Board of Scientific Counselors
National Toxicology Program

Summary Minutes
from
NTP/HEI (Health Effects Institute) Collaborative Ozone Studies
and
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 16-17, 1993

Research Triangle Park, NC

The meeting began at 8:00 a.m. on November 16 and 8:30 a.m. on November 17 in the Conference Center, Building 101, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. Members of the Subcommittee are: Drs. Curtis Klaassen (Chairperson), Paul Bailey, Arnold Brown, Louise Ryan, Robert Taylor, Matthew van Zwieten, and Jerrold Ward. Dr. Brown was unable to attend the meeting. 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 21 and 22, 1994, in Research Triangle Park, North Carolina. For information, contact Dr. Hart, 919/541-3971.

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Peer Review of Draft Technical Reports

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NTP TECHNICAL REPORTS REVIEW SUBCOMMITTEE MEETING
November 16-17, 1993

NTP/HEI (Health Effects Institute) Collaborative Ozone Studies

Introduction — Dr. Gary Boorman, NIEHS, reported that most of the day would be devoted to presentation of findings from extensive mechanistic studies performed in-house, on contract or by investigators around the country under the auspices of the Health Effects Institute (HEI) that examined effects of ozone on pulmonary function, structure and morphometry, and examination of biochemical markers. He commented that the project had been conceived only five years ago and came to completion so quickly because of the productive collaboration among NIEHS, HEI and Battelle Pacific Northwest Laboratories (Battelle) and the design whereby additional animals were added to the exposures at Battelle and then made available to investigators for mechanistic studies. For most of the HEI managed studies, twenty months was selected as the maximum exposure to minimize confounding influences of naturally occurring degenerative and neoplastic processes in older animals. Dr. Debra Kaden, HEI, emphasized the partnership aspect of the effort and then briefly commented on the types of studies to be presented. She noted that those under the auspices of HEI were currently being evaluated by the Institute Health Review Committee.

Exposure and Monitoring of Ozone Studies — Dr. John Decker, Battelle, described the study design and exposure and monitoring systems for inhalation exposures with ozone in 2-year cocarcinogenicity studies in male F344 rats, 2-year toxicology and carcinogenesis studies in male and female $B6C3F_1$ mice and F344 rats, and 124-week studies in rats and 130-week studies in mice. The major problem was in maintaining uniform concentrations of ozone within the exposure chambers. He explained how they resolved this using recirculating fans.

Pulmonary Function

Respiratory Function Alterations Following Chronic Ozone Inhalation — Dr. Joe Mauderly, Inhalation Toxicology Research Institute, said they had used well defined pulmonary function measurements in animals that might be interpreted in terms of human lung performance. Measures were made of dynamic lung compliance and total pulmonary resistance. In male rats there was a significant reduction in maximal expiratory flow volume and in females there was a reduction in residual volume and a concurrent increase in vital capacity as a fraction of total lung capacity. He speculated that thickening of the bronchoalveolar junctions may have played a role in these changes. When values for males and females are normalized to 100% of total lung capacity, he opined that the most striking results were that there was so little change in lung function in exposed animals.

Mechanical and Pharmacological Properties of Airways Isolated from Ozone-exposed Rats — Dr. John Szarek, Marshall University School of Medicine, began by noting that earlier studies of acute effects of ozone exposure had shown an increase in airway responsiveness both in humans and animals *in vivo*, and in isolated airways from animals. He described their experimental setup with large and small airways from rats for measuring contractile responses to pharmacological stimuli and mechanical abrasion

of the luminal epithelium. They also measured tissue levels of eicosanoids in response to ozone. Dr. Szarek concluded that from their studies there appeared to be an increase in sensitivity in the large airways and a decrease in responsiveness in small airways. Further, smooth muscle function appeared to be reduced especially in small airways after ozone exposure and/or inhibitory influences of the epithelium may be enhanced. Finally, the cyclooxygenase enzymatic cascade may be enhanced.

Discussion — Questions directed at Drs. Mauderly and Szarek centered on the apparent lack of dose response as evidenced by diminished effects of ozone at the high dose, 1.0 ppm, compared with the lower doses, 0.12 and 0.5 ppm, and attempts to explain it. Dr. Szarek commented that there may be secretory or structural changes reported in subsequent presentations that may aid in interpretation.

Biochemical Markers

Lung Collagen Content and Crosslinking in Fischer 344 Rats Chronically Exposed to Ozone — Dr. Jerrold Last, California Primate Research Center, said the objectives were to determine whether accumulation of collagen in the lungs of experimental animals could be detected and whether the relative abundance of hydroxyproline derived at crosslinks was altered. He noted that in animals acutely exposed to ozone, there was an increased rate of collagen synthesis. In the 20-month animals, there appeared to be gender differences in that there were no increases in hydroxyproline in males but a dose dependent increase in females expressed on a per lung lobe basis. However, if these data were normalized to the weight or size of the lungs this difference disappeared. Dr. Last said their studies would not tell where in the lung the new collagen is located and how much corresponds to the collagen of fibrosis vs. that corresponding to the collagen of the structural matrix, although the known increased bronchiolarization following ozone exposure suggests that some of the new airway material would contain collagen.

Effects of Chronic Ozone Inhalation on Complex Carbohydrates of Lung Connective Tissue — Dr. Bhandaru Radhakrishnamurthy, Tulane University School of Public Health and Tropical Medicine, said the complex carbohydrates of interest were the proteoglycans and their constituents, the glycosaminoglycans (GAG). He reviewed the structures and tissue locations of the various proteoglycans, the biologic properties of the GAG, and the analytical procedures used to isolate the GAG from lung tissue and quantitate the various GAG. Because of wide differences in weights of lobes in these studies, the results were expressed in terms of concentration. The observations in the current studies clearly indicate that chronic exposure of rats to ozone at concentrations greater than 0.5 ppm altered concentrations of lung proteoglycans. Whether this was due to decreased synthesis or increased catabolism is not known. Dr. Radhakrishnamurthy stated that it was likely that ozone is toxic to fibroblasts which synthesize most of the proteoglycans.

Extracellular Matrix Expression in Ozone-exposed Lungs — Dr. William Parks, Jewish Hospital at Washington University (St. Louis), said that lung extracellular matrix is composed primarily of insoluble molecules synthesized mostly during the perinatal period and other active periods of growth. Previous reports have shown that high doses of ozone increase both synthesis and deposition of matrix molecules, while low doses result in decreased deposition. He reported that *in situ* hybridization techniques were used to detect the messenger RNA for the expression of various interstitial matrix proteins, and

he described the biochemical and immunohistochemical techniques employed to study extracellular matrix protein. Dr. Parks reported that ozone mediated a transient expression of structural proteins such as elastin and collagen after two months exposure but there was no increased production in animals exposed for 20-months.

Comparisons Between Rats and Mice: Acute O_3 Toxicity, $^{18}O_3$ Dose, and Tissue Antioxidants — Dr. Gary Hatch, Health Effects Research Laboratory, U. S. EPA, said an objective was to perform short-term studies that might help explain why there were differences between rats and mice in sensitivity to chronic ozone exposure. Examined was lung tissue dosimetry using radiolabelled ozone and using bronchoalveolar lavage to assess whether there were differences between the species in tissue antioxidant concentrations and possibly to measure reaction products of ozone with biomolecules. Dr. Hatch reported that studies to date do not show large differences between rats and mice in dosimetry to the lungs. He said they may not have looked at the target tissue, and the airways may be where they need to be looking at the dose. With regard to antioxidant levels, he said there was not much difference between rats and mice in whole lung concentrations of uric acid, ascorbic acid, and glutathione; however, for the latter two, there were rather large differences in concentrations in lavage fluids which might be related to differences in susceptibility during long-term exposure.

Discussion — Dr. Robert Taylor, Howard University, asked whether there was evidence of free radical generation or induction of superoxide dismutase. Dr. Hatch said that in studies with NIEHS, spin trapping of free radicals in lung homogenate after ozone exposure showed an increase but these were at high levels of ozone and he doubted this could be detected with ozone exposures of 1 ppm or less.

Respiratory Structure and Morphometry

Ozone-induced Nonneoplastic Lesions at 20, 24, and 30 Months Exposure — Dr. Paul Mellick, Battelle, reported that they had conducted the histopathology for all the studies. He said that in both rats and mice, the lesions attributable to ozone exposure were limited to the respiratory system, and in the lungs, the lesions were located in the central acinar area where there was a definite thickening in the junction between the terminal bronchiole and the alveolar duct. The lesion was termed alveolar epithelial metaplasia. In rats, there was increased interstitial thickening or fibrosis in the alveolar ducts. In both species, there was an increase in numbers of alveolar macrophages in the centriacinar area. In both species, there were similar hyperplastic or metaplastic lesions in the nasal cavity and turbinates, and in the olfactory epithelium. Dr. Mellick said there was a definite dose-response with only minimal effects at 0.12 ppm, and there were differences in nature or severity of lesions among rats exposed at 20, 24, or 30 months, at least at the light microscopic level.

Effects of Chronic Ozone Exposure on the Nasal Mucociliary Apparatus in the Rat — Dr. Jack Harkema, Inhalation Toxicology Research Institute, noted that chronic bronchitis or asthma in humans is characterized by excess mucus in the airways. He reported that in rats chronically exposed to ozone at 0.5 and 1.0 ppm, there were slower mucous flow rates in the anterior nasal airways, there was marked ozone-induced mucous cell metaplasia with large amounts of intraepithelial mucosubstances in the nasal mucosa, there was continual inflammation, and a marked reduction in bone as marked by increased numbers of bone resorption cells in the maxilloturbinates. There was only one

physiological alteration in animals exposed to 0.12 ppm, and that was an increase in mucous flow rates significantly greater than in control animals.

Health Effects of Chronic Ozone Inhalation: Changes in Tracheobronchiolar Epithelium and Antioxidant Enzyme Studies — Dr. Charles Plopper, University of California, Davis, said airways examined in male rats ranged from the trachea through major daughter bronchi and small bronchi to terminal bronchioles. With regard to stored mucus, there were increases in mucus in some airways, no change in other airways, and a dose-related decrease in the trachea. In evaluating epithelial thickness, only in the caudal bronchus was there a change. Both the proportion and mass of nonciliated cells in bronchiolar epithelium were significantly greater in exposed rats. Dr. Plopper described changes in antioxidant enzymes - superoxide dismutase, catalase, glutathione peroxidase and glutathione transferase. Like epithelial changes, changes in antioxidant enzyme activities were very much site specific.

Morphometric Analysis of Structural Alterations in Rat Lungs Chronically Exposed to Ozone — Dr. Margaret Menache, Duke University Medical School, said the objectives of the studies were to examine dose/response relationships from chronic ozone exposure on development of injury such as interstitial disease through looking at tissue volumes and surface areas in three sites, the proximal alveolar region, the terminal bronchiole, and sections representing the distal alveolar region. Their findings showed that for the proximal alveolar region, there were no statistically significant effects at 0.12 ppm, while following exposure at 0.5 and 1.0 ppm ozone, there was epithelial metaplasia and interstitial thickening. No major ozone effects were observed for the distal alveolar region, while terminal bronchioles were not affected at the lower doses but at 1.0 ppm there was a loss of ciliated cells and a proliferation of Clara cells. Dr. Menache said that overall the results indicate that ozone is associated with fibrosis, as exhibited by collagen deposition, but even at high doses, this effect is restricted and very focal in nature.

Health Effects of Chronic Ozone Inhalation: Morphometric Studies — Dr. Kent Pinkerton, University of California, Davis, said he would discuss effects of ozone exposure on the gas exchange region of the lungs, the parenchyma and its functional units, the acini, and then described how they isolated and visualized the ventilatory unit on a computer where they could then subdivide the average 1200 micron linear distance of the alveolar duct into 100 micron intervals. He said their analysis showed that at 200 microns, changes were all above control but similar for all three doses but deeper into the duct one saw a dose-response in such things as alveolar macrophage density and epithelial tissue density.

Discussion — Dr. Kaden asked whether there was a dose-response in the percentage of bronchiolar alveolar ducts affected by ozone. Dr. Pinkerton said that at 1.0 ppm essentially all were affected while at 0.12 ppm about 30-60% were affected.

Biostatistics

The NTP/HEI Collaborative Project: A Combined Analysis — Dr. Louise Ryan, Harvard School of Public Health and Dana-Farber Cancer Institute, said that in their analysis, they combined experimental outcomes related to a disease process that might be relevant to humans. The three types of disease processes of interest were interstitial fibrosis, airways disease, and accelerated aging. She introduced Dr. Paul Catalano, also from Harvard School of Public Health and Dana-Farber Cancer Institute, to talk about

the variables associated with one of these processes, interstitial fibrosis. Dr. Catalano said that in collaboration with the investigators they had agreed on eleven different specific endpoints that people have measured that are thought to be related to fibrosis. These could be grouped under collagen production and biochemistry, morphometric measurements, and respiratory measurements. He described the statistical techniques used to derive summary scores for each animal and how these scores varied with ozone concentration, gender and other study variables.

NTP Ozone Studies

Proliferative Lesions and Cell Proliferation in Ozone-exposed Mice — Dr. Ronald Herbert, NIEHS, said that there was a suggestion of an increase in lung tumors in $B6C3F_1$ mice at 24 months but no comparable increase in F344 rats. The first objective was to characterize the lung neoplasms in exposed mice and whether there were any unique morphologic differences with neoplasms in control mice. Second, the immunohistochemical staining characteristics of tumors from control and exposed animals were compared. Conclusions were that lung neoplasms in mice exposed to varying concentrations of ozone appeared to be similar to those observed in control mice based on size, distribution within the lung (primarily peripheral), histomorphologic patterns of the neoplasms, in the degree of cellular or nuclear atypia, and in immunohistochemical staining characteristics. Cell proliferation studies using the PCNA technique to determine labeling indices in the centriacinar region of the lung in exposed and control animals showed no differences.

Molecular Analysis of Neoplastic Lesions following Ozone Exposure — Dr. Robert Sills, NIEHS, said the objective was to characterize genetic alterations in the K-*ras* oncogene and the P53 tumor suppressor gene in lung neoplasms of $B6C3F_1$ mice following ozone exposure. He noted that mutations and chromosomal losses in the retinoblastoma and P53 tumor suppressor genes and mutations in the K-*ras* oncogene have been shown to be important in early phases of human lung cancer, and similar genetic events may occur in early and late phases of mouse lung cancer. Dr. Sills described the techniques used to isolate and characterize the mutations in these genes. Results were that the mutation spectrum for the K-*ras* gene and lung neoplasms from ozone exposed animals were similar to the mutation spectrum seen in control $B6C3F_1$ mice; however, the percentage of tumors with K-*ras* mutations in the ozone study was greater than that of concurrent or historical controls. Generally, specific K-*ras* mutations did not appear to correlate with the size or morphology of these neoplasms. Neoplasms screened by immunohistochemical methods for the mutated P53 protein were negative, a finding consistent with other NIEHS studies where genetic alterations in the P53 tumor suppressor gene appear to occur less frequently in neoplasms of $B6C3F_1$ mice.

Ozone. Dr. Boorman said there were four basic studies: (1) a four-week study in rats and mice; (2) standard two-year studies in rats and mice; (3) 30-month studies in rats and mice; and (4) two-year cocarcinogenesis or promotion studies in male rats with NNK, a known carcinogen and tobacco-specific nitrosamine. He reported on survival and body weight effects and commented on the lack of neoplastic effects in male or female rats in two-year or 30-month studies, and on compound-related neoplastic lesions in male and female mice in two-year or 30-month studies. Dr. Boorman discussed factors supporting or arguing against a compound-related carcinogenic effect in male and female mice. The proposed conclusions for the studies were that:

> Under the conditions of these two-year and lifetime inhalation studies, there was **no evidence of carcinogenic activity** of ozone in male or female F344/N rats receiving 0.12, 0.5, or 1.0 ppm. There was **equivocal evidence of carcinogenic activity** of ozone in male $B6C3F_1$ mice based on increased incidences of alveolar/bronchiolar adenomas or carcinomas. There was **some evidence of carcinogenic activity** of ozone in female $B6C3F_1$ mice based on increased incidences of alveolar/bronchiolar adenomas or carcinomas.
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> There was no evidence that exposure to 0.5 ppm ozone enhanced the incidence of NNK-induced pulmonary neoplasms in male rats.
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> Exposure of male and female rats to ozone for two years or 124 weeks was associated with goblet cell hyperplasia and squamous metaplasia in the nose, squamous metaplasia in the larynx, and metaplasia (extension of bronchial epithelium into the centriacinar alveolar ducts) and interstitial fibrosis in the lung. Exposure of male and female mice to ozone for two years or 130 weeks was associated with hyperplasia and squamous metaplasia in the nose and inflammation (histiocytic infiltration) and metaplasia (extension of bronchial epithelium into the centriacinar alveolar ducts) of the lung.

Dr. van Zwieten, a principal reviewer, agreed with the conclusions. He said the Abstract should summarize pathology findings from the 4-week studies. He added that since the report documents a comprehensive series of studies with ozone, consideration should be given to including photomicrographs of ozone-induced lesions in the respiratory tract of rodents. Dr. Boorman agreed.

Dr. Bailey, the second principal reviewer, agreed with the conclusions. He said the report indicated that "hypoactivity was observed in male and female rats exposed to ozone," and asked if the hypoactivity was seen only during exposure and immediately afterwards. Dr. Boorman said that would be clarified.

Dr. Taylor, the third principal reviewer, stated that prior to the meeting he thought **equivocal evidence of carcinogenic activity** was more appropriate for female mice based on the relatively flat dose-response curve in the lifetime studies. However, after looking at the combined data from two-year and lifetime studies, he supported the conclusion in the report for female mice as well as the other conclusions. Dr. J. Haseman, NIEHS, said there were two primary factors supporting **some evidence** in female mice. One was that in the two-year study there were 16 tumor-bearing animals in

the top dose group which was more than double the maximum seen historically in inhalation study controls. Second, in the combined analyses, the trend and the high dose effect were an order of magnitude more significant in females than in male mice. Dr. Taylor thought the discussion of genetic toxicology would be better summarized in a table rather than in a generic statement.

Dr. Ward questioned combining the conclusions in mice particularly since the incidence of tumors was higher in the two-year study than in the lifetime study. Dr. Haseman responded that the combined analyses have the advantage of using all the data and, because survival adjusted methods are used, animals are being compared to animals of equivalent age. Dr. Y. Vostal, Environmental Health Consultants, commented that the statement in the Introduction indicating that the primary source of ozone in urban areas was automotive emissions was incorrect.

Dr. Kaden reported that the Health Effects Institute planned to publish their reports along with commentaries as a single volume. Dr. Boorman said he hoped to be able to reference these reports in the Technical Report.

Dr. van Zwieten moved that the Technical Report on ozone be accepted with the revisions discussed and with the conclusions as written for male and female rats, **no evidence of carcinogenic activity,** for male mice, **equivocal evidence of carcinogenic activity**, and for female mice, **some evidence of carcinogenic activity**. Dr. Taylor seconded the motion, which was accepted by four yes votes with one abstention (Dr. Ryan).

o-Benzyl-p-Chlorophenol. Dr. W.C. Eastin, NIEHS, introduced the initiation/promotion study of *o*-benzyl-*p*-chlorophenol (BCP) in Swiss CD-1 mice by discussing the properties and uses of the chemical, describing the experimental design, and commenting on the compound-related skin lesions in male and female mice. The proposed conclusions for the studies were that:

> Under the conditions of this one-year mouse skin initiation/promotion study in Swiss (CD-1®) mice, *o*-benzyl-*p*-chlorophenol was a cutaneous irritant and a weak promoter relative to strong promoters such as TPA. BCP had no activity as an initiator or as a complete carcinogen.

Dr. Eastin reviewed the conclusions for the 2-year gavage study of BCP in rats and mice that were approved by the Subcommittee in December 1992. In that study, there was **no evidence of carcinogenic activity** in male F344 rats or female $B6C3F_1$ mice; there was **equivocal evidence of carcinogenic activity** in female rats with the target site being the kidney; amd there was **some evidence of carcinogenic activity** in male mice with the target site being the kidney.

Dr. Ward, a principal reviewer, agreed in principle with the conclusions except that he thought the words "skin tumor" should be added between "weak" and "promoter" in the first sentence of the Conclusions. He suggested that the weak promoting activity of BCP may be due to skin wounding (irritation) rather than the effects of BCP on skin carcinogenesis/promotion, and provided copies of journal articles describing such effects. Dr. Eastin said he was familiar with the papers and would add information on wounding and skin irritation as well as discussion on other weak promoters.

Dr. Taylor, the second principal reviewer, said the conclusion that BCP was a weak promoter was overstated considering that the DMBA/acetone groups of mice developed many, if not more neoplasms than did the DMBA/BCP groups. Further, the inconclusiveness of the findings was compounded by the degree of irritation and skin wounding that occurred. Dr. Taylor thought that more discussion was needed.

Dr. Ryan, the third principal reviewer, commented that although the conclusions were in line with the findings, she was concerned with the ambiguity of some of the results, such as the fact that higher incidences of some lesions were seen with DMBA/acetone than with DMBA/BCP. In response to Dr. Taylor and Dr. Ryan, Dr. Eastin said that the incidences of skin papillomas were clearly elevated in the top dose DMBA/BCP group relative to DMBA/acetone. The designation of "weak promoter" derived from a comparison of DMBA/BCP with the DMBA/TPA reference controls with TPA representing a strong or potent promoter. Dr. Ryan wondered how to interpret the results of this study in light of the gavage study, and asked whether there had been studies of absorption of BCP following dermal administration. Dr. Eastin said that NIEHS studies have shown that BCP is well absorbed after dermal administration. Dr. Ryan said that because of the various experimental groups, a summary table in the text would be helpful. Dr. Eastin agreed and said a table would be added at the end of the Abstract.

Dr. J. Haartz, NIOSH, reported that she would supply data from the National Occupational Exposure Survey System (NOESS) indicating over 300,000 workers potentially exposed to BCP, while noting that a large majority are female.

Dr. Ward moved that the Technical Report on *o*-benzyl-*p*-chlorophenol be accepted with the revisions discussed and the conclusions as written with the addition of "skin tumor" to read as follows: Under the conditions of this one-year mouse skin initiation/promotion study in Swiss (CD-1®) mice, *o*-benzyl-*p*-chlorophenol was a cutaneous irritant and a weak skin tumor promoter relative to strong promoters such as TPA. BCP had no activity as an initiator or as a complete carcinogen. Dr. Taylor seconded the motion, which was accepted unanimously with five votes.

Diethyl Phthalate and Diethyl Phthalate/Dimethyl Phthalate. Dr. D.S. Marsman, NIEHS, introduced the toxicology and carcinogenesis studies of diethylphthalate (DEP) and the initiation/promotion studies of diethylphthalate with dimethylphthate (DMP). He discussed the uses of the chemical and the rationale for both studies, described the experimental designs, reported on survival and body weight effects, and commented on compound-related nonneoplastic lesions in male and female rats and male mice in the initiation/promotion study, and compound-related neoplastic lesions in male and female mice in the two-year studies. The proposed conclusions for the studies were that:

> Under the conditions of these two-year dermal studies, there was **no evidence of carcinogenic activity** of diethylphthalate in male or female F344/N rats receiving 100 or 300 μL. The sensitivity of the male rat study was reduced due to low survival in all groups. There was **equivocal evidence of carcinogenic activity** of diethylphthalate in male and female $B6C3F_1$ mice based on the increased incidences of hepatocellularneoplasms, primarily adenomas.
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> In an initiation/promotion model of skin carcinogenesis, there was no evidence of initiating activity of diethylphthalate or dimethylphthalate in male Swiss (CD-1®) mice. Further, there was no evidence of promotion activity of diethylphthalate or dimethylphthalate in male Swiss (CD-1®) mice. The promoting activity of TPA following DMBA initiation was confirmed in these studies.
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> Minor dermal acanthosis was observed following dermal application of diethylphthalate in male or female F344/N rats dosed for two years and in male Swiss (CD-1®) mice dosed for one year. Minimal evidence of dermal acanthosis was observed for dimethylphthalate in male Swiss (CD-1®) mice dosed for one year.

Dr. Bailey, a principal reviewer, agreed with the conclusions. He said more rationale should be given for dermal application since the main routes of exposure for humans appear to be ingestion and inhalation. Dr. Marsman said a four-week diet study was done and 2-year diet study designed but the dermal route was considered to be the most important route for humans. Dr. Bailey said a comment should be added in the discussion concerning the possibility of ingestion of DEP from grooming after dermal application. Dr. Marsman said this was an oversight and grooming might have resulted in systemic availability of chemical.

Dr. van Zwieten, the second principal reviewer, agreed with the conclusions. He said a comment was needed as to why four-week studies were done in rats and mice instead of the customary 13-week studies which might have better predicted doses for the first 2-year study in mice. Dr. Marsman said a four-week prechronic regimen for dermal studies was preferred at the time these studies were initiated, and agreed that 13-week studies might have been more helpful in setting doses for the 2-year mouse studies. Dr. van Zwieten asked for discussion about whether an increased incidence of pituitary tumors might help explain the reduced survival in male rats. Dr. R. Hailey, NIEHS, commented that many of these tumors in males were quite large and could have contributed in an additive way to decreased survival along with nephropathy which is much more severe in male rats.

Dr. Ryan, the third principal reviewer, had similar questions about choice of the dermal over other routes of exposure, and why four-week instead of 13-week studies were done. She thought the dose finding aspects for the 2-year studies to be less stringent than usual,

expressing doubts that a Maximal Tolerated Dose (MTD) was reached for either rats or mice.

Dr. Ward asked whether there was evidence of peroxisome proliferation in livers of any of the studies. Dr. Marsman replied that this was not measured although hepatomegaly present could be suggestive of such an effect. In comments from the public, Dr. Raymond David, Eastman Kodak Company, stated that they agreed with the conclusions in rats but thought the conclusions in mice should have been **no evidence** based in part on the incidence of hepatocellular tumors in treated male mice being within the historical control range, and on the lack of a dose-response for liver tumors in female mice. Dr. Dean Fenning, Eastman Kodak, said that he wished to point out that the Use and Human Exposure section in the Introduction stated that DEP and DMP are used in a variety of plasticized consumer products, but in his experience a number of these products contain neither phthalate.

Dr. Bailey moved that the Technical Report on diethylphthalate and diethylphthalate/ dimethylphthalate be accepted with the revisions discussed and with the conclusions as written in the 2-year studies for male and female rats, **no evidence of carcinogenic activity**, and for male and female mice, **equivocal evidence of carcinogenic activity**, as well as the conclusions that there was no evidence of initiating or promoting activity of DEP or DMP in male Swiss (CD-1®) mice. Dr. Ward seconded the motion, which was accepted unanimously with five votes.

Short-Term Study

Isoprene. Dr. R.L. Melnick, NIEHS, introduced the short-term toxicity studies of isoprene by reviewing the uses and rationale for study. Studies were undertaken on isoprene as part of the butadiene initiative developed in the 1980s. Isoprene and chloroprene were put on study because they are high production chemicals and are structurally related to butadiene. Dr. Melnick detailed the metabolism of isoprene and compared it with that for butadiene. Two-week and 13-week inhalation toxicology studies were conducted in male and female F344/N rats and $B6C3F_1$ mice at concentrations ranging up to 7000 ppm. Male rats and male mice were also exposed to isoprene vapors for 6 months followed by a 6-month recovery period to determine if isoprene produces a carcinogenic response similar to that of 1,3-butadiene after intermediate exposure durations. In addition to histopathology, evaluations included clinical pathology, tissue glutathione, forelimb and hindlimb grip strength, and sperm motility and vaginal cytology. Data from inhalation teratology studies as well as from *in vitro* and *in vivo* genetic toxicity studies were also reported.

Dr. Melnick concluded that the studies demonstrated that isoprene was toxic to the testes of rats inducing interstitial cell hyperplasia after 6 months exposure, and the marginal increased incidence of testicular adenomas seen after the 6-month recovery period may have been related to isoprene administration. Exposure to isoprene for 13-weeks produced no discernible toxicologic effects in rats. In mice, isoprene caused a nonresponsive macrocytic anemia, similar to that seen with butadiene, and a decrease in hindlimb grip strength. Isoprene was also toxic to the forestomach, nasal cavity, testes, and spinal cord. Isoprene induced increases in the frequency of sister chromatid exchanges in bone marrow cells and in the frequency of micronucleated erythrocytes in peripheral blood. No-observable-adverse-effect levels (NOAELs) were determined for most of the toxic lesions in mice.

Isoprene was carcinogenic to the liver, lung, forestomach and harderian gland of mice. Inhalation teratology studies did not show an effect in rats, but in CD-1 mice exposed to isoprene there were lower fetal weights and a higher percentage of fetuses per litter with supernumerary ribs at exposures which were not maternally toxic. Most of the toxic and carcinogenic effects seen with isoprene were also caused by 1,3-butadiene in mice.

Dr. Taylor, a principal reviewer, thought the report well written, study design rigorous and highly focused, and the metabolism section quite informative. He wondered whether there had been characterization of the cytochrome P450 isozymes associated with isoprene metabolism. Dr. Melnick said that 2E1 has been shown to be a major contributor to the oxidation of butadiene to its monoepoxide but whether this or another isozyme contributes to isoprene metabolism is not known. Dr. Taylor suggested that there be more discussion of the tumors, especially in terms of multiplicity, location within a site, and morphology. Dr. Melnick agreed to add more detail. Further, recognizing this was not a typical 2-year study, Dr. Taylor said some consideration might be given to assigning a level of evidence for carcinogenicity in mice.

Dr. Ward, a second principal reviewer, also thought that consideration should be given to assigning a level of evidence in mice, in this case, **clear evidence of carcinogenic activity**. Dr. Melnick said the 6-month stop study was adequate to evaluate a carcinogenic effect but not the full carcinogenic potential of isoprene in mice. Dr. Ward commented on the high incidence of liver, Harderian gland, and lung tumors in control

animals at 12 months, and wondered whether such findings were typical for inhalation studies. Dr. Melnick responded that he couldn't answer the question since there is little NTP historical data for sacrifices made at 12 months. Dr. Bailey asked whether there were plans to conduct a 2-year study. Dr. Melnick said a 2-year study was underway in rats but a decision had not been made as to whether a 2-year study in mice was warranted. This concluded the discussion of the isoprene report.