Board of Scientific Counselors
National Toxicology Program

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

on

October 30, 1998

Research Triangle Park, NC

The meeting began at 9:00 a.m. on October 30, 1998 in the Conference Center, Building 101, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina Members of the Subcommittee are: Drs Gary Carlson (Chairperson), John Bailer, Steven Belinsky, James Bus, Linda Chatman, John Cullen, Susan Fischer, Thomas Goldsworthy, Stephen Hecht, Michele Medinsky, and Jose Russo. Drs. Chatman, Fischer, Goldsworthy, and Russo were not present. These minutes have been reviewed and approved by the Chairperson. They were written by Dr. Larry G. Hart, Executive Secretary.

When available, a final NTP Technical Report for the studies may be obtained through the Environmental Health Information Service (EHIS). Call 919-541-3841, Fax 919-541-0273, e-mail at ehis@nih.gov, or subscribe on line at ehis@niehs nih.gov.

The next NTP Technical Reports Peer Review meeting will be held May 21, 1999, in Research Triangle Park, North Carolina For information, contact Dr. Hart, 919-541-3971.

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NTP Technical Report Reviews

Triethanolamine. Dr. J.R. Bucher, NIEHS, introduced the toxicology and carcinogenesis studies of triethanolamine by discussing the rationale for rereviewing the draft Technical Report, which had been reviewed by the Subcommittee in December 1994. The triethanolamine studies were the first to give evidence of *Helicobacter hepaticus* infection in B6C3F$_1$ mice, so the decision was made to intensively analyze the mouse studies following guidelines proposed in December 1996 at a meeting of the Subcommittee. It had been reported that *Helicobacter* infection tended to increase the incidence of mouse liver tumors, especially in males. Dr. Bucher noted that mice, both controls and dosed animals, had been affected by *Helicobacter* infection in eight other NTP studies and the draft Reports of the two-year bioassays for these studies had been reviewed previously. He reviewed the clinical and morphological signs which include the characteristic liver lesions associated with hepatitis, primarily again in males, and the hepatitis seemed to be driving the increases in liver tumors. The guidelines are found in an appendix to the technical report and state that in infected animals where greater than 10 % of animals display the characteristic lesions, the interpretation of increased incidences of hepatocellular neoplasms and hemangiosarcomas in the liver of male mice is considered potentially confounded. For female mice, because hepatitis is observed only rarely, interpretation of chemically-induced neoplastic effects in the liver is not considered confounded.

Using these guidelines, Dr. Bucher turned to the triethanolamine studies, describing the study design, reporting on survival and body weight effects, and commenting on compound-related neoplastic lesions in mice and possibly in male rats, and nonneoplastic lesions in rats and mice. The conclusions for the two-year studies in rats and mice 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 the incidence of renal tubule cell adenoma. There was **no evidence of carcinogenic activity** in female F344/N rats receiving 63, 125, or 250 mg/kg of body weight. The study in male B6C3F$_1$ mice was considered an **inadequate study of carcinogenic activity** because the presence of a *Helicobacter hepaticus* infection complicated interpretation of the relationship between triethanolamine administration and liver neoplasms in these animals. 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 acanthosis and inflammation, and dosed rats had ulceration, at the site of skin application.

Dr. Bucher pointed out that the change in the conclusions from the original review was to change the conclusions in male mice from **equivocal evidence of carcinogenic activity** to **inadequate study** because of the presence of the *Helicobacter hepaticus* infection.

Dr. Belinsky, a principal reviewer, agreed in principle with the conclusions in rats and male mice while wanting to reserve judgement on female mice until after more discussion about the possible confounding by *Helicobacter*. He thought there should be discussion about the

significance of the findings of renal tubule adenomas in male rats, and suggested that to the sentence in the conclusions: "there was **equivocal evidence of carcinogenic activity** of triethanolamine in male F344/N rats based on a marginal increase in the incidence of renal tubule cell adenoma", should be added "that was not dose related."

Dr. Hecht, the second principal reviewer, agreed with the conclusions in rats and male mice but wanted to reserve judgement on the conclusions in female mice. He was concerned about the possible presence of nitrosodiethanolamine, a rodent carcinogen, as a contaminant. This chemical can be readily formed from triethanolamine in the presence of nitrite. Dr. Bucher responded that the positive cancer findings with nitrosodiethanolamine were in rats and there was no component of the response with triethanolamine that resembled responses seen with nitrosodiethanolamine so this chemical was not believed to be a contributor to findings with triethanolamine.

Dr. Fischer, the third principal reviewer, was unable to attend the meeting but had submitted her review, which Dr. L. Hart, NIEHS, read into the record. Dr. Fischer agreed with the conclusions. She questioned the decision to set doses for the 2-year study in female mice at only half of the doses set for male mice, noting that in the 13-week studies the incidence of chronic inflammation is actually less in females with the exception of one animal at the highest dose (2,000 mg/kg) than in males with the exception of one female mouse in the high dose group. Dr. Bucher said it was that one female mouse that influenced choice of 1,000 mg/kg as high dose for female mice in the chronic study.

Dr. Bailer said that given that we were revisiting the study regarding hepatic tumors in mice it would be appropriate to examine other tumors such as alveolar/bronchiolar adenomas and carcinomas in female mice. Dr. J. Haseman, NIEHS, commented that these tumor rates fell within the historical control range and reflected normal variation one would expect to see in a large study Dr. Cullen noted a recently published study reporting an increased rate of hepatocyte cell replication in *Helicobacter* infected female A/JCr mice and wondered how that related to the findings in female mice in the current study. Dr. J. Hailey, NIEHS, responded that in the published study, there were no statistically significant increases in hepatic cell proliferation in females at any time point but there were significant increases in males at all time points. Dr. Haseman added that the hepatic tumor effect in female mice was not subtle, the 82 % incidence in the top dose group was well above control rates for most of the NTP studies

Public Comment: Dr. Raymond Papciak, Huntsman Corporation, representing the Alkanolamines Panel of the Chemical Manufacturers Association (CMA), stated that both the NTP and the CMA have designed protocols for a repeat dermal chronic study of triethanolamine in male and female mice. He said the primary disagreement between them had to do with whether or not the findings in female mice were compromised by the infection. Dr. Papciak reported that Dr James Fox, MIT, a consultant to the CMA, in analyzing the mouse liver data concluded that increased tumor incidences in male and female mice demonstrate a similar response when one looks at true infection and not hepatitis. Thus, he urged reclassification of the findings in female mice.

Dr. Linda Loretz, Cosmetic, Toiletry, and Fragrance Association (CTFA), stated that there was good agreement that the female mice in the triethanolamine studies were infected with *Helicobacter* and it was clear that the hepatitis did not correlate with the infectious status of the animals. She said the differences between males and females could not be explained solely on the basis of hepatitis. Dr.Loretz also urged that the study in female mice be reclassified as an inadequate study.

Dr. Frank Mirer, International Union of United Auto, Aerospace and Agricultural Workers of America (UAW), had submitted public comments which Dr. Hart read into the record. Dr. Mirer reported that triethanolamine is an important component of synthetic and semi-synthetic metalworking fluids. NIOSH estimates that up to one million American workers have occupational exposure and these fluids are subject of a current OSHA Standards Advisory Committee. He said the UAW believes that appearance of tumors remote from site of application is very strong evidence for public health risk. The UAW concurred with the conclusions in female mice and believed a higher top dose should have been used in the rat studies. Further, they believed an inhalation study would have given clearer evidence of hazards in the workplace.

Dr. Hailey said he wanted to clarify the NTP position on the interpretation of the liver pathology in mice. The NTP position is that while males and females may be equally infected, males are clearly more susceptible to development of liver disease. He said that all of the reports to date indicate that the increased incidence of liver neoplasms in association with *Helicobacter* have been in male, not in female mice. Dr. Haseman commented that when comparing tumor rates in infected studies and uninfected studies, it is important to consider that in the context of a generally increasing tumor trend over time, the infected studies are by and large more recent studies and uninfected studies are earlier studies where you would expect a lower tumor rate. Thus, he said if tumor rates in studies of equivalent time frame are compared, the apparent elevation of liver tumors in female mice is much less impressive. Dr. Bailer observed that if one accounts for all of the animals with infection, there are still concentration-related increases in liver tumors. Dr. Bus commented that there is the possibility here that a subclinical infection, i.e., *Helicobacter*, might somehow promote chemical tumorigenesis, and this will only be resolved by repeat studies that are uncontaminated with the organism. Dr. Bucher asked the members as a parting consideration to consider the strength of the data that relates liver tumor increases with *Helicobacter* induced hepatitis versus the strength of the evidence they have seen today that relates to an increase of liver tumor incidence with the presence of the organism.

Dr. Belinsky moved that the Technical Report on triethanolamine be accepted with revisions discussed and the conclusions as written for male rats, **equivocal evidence of carcinogenic activity** based on a marginal increase in the incidence of renal tubule cell adenoma with the addition of the phrase "that was not dose related." He moved that the conclusions be accepted as written for female rats, **no evidence of carcinogenic activity**, and for male mice, **inadequate study of carcinogenic activity**, and that the conclusion for female mice be changed to **inadequate study of carcinogenic activity**. Dr. Hecht seconded the motion. In discussion, Dr. Cullen suggested that equivocal evidence indicates there is no dose relationship and adding the phrase to the basis for the conclusion in male

rats is unnecessary. Dr. Belinsky agreed and the phrase was dropped from the motion. Dr. Cullen also thought the evidence presented made it likely that there was no effect of the organism in female mice so he would support the conclusion as written for female mice, **some evidence of carcinogenic activity.** Dr. Belinsky's motion was accepted by three yes votes (Belinsky, Hecht, Medinsky) to two no votes (Bailer, Cullen) with one abstention (Bus). Dr. Bus abstained for reasons of company affiliation.

Methyleugenol. Dr. K. M. Abdo, NIEHS, introduced the toxicology and carcinogenesis studies of methyleugenol by discussing the uses and rationale for study, describing the experimental design in mice and rats, 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 two-year studies in rats and mice were that.

> Under the conditions of these 2-year gavage studies, there was **clear evidence of carcinogenic activity** of methyleugenol in male and female F344/N rats based on the increased incidences of liver neoplasms and neuroendocrine tumors of the glandular stomach in male and female rats and the increased incidences of kidney neoplasms, malignant mesothelioma, mammary gland fibroadenoma, and subcutaneous fibroma and fibroma or fibrosarcoma (combined) in male rats. A marginal increase in squamous cell neoplasms of the forestomach may have been related to methyleugenol administration in female rats. There was **clear evidence of carcinogenic activity** of methyleugenol in male and female $B6C3F_1$ mice based on the increased incidences of liver neoplasms in males and females. Neuroendocrine tumors of the glandular stomach in male mice were also considered related to exposure to methyleugenol.
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> In male and female rats and mice, methyleugenol administration caused significant increases in non-neoplastic lesions of the liver and glandular stomach.

Dr. R. A. Herbert, NIEHS, characterized the spectrum of lesions in the fundic region of the glandular stomach associated with methyleugenol administration in both sexes of both species These included atrophy, neuroendocrine cell hyperplasia, and benign and malignant neuroendocrine tumors; tumors which are rare in rats and mice both as spontaneous or chemically induced lesions. Dr. Herbert described a series of short-term studies (14, 30 and 90-day) that provided data which seemed to support the hypothesis that parietal cell cytotoxicity with subsequent mucosal atrophy, increased intragastric pH, and increased circulating gastrin (hypergastrinemia) is probably the mode of action through which methyleugenol produces neuroendocrine tumors in the glandular stomach.

Dr. M.L. Cunningham, NIEHS, presented data from work in progress that described *in vivo* and *in vitro* studies of methyleugenol metabolism in rodents and some recent results from human model systems. He began by describing the more widely studied metabolism of the close structural analogue and hepatocarcinogen, safrole, and contrasted the results with those obtained for methyleugenol. The findings to date indicate that methyleugenol can undergo a variety of Phase 1 oxidation reactions, metabolites can be further metabolized through Phase 2 conjugations to yield reactive sulfonoxy metabolites, and human tissue preparations are capable of metabolizing and bioactivating the chemical. The genetic

toxicity of methyleugenol is very similar to safrole and for both compounds appears to be dependent on both Phase 1 and Phase 2 metabolic activation.

Dr. T. R. Devereux, NIEHS, said she would provide information on molecular alterations in tumors from the NTP study to better understand the tumor response to chemical treatment, concentrating on the mouse liver and lung tumors for which there is a large database of genetic information. She said their focus was on the APC/ß-catenin-Wnt signaling pathways that have been implicated in various human and rodent cancers. In tumor cells, either a mutation in the APC gene or in ß-catenin can upregulate ß-catenin and the Wnt signaling pathway leading eventually to cell proliferation. ß-Catenin mutations were found in about half of the methyleugenol mouse liver tumors compared with mutations in only five per cent of spontaneous tumors. Mutations were found at the same sites as those in human hepatocellular carcinomas suggesting similar carcinogenic pathways. Genetic alterations were not found in H-*ras* or p53 suggesting these genes are not involved in methyleugenol induced mouse liver carcinogenesis.

Dr. G. M. Blumenthal, NIEHS, discussed the development of physiologically based pharmacokinetic models (PBPK) to describe and simulate the toxicokinetics of methyleugenol in rats and humans. Animal data were obtained from single dose intravenous administration to rats at the low study dose and by gavage at all three study doses. Human data were obtained from an in-house study where volunteers ate 12 ginger snaps and blood samples were collected prior to and at 15 and 30 minutes, and one and two hours afterwards. Data was also obtained from the NHANES database collected by the Centers for Disease Control and Prevention (CDC). The studies to date showed that absorption of methyleugenol was rapid in rats and humans with a large first pass effect in rats, and assumed in humans. Metabolism is saturating at all study doses in rats, while a slower metabolism is predicted in humans. Over 90% of the doses are metabolized within 24 hours in rats, and as modeled also in humans More studies are in process which should eventually lead to an entire dose response characterization.

Dr. Hecht, a principal reviewer, agreed with the conclusions. He wondered why considering the structural similarity to safrole and human exposure to methyleugenol that the NTP had not considered this chemical for study a lot earlier.

Dr Cullen, the second principal reviewer, agreed with the conclusions. He thought the study was remarkable because of the presence of two unusual kinds of neoplasms. In the liver, mixed neoplasms composed of cholangiocellular and hepatocellular elements are very unusual and suggest a potent carcinogenic effect that affects both biliary and hepatic cell lineage or, possibly, a stem cell population. He said that gastric neuroendocrine neoplasms are also very uncommon and thought it prudent that immunohistochemical and histochemical stains were done to establish the cell type. Dr. Herbert noted that within some of the liver tumors there seemed to be a hepatocellular component and a biliary cell component, and, thus, the diagnosis of hepatocholangiocellular tumor was most descriptive. Dr. Cullen said the dose related increases in oval cell hyperplasia in mice suggested further discussion of the possibility of a synergistic effect with the presence of *Helicobacter* and this lesion.

Dr. Bus, the third principal reviewer, agreed with the conclusions. He complimented the NTP on the extensive toxicokinetic and disposition studies including information on how disposition may change with time and age of the animals. Noting that the low dose in the rodent studies, 37 mg/kg, was metabolically saturating, and likely not a No Observed Effect Level (NOEL), Dr. Bus suggested that there were lessons here for future protocol designs to provide data more valuable for future risk assessment purposes. Dr. Bucher commented that methyleugenol is a Generally Recognized as Safe (GRAS) listed substance in the U.S. , and although there is a 5 mg/kg limit in Europe, there are not large differences between levels permitted in foods and the low dose in rodents. Palatability may be the limiting factor.

Dr. Medinsky cautioned that bioavailability of a compound is less relevant when a metabolite is the active/toxic form. Dr. Bailer commented that he was a bit uncomfortable with possible utility of data for risk assessment purposes when the lowest animal dose is ~ 37, 000 times the human dose (the ginger snap study). Dr. Lucier pointed out that the blood levels from the NHANES study were only about 1,000 fold greater than the rat blood levels.

Public Comment. Dr. Tim Adams, Flavor and Extract Manufacturers Association (FEMA), stated that actual exposure to methyleugenol has substantially decreased over the last 30 years with most coming from fruits and spices. Further, their estimate is that exposure from the diet exceeds intentional addition by a factor of at least 100. With regard to the neuroendocrine lesions in the stomach, he wanted to note that the agent was given by gavage in a microencapsulated form perhaps allowing for prolonged exposure in the stomach. Dr. Bucher said the dose material was in methylcellulose and not microencapsulated.

Dr. Hecht moved that under the conditions of this study the Technical Report on methyleugenol be accepted with revisions discussed and the conclusions as written for male and female rats and mice, **clear evidence of carcinogenic activity**. Dr. Cullen seconded the motion, which was accepted unanimously with six yes votes.

2-Butoxyethanol (Ethylene Glycol Monobutyl Ether) Dr. J H. Roycroft, NIEHS, introduced the toxicology and carcinogenesis studies of 2-butoxyethanol (2-BE) by discussing the uses and rationale for study, describing the experimental design in mice and rats, reporting on survival and body weight effects, and commenting on compound-related neoplastic lesions in male and female mice and non-neoplastic lesions in male and female rats and mice. In addition to the standard core study, because of important hematological effects, a number of animals were assessed for hematological parameters and bone marrow cellularity and myeloid to erythroid ratios at 3, 6, and 12 months. Additionally, a number of animals were included in the design for toxicokinetic measures of 2-BE and its principal metabolite 2-butoxyacetic acid (2-BAA). Hemolytic effects of 2-BE have been attributed to 2-BAA. The conclusions for carcinogenic activity for the two-year studies in rats and mice were that:

> Under the conditions of these 2-year inhalation studies, there was **no evidence of carcinogenic activity** of 2-butoxyethanol (2-BE) in male F344/N rats exposed to

31.2, 62.5 or 125 ppm. There was **equivocal evidence of carcinogenic activity** 2-BE in female F344/N rats based on the increased combined incidences of benign or malignant pheochromocytoma (mainly benign) of the adrenal medulla. There was **some evidence of carcinogenic activity** of 2-BE in male $B6C3F_1$ mice based on increased incidences of hemangiosarcoma of the liver. A marginal increase in the incidences of forestomach squamous cell papilloma may have been exposure related. There was **some evidence of carcinogenic activity** of 2-BE in female $B6C3F_1$ mice based on increased incidences of forestomach squamous cell papilloma or carcinoma (mainly papilloma). Increased incidences of forestomach neoplasms in male and female mice occurred in groups in which ulceration and and hyperplasia were also present.

Exposure to 2-BE caused a regenerative anemia and effects secondary to the anemia.

Dr. Roycroft reviewed some of the findings from the toxicokinetic studies performed during the course of the 2-year studies. The area under the curve (AUC) for 2-BE increased proportionately with increased exposure concentrations in rats and mice, while the AUC for 2-BAA increased non-proportionately. 2-BE is rapidly cleared from the blood of both species and half lives are comparable to those measured by other labs. Mice eliminate 2-BE and 2-BAA faster than rats, and female rats elimnate 2-BAA slower than male rats. Female mice have slightly higher 2-BAA blood concentrations than male mice; however, females eliminate 2-BAA slightly faster than males, so there wasn't much difference in overall kinetics, and also in hematological effects. Finally, with repeated exposures, 2-BE and 2-BAA elimination becomes slower in both species.

Dr. Medinsky, a principal reviewer, agreed in principle with the conclusions. Her concern was that the conclusions on carcinogenicity for male mice and female rats were made based not on differences in the response of the test animals from their respective controls but rather based on differences in response compared with historical control values. She wondered what objective statistical measure of differences was used. Dr. Roycroft responded that we don't as a rule compare tumor data with historical control data statistically as there are so many factors that can vary from study to study. The concurrent controls are still considered the most appropriate control group. Dr. Haseman said there are a host of things, e.g., were there increases in preneoplastic lesions, factored into a decision. Dr. Medinsky commented that one of the strengths of the report were the comprehensive sections on the chemical disposition and toxicokinetics of 2-BE and 2-BAA, however, for the average reader, a summary paragraph would be helpful which attempts to synthesize and distill the information into a few main points highlighting the importance of these studies for understanding toxicity results. Dr. Roycroft agreed to try and provide a summary of the PBPK and chemical disposition data

Dr Bailer, the second principal reviewer, agreed with the conclusions for rats but not for mice. He thought that not enough consideration was given to the strong significant and dose-related trends in analyzing the tumor data in mice. Thus, Dr. Bailer asked for clarification as to why the findings did not support a level of **clear evidence** in the mice. With regard to male mice, Dr Roycroft said benign and malignant tumors are analyzed

independently and combined with the most important being the combined, which for liver adenomas and carcinomas gave no increases. Increases in carcinomas alone were within the historical control rates. Dr. Hailey noted that whether or not more emphasis is given to the combined tumor incidence depends somewhat on the tumor type. With liver tumors, there is a morphological and biological continuum of progression from foci to adenomas and to carcinomas. Further, it is often difficult to discern between a benign and malignant neoplasm.

Dr. Cullen, the third principal reviewer, agreed with the conclusions. He said the reliance on the historical control incidence and lack of concordant increase in preneoplastic and benign lesions support the view that there is less than **clear evidence** in the liver of male mice but the data appear to reflect at least **equivocal evidence**. In female mice, the conclusions regarding squamous papillomas are appropriate. Dr. Cullen commented that some of the toxic effects attributed directly to action of the chemical might be addressed as secondary responses due to other insults created by the chemical, e.g., anemia in response to blood loss from gastric ulceration in female, and perhaps, male mice. He thought a table giving a range of normal values for various hematologic parameters would be helpful.

Public Comment. Dr. Tipton R. Tyler, Chemical Manufacturers Association (CMA) Ethylene Glycol Ethers Panel, stated that it has long been recognized that the most characteristic toxicological effect of 2-BE is as a hemolytic agent with humans being less susceptible than rodents. With regard to the forestomach tumors in female mice, they believed that **some evidence** was probably correct, but likely has no relevance since there is no such organ in humans. With regard to pheochromocytomas in female rats, he asked the Subcommittee to reconsider the designation of **equivocal evidence**, as there were no statistically significant pairwise comparisons, the incidence was barely outside the historical control range, and there was no indication of increased incidence in males.

Dr. Rodney Boatman, Eastman Chemical Company, said he wanted to comment on the findings for hemangiosarcomas of the liver in male mice, and propose that this represents a marginal or equivocal finding. He compared the results for this study with those from the NTP bioassay of p-nitroaniline, for which very similar incidences of hemangiosarcomas of the liver in male mice were classified as **equivocal evidence**. Further, he stated that the possibility that the study was compromised by *Helicobacter* infection cannot be ruled out. Dr. Medinsky asked for staff comment on the *p*-nitroaniline study. Dr. Roycroft responded that the p-nitroaniline study was a gavage study and the historical control range and high incidence for gavage studies at the time were slightly higher than the high incidence and range for the current inhalation study.

Dr. Medinsky moved that under the conditions of this study the Technical Report on 2-butoxyethanol be accepted with revisions discussed and the conclusions as written for male rats, **no evidence of carcinogenic activity**, for female rats, **equivocal evidence of carcinogenic activity**, and for male and female mice, **some evidence of carcinogenic activity**. Dr. Bailer seconded the motion Dr. Cullen asked whether a sentence could be added to the conclusion for male mice that there was a dose-related increase in malignant hepatocellular tumors. Dr. Roycroft noted that there already was a sentence on the marginal increases in the incidences of forestomach tumors and mention of the male mouse

liver tumors could be added into that sentence. There was consensus that addition would be acceptable. The motion was accepted with five yes votes and one abstention (Bus). Dr. Bus abstained for reasons of company affiliation.

Glutaraldehyde. Dr. A. van Birgelen, NIEHS, introduced the toxicology and carcinogenesis studies of glutaraldehyde by discussing the uses and rationale for study, describing the experimental design in mice and rats, reporting on survival and body weight effects, and commenting on compound-related non-neoplastic lesions in male and female rats and mice. The conclusions for the two-year studies in rats and mice were that:

> Under the conditions of these 2-year inhalation studies, there was **no evidence of carcinogenic activity** of glutaraldehyde in male or female F344/N rats exposed to 250, 500, or 750 ppb. There was **no evidence of carcinogenic activity** in male or female B6C3F$_1$ mice exposed to 62.5, 125, or 250 ppb.
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> Incidences of non-neoplastic lesions of the nose were significantly increased in male and female rats and mice.

Dr. Belinsky, a principal reviewer, agreed with the conclusions. He commented that given the high reactivity of this chemical, it was unlikely that any significant amount reached the other organs of either species. If the concern for human exposure is truly restricted to inhalation then the studies are probably adequate; however, if dermal exposure is also an issue then one would have to consider other routes. Dr. van Birgelen said that it was plausible that the chemical does not get beyond the nose but lacking toxicokinetic data we can't be sure. Dr. Belinsky's other concern was the apparent inadvertent caloric restriction. Both this issue and that of tissue distribution need be incorporated further in the discussion. Dr. van Birgelen said that mild decreases in body weight gain , especially in female rats, may be related to decreases in mammary gland and pituitary gland tumors.

Dr. Bus, the second principal reviewer, agreed with the conclusions. He disagreed with the conclusions for genetic toxicity in that positive findings were reported for *Salmonella*, sister chromatid exchange induction in Chinese hamster ovary cells, and chromosomal aberrations in mouse bone marrow *in vivo* He said that inconsistencies and lack of dose response supported an equivocal conclusion for all. Dr. van Birgelen explained how the genotoxicity results are arrived at, noting that test results from different laboratories are not combined. She said the results on all three types of assays supported a positive finding but agreed that the conclusion for chromosomal aberrations should be changed to weakly positive. Dr. Bus commented that the section attempting to make delivered dose comparisons between glutaraldehyde and formaldehyde might not be valid in the absence of comparative distribution data.

Dr. Medinsky, the third principal reviewer, agreed with the conclusions. She noted that structure activity relationships are an important component of toxicology research in helping to explain why similar chemicals may have different toxic/carcinogenic endpoints. Dr. Medinsky said the observation that the more reactive glutaraldehyde is primarily deposited in the anterior portion of the nose while formaldehyde deposition deeper in the upper respiratory tract at least in part explains the marked differences in carcinogenic

activity, and there should be more discussion about this. Dr. van Birgelen agreed that there could be more discussion on the comparisons between glutaraldehyde and formaldehyde, and this would be added.

Public Comment. Ms. Janet Kenepp and Ms. Sharon Sowers, operating room nurses from New Holland, Pennsylvania, spoke on behalf of a chemical injury support group called WASTE (Workers Against Senseless Toxic Exposure). Ms. Kenepp stated that hundreds of healthcare professionals had been exposed to glutaraldehyde in its use as a cold sterilant while not being warned of its toxic effects or being trained in its proper use and disposal. She described many health effects that she ascribed to exposure including increased sensitivity to effects of other chemicals. Ms. Sowers listed several unanswered questions pertaining to lack of regulations or controls on the use of glutaraldehyde in the workplace. She noted concerns raised by the NIH and EPA regarding possible health effects and the need for more research on toxic and carcinogenic effects in humans.

Dr. Bus moved that under the conditions of this study the Technical Report on glutaraldehyde be accepted with revisions discussed and with the conclusions as written for male and female rats and mice, **no evidence of carcinogenic activity**. Dr. Medinsky seconded the motion, which was accepted unanimously with five yes votes.

Oxymetholone. Dr. W. Eastin, NIEHS, introduced the toxicology and carcinogenesis studies of oxymetholone by discussing the uses and rationale for study, describing the experimental design in rats, reporting on survival and body weight effects, and commenting on compound-related neoplasms and non-neoplastic lesions in male and female rats. Dr. Eastin also discussed 16-day and 13-week studies in male and female $B6C3F_1$ mice. The results of the 13-week oral gavage studies were generally similar in rats and mice, but rats were much more sensitive to oxymetholone. Because there were data available in humans and because it was considered not likely that a long-term mouse study would provide significant additional toxicity information, the NTP decided to conduct a two-year study in rats only. The conclusions for the two-year studies in rats were that:

> Under the conditions of this 2-year gavage study, there was **equivocal evidence of carcinogenic activity** of oxymetholone in male F344/N rats based on increased incidences of subcutaneous tissue fibroma and fibroma or fibrosarcoma (combined) of the skin, variably increased incidences of benign and benign or malignant pheochromocytoma (combined) of the adrenal gland, and increased incidences of renal tubule adenoma. There was **clear evidence of carcinogenic activity** of oxymetholone in female F344/N rats based on increased incidences of hepatocellular neoplasms. Increased incidences of alveolar/bronchiolar neoplasms and skin neoplasms in female rats were also related to oxymetholone administration.
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> Decreased incidences of testicular adenoma in males; uterine stromal polyp or stromal sarcoma (combined), mammary gland neoplasms, and pituitary gland pars distalis adenoma in females; and mononuclear cell leukemia in males and females were related to oxymetholone administration.

In addition, gavage administration of oxymetholone to male and female F344/N rats resulted in a spectrum of nonneoplastic effects frequently reported with administration of synthetic anabolic androgens.

Dr. Fischer, a principal reviewer, was unable to attend the meeting but had submitted her review, which Dr. Hart read into the record. Dr. Fischer agreed with the conclusions. She thought the comparison of the rodent results with studies in humans was thorough and enhanced confidence in the conclusions. Dr. Fischer questioned that increased incidence of lung neoplasms in the 30 mg/kg group of females be considered treatment related when there was no significant increase in these tumors in the 100 mg/kg group.

Dr. Bailer, the second principal reviewer, agreed with the conclusions. Since a complete necropsy and microscopic examination was performed on all rats, he wondered if all rat data, including interim sacrifice data, should be included in test of tumorigenic trends, i.e., should this be a routine default analysis? Dr. Haseman responded that indeed we do carry out statistical analyses that include the interim sacrifice data but do not usually include it in the report unless it affects the overall interpretation of the data, as it does present some difficult problems in terms of data presentation and comparison with historical control data based on a regular two-year study. Further, interim sacrifices have few if any tumors. Dr. Bailer noted the statement that "there is a strong correlation between a chemical's electrophilicity, mutagenicity in *Salmonella* , and carcinogenicity in rodents" and suggested that while this may be true for some chemical classes, is it true for all classes? Dr. Eastin said we would clarify this and modify the statement if necessary.

Dr. Cullen, the third principal reviewer, agreed in principle with the conclusions. He thought the lack of dose-related response for hepatocellular neoplasms in female rats suggested **some evidence** rather than **clear evidence**. Dr. Eastin commented that as a synthetic anabolic steroid analog of testosterone which has complicated and divergent biological effects, it makes interpretation of tumor responses difficult. The conclusion for liver neoplasms in female rats was based on the rarity of these tumors in female rats, and especially for carcinomas. Dr. Bailer observed that he would not say there is no dose response but rather, there is not a linear dose response. Dr. Cullen said that given the IARC statement that there is limited evidence of human carcinogenicity for anabolic compounds and the fact that there is a paucity of data on carcinogenicity of oxymetholone in animals, it would have been useful to have more information on mice, and especially for mouse liver.

Dr. Bailer moved that under the conditions of this study the Technical Report on oxymetholone be accepted with revisions discussed and with the conclusions as written for male rats, **equivocal evidence of carcinogenic activity**, and for female rats, **clear evidence of carcinogenic activity**. Dr. Hecht seconded the motion. Dr. Cullen said that based on the definition of **clear evidence** and lack of a clear dose-response, he offered an amendment to change the conclusion in female rats to **some evidence of carcinogenic activity**. Lacking a second, the amendment was tabled. Dr. Bailer's original motion was accepted with four yes votes to one no vote (Cullen).