to determine the significance of these studies with regard to human risk.

Dr. Klaassen moved that the Technical Report on nickel oxide be accepted with the revisions discussed and with the conclusions as written for male and female rats, **some evidence of carcinogenic activity**, for male mice, **no evidence of carcinogenic activity**, and for female mice, **equivocal evidence of carcinogenic activity**. Dr. Goldsworthy seconded the motion, which was accepted unanimously with seven votes.

Nickel Subsulfide. Dr. J. K. Dunnick, NIEHS, gave a general introduction for the three nickel compounds for which draft Technical Reports were to be reviewed at this meeting. She reported that excess risks for respiratory cancers have been reported in the nickel industry in several countries over the past 60 years but because of exposure often being to multiple nickel compounds as well as other chemicals, it has not often been possible to characterize risks of cancer from exposures to specific nickel compounds. Dr. Dunnick introduced the toxicology and carcinogenesis studies of nickel subsulfide by describing the experimental design, reporting on survival and body weight effects, and commenting on possible compound-related neoplastic lesions in rats and compound-related non-neoplastic lesions in rats and mice. The conclusions for the studies were that:

> Under the conditions of these two-year inhalation studies, there was **clear evidence of carcinogenic activity** of nickel subsulfide in male F344/N rats based on increased incidences of alveolar/bronchiolar adenomas, carcinomas, and adenomas or carcinomas (combined) and increased incidences of benign, malignant, and benign or malignant (combined) pheochromocytomas of the adrenal medulla. There was **clear evidence of carcinogenic activity** of nickel subsulfide in female F344/N rats based on increased incidences of alveolar/bronchiolar adenomas or carcinomas (combined) and an increased incidence of benign pheochromocytomas of the adrenal medulla. There was **no evidence of carcinogenic activity** of nickel subsulfide in male or female $B6C3F_1$ mice exposed to 0.6 or 1.2 mg/m^3.
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> Exposure of rats to nickel subsulfide by inhalation for two years resulted in inflammation, hyperplasia, and fibrosis in the lung; inflammation and atrophy of the olfactory epithelium in the nose; and hyperplasia in the adrenal medulla (females). Exposure of mice to nickel subsulfide by inhalation for two years resulted in inflammation, bronchialization, hyperplasia, and fibrosis in the lung and inflammation and atrophy of the olfactory epithelium in the nose.

Dr. Reddy, a principal reviewer, was unable to attend the meeting but had submitted his review which Dr. Hart read into the record. Dr. Reddy agreed with the conclusions.

Dr. Klaassen, the second principal reviewer, agreed with the conclusions. He commented that the wide dose range in rats was appropriate but needed to be discussed. Dr. Dunnick explained that the high dose was selected because there had been one other rat study at that exposure level, while the low dose represented an effect level where there was a minimal to mild lung lesion. Dr. Klaassen said there needed to be more discussion of the observation that nickel subsulfide exposure increases alveolar/bronchiolar adenomas and carcinomas in rats while resulting in decreases in these lesions in mice. Dr. Haseman commented that there was a consistent trend in the three nickel studies of an association in mice but not in rats between higher lung weights at the 15-month interim evaluation and lower lung tumor incidences at 2-years. He did not know whether or not this association was biologically significant.

Dr. Ryan, the third principal reviewer, agreed with the conclusions. She thought it would be useful to state more clearly up front why inhalation was chosen as the route of exposure since intake from food is likely to be much higher in humans. Dr. Dunnick agreed noting that the inhalation route was chosen because this is the predominant route of exposure in the workplace. Dr. Ryan asked why there were only two dose groups

instead of the usual three. Dr. Dunnick replied that when nickel subsulfide was included for this series of studies it was considered to be a positive control, based on results of a previously published study. To reduce the number of animals and exposure groups and costs, the nickel subsulfide study was limited to two exposure groups. The highest exposure concentration was selected to repeat that used in the previous study. The lower exposure was selected based on the presence of a similar spectrum and severity on histopathologic changes in the 13-week study that were present in the exposures selected for the highest dose groups in the nickel oxide and nickel sulfate studies. Dr. Goldsworthy suggested that a more appropriate way to have set doses here that might have yielded mechanistic information would have been to choose a top dose that affects pulmonary clearance compared to a low dose that does not.

Dr. Klaassen moved that the Technical Report on nickel subsulfide be accepted with the revisions discussed and with the conclusions as written for male and female rats, **clear evidence of carcinogenic activity**, and for male and female mice, **no evidence of carcinogenic activity**. Dr. Ryan seconded the motion, which was accepted unanimously with six votes (Dr. Ward was not present).

Nickel Sulfate Hexahydrate. Dr. J. K. Dunnick, NIEHS, gave a general introduction for the three nickel compounds for which draft Technical Reports were to be reviewed at this meeting. Dr. Dunnick introduced the toxicology and carcinogenesis studies of nickel sulfate hexahydrate by describing the experimental design, reporting on survival and body and lung weight effects, and commenting on possible compound-related non-neoplastic lesions in rats and mice. The conclusions for the studies were that:

> Under the conditions of these two-year inhalation studies, there was **no evidence of carcinogenic activity** of nickel sulfate hexahydrate in male or female F344/N rats exposed to 0.12, 0.25, or 0.5 mg/m^3 (0.03, 0.06, or 0.11 mg nickel/m^3). There was **no evidence of carcinogenic activity** of nickel sulfate hexahydrate in male or female B6C3F_1 mice exposed to 0.25, 0.5, or 1.0 mg/m^3 (0.06, 0.11, or 0.22 mg nickel/m^3).
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> Exposure of rats to nickel sulfate hexahydrate by inhalation for two years resulted in increased incidences of chronic active inflammation, macrophage hyperplasia, alveolar proteinosis, and fibrosis of the lung; lymphoid hyperplasia of the bronchial lymph node; and atrophy of the olfactory epithelium.
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> Exposure of mice to nickel sulfate hexahydrate by inhalation for two years resulted in increased incidences of chronic active inflammation, bronchialization (alveolar epithelial hyperplasia), macrophage hyperplasia, interstitial infiltration, and alveolar proteinosis of the lung; lymphoid and macrophage hyperplasia of the bronchial lymph node; and atrophy of the olfactory epithelium.

Dr. Taylor, a principal reviewer, agreed with the conclusions. He said one could argue that the exposure levels could have been slightly higher.

Dr. Goldsworthy, the second principal reviewer, agreed with the conclusions although he thought the doses selected might have been higher, especially in mice, and observed that doses selected for minimal to mild responses at 13-weeks resulted in minimal to mild changes at two years, including no target tissue weight changes in some circumstances. Dr. M. Elwell, NIEHS, commented that the high dose was based on the morphologic appearance of the lungs being similar to that in high dose animals in the nickel oxide study. Based on body weight decrements, he believed that a higher dose might have resulted in exceeding the MTD. Dr. Goldsworthy said that target tissue (lung) nickel concentrations were not observed at any exposure levels in mice, and, thus, exposure-dose-response linkages could not be made, limiting extrapolation of data and comparison to the other nickel studies. Dr. J. Bucher, NIEHS, explained that lung burden information would have been used if in the prechronic studies there had been a non-linearity in the increase, i.e., an overload condition was reached with a particular dose. This didn't occur, so in this case all doses were selected based on inflammatory changes in the lung and decrements in body weight gain.

Dr. Russo, the third principal reviewer, agreed with the conclusions. She stated that the lymph node hyperplasia should be documented in order to prove that the lesions represented a reactive process, either a reactive hyperplasia or a granulomatous reaction, versus monoclonal proliferation or early lymphoma.

Dr. Klaassen expressed surprise in view of the epidemiological data that the nickel compounds did not provide stronger evidence of carcinogenic activity in the NTP animal studies by the inhalation route. Dr. Dunnick noted the evidence for multiple exposures in the workplace and speculated that this could result in concurrent biologic events occurring that might enhance cancer development. Dr. Goldsworthy commented again that since toxicity did not predict or relate to carcinogenicity a good lesson for future studies with metals and inhaled toxicants should be more concern with pulmonary function. Dr. G. Lucier, NIEHS, said that the discussions regarding dose selection and how one compares studies across a class of chemicals illustrate why the NTP in its more recent study designs is incorporating mechanistic markers or toxicokinetic profiles to enable better comparisons across organs and species.

Dr. Taylor moved that the Technical Report on nickel sulfate hexahydrate be accepted with the revisions discussed and with the conclusions as written for male and female rats and mice, **no evidence of carcinogenic activity**. Dr. Klaassen seconded the motion, which was accepted unanimously with seven votes.

Triethanolamine. Dr. J. R. Bucher, NIEHS, introduced the toxicology and carcinogenesis studies of triethanolamine by discussing the uses of the chemical, describing the experimental design, reporting on survival and body weight effects, and commenting on possible compound-related neoplastic lesions in male rats and male and female mice and compound-related non-neoplastic lesions in rats and mice, most notably at the site of application including acanthosis, inflammation, and erosion, and in rats ulceration. The conclusions for the studies were that:

> Under the conditions of these dermal studies, there was **equivocal evidence of carcinogenic activity** of triethanolamine in male F344/N rats based on a marginal increase in renal tubule cell adenomas. There was **no evidence of carcinogenic activity** in female F344/N rats receiving 63, 125, or 250 mg triethanolamine/kg body weight. There was **equivocal evidence of carcinogenic activity** in male $B6C3F_1$ mice based on a marginal increase in hepatocellular adenomas and hepatoblastomas. There was **some evidence of carcinogenic activity** of triethanolamine in female $B6C3F_1$ mice based on increased incidences of hepatocellular neoplasms.
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> Dosed rats and mice had varying degrees of inflammation and acanthosis, and exposed rats had ulceration at the site of application. The presence of an infection of male mice with *Helicobacter hepaticus* confounded interpretation of the relationship of triethanolamine with liver neoplasms in these animals.

Dr. Bucher noted that the *Helicobacter* had first been described in the literature in 1994 by member of the Subcommittee, Dr. Ward. He said the infection is associated with a chronic active hepatitis in many strains of mice and appears to affect males more than females. It causes a focal necrosis and inflammation progressing to hepatocytomegaly, oval cell hyperplasia and cholangitis, and in male mice this leads to an increase in liver tumors. Dr. R. Hailey, NIEHS, described the histopathologic appearance of liver cells from infected animals and the stain used to identify the bacteria. He reported that infection with *Helicobacter* was suspected or confirmed in four other recent NTP studies in mice and the impact on study interpretation was being assessed. Dr. G. Rao, NIEHS, said the presence of the bacteria has been reported in a number of laboratories and animal production facilities around the country. However, he stated that our management procedure in production colonies of terminating and restarting colonies every two to three years made the problem self-limiting in NTP laboratories and believed that our colonies have been free of *Helicobacter* since 1991 or before.

Dr. Ward, a principal reviewer, agreed with the conclusions. He suggested that because of the infection, information on complete sources of mice including foster mothers should be added to the discussion and would help to indicate that the infection is limited to certain sources of mice. Dr. Ward thought the rationale for the dermal route was all right but said the report should indicate that the skin study was approximately equivalent to a low dose oral study because of significant skin absorption. Dr. Bucher said there was not good enough dose-response information on absorption to make quantitative comparisons of oral vs. skin absorption of the chemical.

Dr. Miller, the second principal reviewer, agreed with the conclusions although she said more clarification was needed for the casual reader as to why the level of evidence for

carcinogenicity in female mice was **some evidence** rather than **clear evidence**. Because triethanolamine is used extensively in more than 2500 cosmetics, she said the chemical also may contact mucous membranes, especially around the eyes and mouth, and suggested consideration be given to oral/mucous membrane testing. Dr. Bucher said that Japanese studies using 1% and 2% drinking water solutions did not give any strong indication of carcinogenicity. Dr. Miller wondered how the doses used would compare with doses humans might encounter, e.g., in a 5% cream applied daily to the face. Dr. Bucher estimated from a personal communication that such a human dose would not differ greatly from the dose in rats.

Dr. Karol, the third principal reviewer, was unable to attend the meeting but had submitted her review which Dr. Hart read into the record. Dr. Karol agreed with the conclusions. She said that in view of a statement in the Report that toxicity is thought to be due to the alkalinity of the chemical, a discussion is needed on effects of various solvents on alkalinity of triethanolamine and justification needed for selection of acetone as the solvent for the study. Dr. Bucher said he would add a justification for selection of acetone as solvent for the study. Dr. Karol said in view of reports that the chemical has sensitization potency, the skin lesions and 'active inflammation' should be discussed in connection with possible contact dermatitis. Dr. Bucher agreed that a case could be made for contact dermatitis being associated but in looking at the lesions histologically there wasn't much evidence that an inflammatory process had an allergic response as part of it. There were no perivascular lymphoid infiltrates or edematous reactions with eosinophilic infiltrates which might be expected if there was a contact dermatitis.

There was further discussion about the possible impact of *Helicobacter* in female mice and whether or not infection could be a confounder in the etiology of the liver lesions as in male mice. Dr. Bucher said the diagnosis of oval cell hyperplasia or karyomegaly was seen in only one mid-dose female mouse and although an exhaustive evaluation was not done, the bacteria were not believed to be a factor in female mice.

Dr. Miller moved that the Technical Report on triethanolamine be accepted with the revisions discussed and with the conclusions as written for male rats and mice, **equivocal evidence of carcinogenic activity**, for female rats, **no evidence of carcinogenic activity**, and for female mice, **some evidence of carcinogenic activity**. Dr. Russo seconded the motion, which was accepted unanimously with seven votes.

Short-Term Toxicity Study

1-Nitropyrene. Dr. J. R.. Bucher, NIEHS, opened the discussion by noting that at the last meeting (June 21, 1994), the Subcommittee had voted to defer final action on the draft Technical Report of the short-term toxicity study of 1-nitropyrene to allow for obtaining a third mail review and to allow for public comment, if any. The report is the first where the conclusion was drawn that a chemical is a likely carcinogen in the absence of neoplasms in an NTP study. Dr. P. C. Chan, NIEHS, the study scientist discussed the rationale for study, described the experimental design for nose only inhalation studies in rats and including toxicokinetic studies, and reported on compound-related lesions after 13-weeks, primarily squamous metaplasia and cytoplasmic alterations in the larynx and bronchial epithelium. He related literature reports on the mutagenicity, carcinogenicity and DNA adduct forming activity of the chemical. He said that based on our findings and also the published data by others, the NTP concludes that there is sufficient evidence that 1-nitropyrene is carcinogenic for mice and rats and that two-year nose only studies would not be conducted. Two-year nose only studies would be very costly and technically difficult to conduct. In addition, the International Agency for Research on Cancer (IARC) has determined that 1-nitropyrene is a rodent carcinogen and a possible carcinogen in humans.

Dr. Brown asked whether the IARC conclusion was known at the time that the NTP designed their study. Dr. Bucher said the evidence may have been available at the time but the nomination was for inhalation studies and there had been no demonstration that 1-nitropyrene was carcinogenic by itself when administered by the inhalation route. He stated that the study provided evidence for an appropriate dose range were a two-year study to be conducted. Dr. Brown commented that Subcommittee members had the opportunity to read the four written reviews and he sensed a consensus of approval for the report and the conclusions reached by the Program. Dr. Ryan moved that the Technical Report on 1-nitropyrene with the conclusions as written be accepted. Dr. Klaassen seconded the motion, which was accepted unanimously with seven votes.

There ensued a discussion on the procedures for future Subcommittee peer review of short-term toxicity study reports. At Dr. Taylor's suggestion, Dr. Brown read the motion that had been accepted by the Subcommittee on June 21, as follows: "Dr. Vodicnik moved that for short-term reports where there are predictions, like 1-nitropyrene, there be an initial mail review with three reviewers, two being *ad hoc*. Copies of the three reviews along with the report would be sent to the Subcommittee prior to their next meeting and at the meeting there would be a brief discussion on whether or not to accept the recommendations in the reviews. Dr. Reddy seconded the motion. As clarification, Dr. Brown said short-term reports where there was not a prediction of carcinogenicity would be reviewed by mail as they are now. The motion was accepted unanimously with eleven votes."

Dr. Ward commented that the trouble with the predictive studies is that they do not provide actual definitive data on the carcinogenicity of a chemical which in the absence of other data would have limited use for regulatory purposes. Dr. G. Lucier, NIEHS, said a workshop would be held on January 11-13, 1995, titled 'Mechanism-Based Toxicology in Cancer Risk Assessment: Implications for Research, Regulation, and Legislation' which would address this issue. Dr. Goldsworthy opined that for the reviewers to decide whether they agree with the 'prediction' they may need more than just the 13-week study results. Dr. Miller said that there needs to be a prominent statement in the front of these reports

that says these are not risk assessment documents although they may be used by others in the risk assessment process. Dr. Lucier agreed and said that a paragraph could be added to the "Note to the Reader" on the inside front cover stating what these studies are intended to do and not intended to do.

These Summary Minutes (for the November 29, 1994, meeting) have been read and approved by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee. They are certified by the Chair of that Subcommittee below.

Date	Dr. Arnold Brown, Chair National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee