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

December 5, 1995

Research Triangle Park, N.C.

The meeting began at 8:30 a.m. on December 5 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), Gary Carlson, Thomas Goldsworthy, Robert LeBoeuf, Janardan Reddy, Irma Russo, Louise Ryan, Robert Taylor, Frederick Tyson, and Jerrold Ward. Drs. Reddy and Ward were not present, although written reviews were provided by them and were 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 December 11 and 12, 1996, in Research Triangle Park, North Carolina. For information, contact Dr. Hart, 919/541-3971.

Contents

D & C Yellow No. 11. Dr. W. C. Eastin, NIEHS, introduced the toxicology and carcinogenesis studies of D & C Yellow No. 11 by discussing the uses 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. Dr. Eastin reported that the study of this color additive was part of a larger effort mandated by Congress and undertaken by the FDA to determine the safety of provisionally listed dyes. The study design was not a standard NTP protocol. In discussions with the nominator, FDA, the NTP decided to tailor the protocol to provide perinatal exposure followed by dietary exposure for two years in order to generate data similar to those used by FDA to regulate other color additives. The conclusions for the two-year studies in rats were that:

> Under the conditions of this feed study, perinatal exposure followed by exposure for two more years, there was *some evidence of carcinogenic activity* of D & C Yellow No. 11 in male and female F344/N rats based on increased incidences of hepatocellular adenoma in males, hepatocellular adenoma or carcinoma (combined) in females, increased incidences of renal tubule adenomas and renal tubule adenoma or carcinoma (combined) in males, and squamous cell papilloma and squamous cell papilloma or squamous cell carcinoma (combined) of the oral cavity in males.
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> Exposure of rats in D & C Yellow No. 11 in feed for two years resulted in increased incidences of nonneoplastic liver lesions including clear cell foci, cytologic alterations of hepatocytes, and bile duct, hepatocyte, and Kupffer cell pigmentation in males and females and mixed cell foci in males. In the kidney, there were increased incidences of renal tubule pigmentation and transitional epithelial hyperplasia in males. The severity of nephropathy was increased in exposed males and females.

Dr. Reddy, a principal reviewer, was unable to attend the meeting but had submitted his review, which Dr. Hart, NIEHS, read into the record. Dr. Reddy agreed with the conclusions. He said the Abstract should give the reasons for using rats only in this study.

Dr. Russo, the second principal reviewer, agreed with the conclusions. She also thought there needed to be clarification of why concurrent studies were not done in mice. Dr. Eastin said that in prechronic studies effects on mice were about the same as in rats, though in all endpoints measured, rats were the more sensitive species. To have done both species with the larger perinatal protocol would have diverted resources from studying another chemical. Conducting the study in the more sensitive species would meet the FDA's needs. Dr. Eastin said he would add this information to the report.

Dr. Carlson, the third principal reviewer, agreed with the conclusions. He commented that he had problems with the perinatal protocol from this and other such studies and claims made about effects of *in utero* particularly when there are not groups treated only post weaning for comparison. Dr. Eastin agreed that these groups would have been useful as well as animals treated only *in utero* and followed out for two years. Further, Dr. Carlson said the discussion mentions positive findings, so for balance negative findings should be cited. Dr. Eastin reported that there were only three other NTP studies with prenatal or

perinatal exposures. More discussion will be added on the findings. Dr. Carlson said he was intrigued by the description of head swelling and edema, and asked for more information on etiology. Dr. A. Radovsky, NIEHS, said that they had looked for possible hyperproteinemia secondary to kidney or liver disease or intestinal malabsorption, and vascular or heart lesions. All of these conditions were present in some animals with edema but not all, nor was severity of kidney or liver disease any worse in these animals than in cohorts without the edema. Thus, she said from an anatomic histopathologic perspective, there was no explanation.

Dr. LeBoeuf noted a weight reduction at the top dose in males of about 15%, and wondered if this was typical or acceptable for NTP studies, rather than 10% which he thought was associated with reaching a maximum tolerated dose (MTD). Dr. J. Bucher, NIEHS, responded that it depends on the study outcome. If there is a tumor response in a study that has a 15% decrease, that would be acceptable. Whereas, in a negative study, such a large decrease might have contributed to preventing development of a neoplastic response, and this would be a problem.

Dr. Bucher reported that in top dose female rats a second unusual oral cavity carcinoma was observed. Thus, the NTP proposed to add a sentence to the end of the first paragraph of the conclusions. This sentence would read: 'Uncommon squamous cell carcinoma in the oral cavity of females may have been related to chemical treatment.' This would be considered as equivocal evidence; however, the primary level of evidence in female rats would remain **some evidence of carcinogenic activity** based on the increased incidences of hepatocellular neoplasms.

Dr. Russo moved that the Technical Report on D & C Yellow No. 11 be accepted with revisions discussed and the conclusions as written for male and female rats, including the sentence about the oral cavity neoplasms in females, **some evidence of carcinogenic activity**. Dr. Carlson seconded the motion, which was accepted with six yes votes and one abstention (LeBoeuf). Dr. LeBoeuf abstained based on his company's commercial interest in this chemical.

Molybdenum Trioxide. Dr. P. C. Chan, NIEHS, introduced the toxicology and carcinogenesis studies of molybdenum trioxide by discussing the uses and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related or uncertain neoplastic lesions in male rats and male and female mice, and on compound-related non-neoplastic lesions in rats and mice. The conclusions for the two-year studies in rats and mice were that:

> Under the conditions of these two-year inhalation studies there was **equivocal evidence of carcinogenic activity** of molybdenum trioxide in male F344/N rats based on a marginally significant positive trend of alveolar/bronchiolar adenoma or carcinoma (combined). There was **no evidence of carcinogenic activity** of molybdenum trioxide in female F344/N rats exposed to 10, 30, or 100 mg/m^3. There was **some evidence of carcinogenic activity** of molybdenum trioxide in male B6C3F$_1$ mice based on increased incidences of alveolar/bronchiolar carcinoma and adenoma or carcinoma (combined). There was **some evidence of carcinogenic activity** of molybdenum trioxide in female B6C3F$_1$ mice based on increased incidences of alveolar/bronchiolar adenoma and adenoma or carcinoma (combined).
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> Exposure of male and female rats to molybdenum trioxide by inhalation resulted in an increased incidence of chronic alveolar inflammation, hyaline degeneration of the respiratory epithelium, hyaline degeneration of the olfactory epithelium (females), and squamous metaplasia of the epiglottis.
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> Exposure of male and female mice to molybdenum trioxide by inhalation resulted in an increased incidence of metaplasia of the alveolar epithelium, histiocyte cellular infiltration (males), hyaline degeneration of the respiratory epithelium, hyaline degeneration of the olfactory epithelium (females), squamous metaplasia of the epiglottis, and hyperplasia of the larynx.

Dr. Taylor, a principal reviewer, agreed with the conclusions. His major criticism concerned the failure to select higher doses for the two-year study. While some of the outcomes, e.g., tumor promoter activity, were detected at doses used, other toxicities and tumors at other sites might have been induced by higher doses, perhaps up to 200 mg/m^3. Dr. Chan said the dose selection was based on body weight effects in the 14-day and 13-week studies but agreed that the top dose could have been higher. Dr. Taylor noted a statement in the report that male rats exhibited higher blood molybdenum concentrations and greater variability than females. He wondered whether this was related to body weight or pharmacokinetic considerations and asked for discussion. Dr. Chan responded that pharmacokinetic data was not available and we have no explanation for the blood level differences.

Dr. Russo, the second principal reviewer, agreed with the conclusions. She inquired as to the criteria used to designate the lung tumor findings in male rats as 'uncertain neoplastic effects.' Dr. Bucher said that in the summary table in the Abstract, equivocal responses are generally listed under 'uncertain effects.' These are neoplastic responses that may or may not be chemically-related, and we do not want to discount them.

Dr. Brown initiated a considerable discussion about the severity grades assigned to the non-neoplastic lesions of the respiratory system in rats and mice and given in the tables.

The grades range from 1 (mild) all the way to 4 (marked). Dr. Brown noted that many of the lesions are listed as 2 (minimal) or below and wondered about the significance of such apparently slight changes from normal. Dr. Carlson said he would find the inclusion of ranges of severity within a group to be helpful. Dr. J. R. Hailey, NIEHS, commented that perspective is provided, and if, for example, most of the lesions are mild to minimal, the lesions probably would be characterized as minor. Dr. J. Haseman, NIEHS, said reporting the average severity grade primarily is a space-saving device, and for selected lesions the complete distribution could be included. Dr. LeBoeuf asked why the level of evidence for male mice was not **clear evidence**, since malignant lung neoplasms were significantly increased over control at all three dose levels. Dr. Haseman said there were two reasons, one being the lack of a dose response, and secondly, for lung tumors, combined tumor incidence is given primary emphasis, so that when adenomas and carcinomas are combined the top dose group loses significance.

Public Comment: Dr. G. Van Riper, Vice President, Environmental Services, Behre Dolbear & Company, Inc., said he was representing Climax Molybdenum Company which is the largest producer of molybdenum compounds in the country, having produced such compounds since the early 1900s. He stated that during that time there has never been correlation of cancer with employees exposed to the compounds and because of the process used, worker exposure is quite low. Dr. Van Riper said he believed the pure oxide was used in NTP studies and detailed the synthetic processes to its production. With regard to toxicity, he commented that the pure trioxide is quite acidic (pH of 3.5) and speculated that bronchial effects might be an artifact of the low pH. Dr. Van Riper concluded by referring to reports of the anti-carcinogenic effects of molybdenum and the need for more discussion of such effects. Dr. Brown asked if there was any comment on effects of inhalation of other materials with such a low pH. Dr. Bucher said there have been epidemiology studies of certain refinery populations where there had been fairly high sulfuric acid levels in the air, and there was an association with increased lung cancer incidence among the workers. Dr. Brown asked for any comment on the purported anticarcinogenic effects. Dr. Bucher said selenium was another example whereby low levels appeared to be anti-carcinogenic and high levels carcinogenic. Dr. LeBoeuf agreed and said that where such data might be used in the risk assessment process considerations of discontinuity in terms of dose and biological activity needed to be discussed.

Dr. Taylor moved that the Technical Report on molybdenum trioxide be accepted with revisions discussed and with the conclusions as written for male rats, **equivocal evidence of carcinogenic activity**, for female rats, **no 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 seven votes.

Nitromethane. Dr. J. H. Roycroft, NIEHS, introduced the toxicology and carcinogenesis studies of nitromethane by discussing the uses, describing the experimental design, reporting on any survival and body weight effects, and commenting on compound-related neoplastic lesions in female rats and male and female mice and non-neoplastic lesions in male and female mice. The conclusions for the two-year studies in rats and mice were that:

> Under the conditions of these two-year inhalation studies, there was **no evidence of carcinogenic activity** of nitromethane in male F344/N rats exposed to 94, 188, or 375 ppm. There was **clear evidence of carcinogenic activity** of nitromethane in female F344/N rats based on increased incidences of mammary gland fibroadenomas and carcinomas. There was **clear evidence of carcinogenic activity** of nitromethane in male $B6C3F_1$ mice based on increased incidences of harderian gland adenomas and carcinomas. There was **clear evidence of carcinogenic activity** in female $B6C3F_1$ mice, based on increased incidences of liver and harderian gland adenomas and carcinomas. Increased incidences of alveolar/bronchiolar adenomas and carcinomas in male and female mice exposed to nitromethane were also considered to be chemical-related.
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> Exposure to nitromethane by inhalation for two years resulted in increased incidences of nasal lesions including degeneration and metaplasia of the olfactory epithelium and degeneration of the respiratory epithelium in male and female mice.

Dr. Russo, a principal reviewer, agreed with the conclusions and found the report otherwise acceptable.

Dr. Ryan, the second principal reviewer, agreed with the conclusions. She noted that high dose female rats weighed more than the controls and wondered whether this could be related to chemical effects on the thyroid, and, further, what impact the weight effect might have had on the increased incidence of mammary tumors. Dr. Roycroft responded that transient thyroid effects were seen early in the 13-week studies but not at the end and were not observed in the two-year study so he did not think the thyroid had an impact. Dr. Haseman described a model developed by Dr. S. Seilkop using NTP historical control data to predict how certain tumors are affected by body weight and how body weights at certain ages are predictive of subsequent tumor development. Using the model, one would predict a 51% incidence of mammary tumors in top dose female rats, while the actual incidence in the study was 82%. Dr. Haseman said in this case the increase in body weights could not account for the increase in tumors.

Dr. LeBoeuf, the third principal reviewer, agreed with the conclusions in principle. He questioned whether the increases in hepatocellular adenomas alone in female mice were sufficient to support the conclusion of **clear evidence**. Dr. Roycroft said the incidences of 51% and 70% in low and high dose groups well exceeded the concurrent control incidence of 28% as well as the highest historical rate of 40% in any of the contemporary inhalation studies and justified their inclusion as support. Dr. LeBoeuf commented that since neurotoxicity was the prime determinant for dose setting for the chronic rat study, there should have been histopathologic examination of sciatic nerve and spinal cord in animals from this study. Dr. Roycroft observed that the 13-week data indicated sciatic nerve degeneration was less severe than in the 16-day study, although there were obvious

clinical observations in the longer study. He noted that our standard protocol calls for cutting sections of sciatic nerve, spinal cord, and other nervous system tissues when neurobehavioral effects are seen clinically but in the nitromethane study such effects were not seen in the two-year study. However, Dr. Roycroft reported that subsequently, sections were taken from high dose and controls and none of the lesions observed in prechronic studies were seen. Words describing this would be added to the report.

Public Comment: Dr. A. Bollmeier, Angus Chemical Company, said Angus was essentially the only manufacturer of nitroparaffins now in this country. He pointed out the variation among the three batches used for the studies and wondered if this might not play a role in differences in toxicology findings among the 16-day vs. 90-day vs. two-year studies. Dr. Bollmeier commented that the potential human exposures estimated by NIOSH were done in 1981-83, while current exposures would likely be much less, being less than 10,000.

Dr. Russo moved that the Technical Report on nitromethane be accepted with the revisions discussed and with the conclusions as written for male rats, **no evidence of carcinogenic activity**, and for female rats and male and female mice, **clear evidence of carcinogenic activity**. Dr. Ryan seconded the motion. Dr. Bucher asked that the wording of the statement supporting the level of evidence for female mice be changed to add 'adenomas' after 'liver.' Dr. Brown said this would not be an amendment but should be kept in mind by the members when voting. Dr. Goldsworthy commented that one could argue for the same change with the harderian gland in female mice as the tumor response in this organ is primarily driven by the adenomas. Dr. Bucher proposed also using the less specific word 'neoplasm.' The revised sentence could read: "There was **clear evidence of carcinogenic activity** in female $B6C3F_1$ mice based on increased incidences of liver neoplasms (primarily adenomas) and harderian gland adenomas and carcinomas." The motion by Dr. Russo was then accepted unanimously with seven votes.

Phenolphthalein. Dr. J. K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of phenolphthalein by describing 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. Dr. Dunnick noted that molecular biology studies in collaboration with NIEHS intramural scientists are in progress or planned, including studies of the chemical in transgenic mouse models. Pharmacokinetic studies are in progress. Dr. J. R. Hailey, NIEHS, presented photomicrographs of lesions of the hematopoietic system, examples of histiocytic sarcoma, malignant lymphoma of thymic origin and associated hyperplasia, ovarian proliferative lesions and testicular atrophy. The conclusions for the two-year studies were that:

> Under the conditions of these two-year feed studies, there was **clear evidence of carcinogenic activity** of phenolphthalein in male F344/N rats based on the markedly increased incidences of benign pheochromocytoma of the adrenal medulla and of renal tubule adenoma and adenoma or carcinoma (combined). There was **some evidence of carcinogenic activity** of phenolphthalein in female F344/N rats based on the increased incidences of benign pheochromocytoma of the adrenal medulla in the 12,000 ppm group and of benign or malignant pheochromocytoma (combined) in the 12,000 and 25,000 ppm groups. There was **clear evidence of carcinogenic activity** of phenolphthalein in male $B6C3F_1$ mice based on the increased incidences of histiocytic sarcoma and of malignant lymphoma of thymic origin. There was **clear evidence of carcinogenic activity** of phenolphthalein in female $B6C3F_1$ mice based on the increased incidences of histiocytic sarcoma, malignant lymphoma of all types, lymphoma of thymic origin, and benign sex-cord stromal tumors of the ovary.
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> Exposure of rats to phenolphthalein in feed for two years resulted in increased incidences of focal hyperplasia of the adrenal medulla in males and in increased incidences and/or severity of nephropathy of the kidney in males and females. Exposure of mice to phenolphthalein in feed for two years resulted in increased incidences of atypical hyperplasia of the thymus in males and females, degeneration of the germinal epithelium of the testis in males, and ovarian hyperplasia in females.
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> Exposure of mice to phenolphthalein in feed for two years resulted in decreased incidences of hepatocellular neoplasms and nonneoplastic lesions in males and females.

Dr. Goldsworthy, a principal reviewer, agreed with the conclusions. He stated that the report should provide assurance that biological responses observed, especially those related to estrogenic effects, are attributable to phenolphthalein and not to lipophilic impurities. Small quantities of such impurities have been shown to account for the estrogenic activity in commercial preparations of the sulfonated analog of phenolphthalein, phenosulfonphthalein or phenol red. Dr. Dunnick responded that the phenolphthalein used in this study was 99% pure. She noted that Dr. M. Shelby, NIEHS, was developing assays for estrogenic activity and anticipated that we could then compare the estrogenic potential of phenolphthalein with that of phenol red and lipophilic impurities. Dr. Goldsworthy said there should be a comprehensive treatment of the estrogenic responses;

for example, discussion on a decreased incidence of liver tumors in mice should mention that this effect may be due to phenolphthalein's estrogenic effects. Dr. Dunnick proposed isolating the 1% impurity and examining its carcinogenic and estrogenic activity in shorter term model systems such as transgenics and MCF-7 cells.

Dr. Ward, the second principal reviewer, was unable to attend the meeting but had submitted his review, which Dr. Hart read into the record. Dr. Ward agreed with the conclusions. He wondered whether the myelofibrosis reported was the typical lesion found in aging $B6C3F_1$ mice, and probably estrogenic in origin. Dr. Hailey said that it was the aging lesion.

Dr. Taylor, the third principal reviewer, agreed with the conclusions. He thought that since pharmacokinetic data on phenolphthalein is rare in the literature the pharmacokinetic data in the Appendix should be placed in the body of the report and a brief discussion undertaken, especially about the lack of a dose-response relationship. Dr. Dunnick said that these studies were limited but she would try to give more emphasis to the findings. Dr. Goldsworthy hoped that follow-up studies would look at tissue dose in organs such as the ovaries. Dr. G. Lucier, NIEHS, reported that follow-up pharmacokinetic studies being done span a very wide dose range and will enable us to better define leveling off of tissue or blood levels in relation to dose.

Dr. Russo commented that there did not seem to any influence of chemical treatment on pituitary or mammary gland lesions, and even a lower incidence of thyroid C-cell lesions than in control animals, all speaking against an estrogenic effect. Dr. Dunnick agreed noting that another chemical with estrogenic activity, zearalenone, had not produced increases in mammary gland tumors in rats or mice. Dr. LeBoeuf asked whether the *in vitro* increases in chromosomal aberrations and *in vivo* increases in the frequency of micronucleated erythrocytes were consistent with other chemicals which may have estrogen-like properties and can also form quinones with metabolism, such as diethylstilbestrol. Dr. Shelby replied that the data base is too small to reach any general conclusion. However, these increases along with the two-year study results are fully consistent with the endocrine disrupting estromimetic compounds. Dr. W. T. Allaben, NCTR/FDA, thanked the NTP, and particularly Dr. Dunnick, for sharing information during the study and review process, and noted that the additional studies mentioned will be helpful to the FDA in its further review toward making an assessment regarding potential human risk. No public comments were received from the floor. Written comments received from the manufacturer prior to the meeting had been provided to Subcommittee members for their review.

Dr. Goldsworthy moved that the Technical Report on phenolphthalein be accepted with the revisions discussed and with the conclusions as written for male rats and male and female mice, **clear evidence of carcinogenic activity**, and for female rats, **some evidence of carcinogenic activity**. Dr. Taylor seconded the motion, which was accepted with six yes votes and one abstention (LeBoeuf). Dr. LeBoeuf abstained based on his company's commercial interest in laxative-type products.

Sodium Xylenesulfonate. Dr. A. Radovsky, NIEHS, introduced the toxicology and carcinogenesis studies of sodium xylenesulfonate by discussing the uses of the chemical, describing the experimental design, reporting on survival and body weight effects, and commenting on possible chemical related non-neoplastic lesions in female rats and male mice. Dr. Radovsky reported that an increased incidence of hepatocellular neoplasms in control and treated male mice could be attributed to infection with *Helicobacter* bacteria. Increased incidences of hepatocellular neoplasms in female mice could not with certainty be associated with *Helicobacter*. Dr. Radovsky suggested adding a sentence to the conclusions indicating that mice were infected with *Helicobacter*. The conclusions in the report for the two-year studies in rats and mice were that:

> Under the conditions of these two-year dermal studies, there was **no evidence of carcinogenic activity** of sodium xylenesulfonate in male or female F344/N rats administered 60, 120, or 240 mg/kg or in male or female B6C3F$_1$ mice administered 182, 364, or 727 mg/kg.
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> Increased incidences of epidermal hyperplasia in female rats and male mice may have been related to exposure to sodium xylenesulfonate.

Dr. Carlson, a principal reviewer, agreed with the conclusions. He commented that he would not have used ethanol as vehicle for application of the chemical.

Dr. Goldsworthy, the second principal reviewer, agreed in principle with the conclusions as long as there was further clarification and documentation on the role of *Helicobacter* in the mouse liver tumor responses. He said the report needs to better address the response in females and the effects observed in both sexes in a comprehensive manner to ensure that the responses are properly interpreted as non-treatment related. Historical control data with *Helicobacter* involvement also should be addressed. Dr. Radovsky responded that several other studies with *Helicobacter* infection were completed and would be reviewed at the next meeting. Hopefully, firmer conclusions then could be drawn about the association of liver tumor response and infection in B6C3F$_1$ mice. Dr. Goldsworthy said the report should more clearly state any potential dose or absorption effects that occurred from changing volumes as well as vehicles from 17-day studies to 14-week and two-year studies, and comment on relevance to human exposures. Dr. Radovsky said sodium xylenesulfonate was more soluble in water than in ethanol but ethanol may have enhanced skin penetration more than water. She opined that relevance to human exposure would be speculative on her part.

Dr. Tyson, the third principal reviewer, agreed with the conclusions. He also questioned the use of ethanol as the vehicle noting the association of dermally applied ethanol with induction of mononuclear cell leukemia in F344 rats.

Dr. Allaben asked for comment on the poor survival in male rats. Dr. Bucher said that male rat survival has declined primarily because of increases in nephropathy, body weight, and incidence of pituitary adenomas. Dr. Rao commented that survival of rats is lower when they are individually as opposed to group-housed. Dr. Haseman said that survival in this study is similar to that in other dermal studies using individual housing. Dr. Ryan asked whether there could be a correlation between mice with *Helicobacter* and those with neoplastic lesions. Dr. Hailey said that all of the animals have not been examined but for

those that have there is a good correlation. Dr. Haseman commented that in the previous study with *Helicobacter*, animals with liver tumors had the more severe non-neoplastic lesions that were indicative of the infection. Dr. Goldsworthy said that we need to say more in the conclusions than that mice were infected but rather that the infection was a confounding factor in interpretation of the liver lesions in mice. Dr. Haseman said that while total liver tumor rates in this study are above expected rates, they are similar in all groups; and we concluded that there was no evidence of a chemically related effect.

Dr. Frank Mirer, Health and Safety Department, United Auto Workers Union, had submitted a statement which, at his request, Dr. Hart read into the record. Dr. Mirer opined that the studies were inadequate to address the carcinogenicity of sodium xylenesulfonate in humans. He based his assessment on: (1) not high enough a dose to approach a maximum tolerated dose (MTD); (2) likely poor absorption of such an ionic material through intact skin; and (3) wrong route of exposure to estimate human risk, i.e., inhalation exposure should have been used.

Dr. Carlson moved that the Technical Report on sodium xylenesulfonate be accepted with revisions discussed and the conclusions as written for male and female rats and mice, **no evidence of carcinogenic activity**. Dr. Tyson seconded the motion. Dr. Goldsworthy offered an amendment that a sentence be added to the conclusions stating that mice were infected with *Helicobacter*. Dr. Carlson agreed to the amendment and the amended motion was accepted by six yes votes to one no vote (Russo). Dr. Allaben asked that a short paragraph be added to the discussion regarding individual animal housing and poor survival.

Tetrafluoroethylene. Dr. J. H. Roycroft, NIEHS, introduced the toxicology and carcinogenesis studies of tetrafluoroethylene by describing the uses of the chemical, 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. With the presence of a number of benign and malignant neoplasms in the kidneys of male and female rats, additional step sections were taken to further evaluate the renal effects. Dr. R. C. Sills, NIEHS, presented data from ongoing molecular biology studies characterizing the H-*ras* codon 61 mutation spectra in hepatocellular neoplasms from control B6C3F$_1$ mice and mice exposed to tetrafluoroethylene for two years. Data were also presented comparing the mutation profiles of hepatocellular neoplasms from the current study with those from the NTP study of tetrachloroethylene. The conclusions for the two-year studies were that:

> Under the conditions of these two-year inhalation studies, there was **clear evidence of carcinogenic activity** of tetrafluoroethylene in male F344/N rats based on increased incidences of renal tubule neoplasms (mainly adenomas) and hepatocellular neoplasms. There was **clear evidence of carcinogenic activity** of tetrafluoroethylene in female F344/N rats based on increased incidences of renal tubule neoplasms, liver hemangiosarcomas, hepatocellular neoplasms, and mononuclear cell leukemia. There was **clear evidence of carcinogenic activity** of tetrafluoroethylene in male and female B6C3F$_1$ mice based on increased incidences of liver hemangiomas and hemangiosarcomas, hepatocellular neoplasms, and histiocytic sarcomas.
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> Slight increases in the incidences of mononuclear cell leukemia and testicular interstitial cell adenomas in male rats may be been related to exposure to tetrafluoroethylene.
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> Exposure of rats to tetrafluoroethylene resulted in increased incidences of renal tubule degeneration and hyperplasia in males and females, increased severity of kidney nephropathy in males, and liver angiectasis and cataracts in females. Exposure of mice to tetrafluoroethylene resulted in increased incidences of renal tubule karyomegaly in males and females, renal tubule dilatation in males, liver angiectasis in males and females, hematopoietic cell proliferation of the liver in females, and splenic hematopoietic cell proliferation in males and females.

Dr. Ryan, a principal reviewer, agreed with the conclusions. She inquired as to whether short burst, high exposures had been considered in study design as being more similar to a typical occupational exposure. Dr. Roycroft replied that this regimen had not been considered; the six-hours-a-day five-days-a-week design used was similar to continuous exposure in a workplace situation. Dr. Ryan asked whether there was information on the degree of human exposure encountered through spills or leakages. Dr. Roycroft noted that tetrafluoroethylene is produced in and maintained in closed-capture systems, no occupational exposure limits have been established, and no information on spills and leaks has been found.

Dr. Carlson, the second principal reviewer, agreed with the conclusions. He observed that the numbers of clinical pathology type measurements appeared to be decreased from past reports and in view of the types of lesions seen wondered why. Dr. Roycroft responded

that, in actuality, there were more as in addition to standard time points and collections at 90-days, clinical pathology was done on animals sacrificed at 15-months. Dr. Carlson said a sentence needed to be added to the Abstract regarding the reason for terminating the mouse study early. Dr. Roycroft agreed.

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 with the conclusions and found the report acceptable.

There was some discussion about mixing of rats and mice in exposure chambers. Dr. Goldsworthy asked if hormonal alterations were seen. Dr. Roycroft said that hormone measurements were not made but if a chemical to be studied was known to affect hormonal levels then separate housing might have to be considered. Dr. G. N. Rao, NIEHS, noted that rats and mice are produced in the same rooms so are used to each others presence, and pointed out that each inhalation chamber is by itself a room and within the chamber rats and mice are not side by side in cages but rather in racks. This provides more efficient and economical use of available space. Dr. LeBoeuf commended the NTP for the molecular biological studies in evaluating the H-*ras* activation profiles and noted the usefulness of such studies for risk assessment purposes.

Dr. Ryan moved that the Technical Report on tetrafluoroethylene be accepted with the revisions discussed and with the conclusions as written for male and female rats and male and female mice, **clear evidence of carcinogenic activity**. Dr. Carlson seconded the motion, which was accepted unanimously with seven votes.