Board of Scientific Counselors
National Toxicology Program

Summary Minutes
from

Peer Reviews of Draft Technical Reports of Long-Term Toxicology and Carcinogenesis Studies by the Technical Reports Review Subcommittee and Panel of Experts

on

October 28, 1983
Research Triangle Park, North Carolina

The review meeting began at 9 a.m. in the Conference Center, Building 101, South Campus, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. Members of the Subcommittee are: Drs. Jerry Hook (Chairperson), Curtis Harper and James Swenberg. Members of the Panel are: Drs. Louis Beliczky, Devra Davis, Robert Elashoff, Seymour Friess, Michael Holland, Robert Scala, Tom Slaga, John Van Ryzin, Stan Vesselinovitch, and Mary Vore. Drs. Slaga, Vesselinovitch and Vore were unable to attend the meeting.

When available, final NTP Technical Reports for the approved studies may be purchased from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, Virginia 22161, (703) 487-4650.

The next NTP technical reports peer review meeting will be held February 29, 1984, in Washington, D.C. For information contact Dr. Larry G. Hart, (919) 541-3971; FTS 629-3971.

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Benzene. Dr. Friess, a principal reviewer for the technical report on the carcinogenesis studies of benzene, essentially agreed with the conclusions that: "Under the conditions of these studies, there was clear evidence of carcinogenicity of benzene for male F344/N rats, female F344/N rats, male B6C3F$_1$ mice, and female B6C3F$_1$ mice. For male rats, benzene caused increased incidences of Zymbal gland carcinomas, squamous cell papillomas and squamous carcinomas of the oral cavity, and squamous cell papillomas and squamous cell carcinomas of the skin. For female rats, benzene caused significantly increased incidences of Zymbal gland carcinomas and squamous cell papillomas and squamous cell carcinomas of the oral cavity. For male mice, benzene caused increased incidences of Zymbal gland squamous cell carcinomas, malignant lymphomas, alveolar/bronchiolar carcinomas and alveolar/bronchiolar adenomas or carcinomas (combined), Harderian gland adenomas, and squamous cell carcinomas of the preputial gland. For female mice, benzene caused increased incidences of malignant lymphomas, ovarian granulosa cell tumors, ovarian benign mixed tumors, carcinomas and carcinosarcomas of the mammary gland, alveolar/bronchiolar adenomas, and alveolar/bronchiolar carcinomas." He added that the findings of leukopenia in rats and mice could be cited in the conclusions and a summary table in the results section would be useful for highlighting the 17 week and two-year studies. To aid reviewers in interpretation of findings where significance shown by life table analysis differs from that shown by the incidental tumor test, Dr. Friess asked that more explanation be given, and perhaps where appropriate, values from the Fisher exact test and Cochran-Armitage trend test could be included in summary tables. Dr. J. Huff, NTP Chemical Manager, noted that the results of the latter two tests are included routinely in the statistical analyses appendix but since emphasis is given by NTP to survival-adjusted methods, only these are generally included in the Results Section. Dr. Friess also suggested that the discussion on the role of benzene metabolites and putative precursors in benzene carcinogenesis should be condensed and made less speculative.

As a second principal reviewer, Dr. Swenberg agreed with the conclusions; however, he indicated that the maximum tolerated dose (MTD) was exceeded in the high dose male rats. Like Dr. Friess, he thought this was an important bioassay of a widely used chemical and there was little question that benzene was carcinogenic in the study. He said that general comments needed to be made to provide a rationale for the route of administration (gavage) since the routes relevant to human exposure were mainly inhalation and dermal. Dr. Swenberg's critique focused on two areas, the first was the need for considerable editing and a more tightly organized manuscript. Second, the hematology data were incompletely presented and were unacceptable. Dr. Huff said that since the hematology data may not have been adequately analyzed, and appeared somewhat inconsistent, he proposed that consideration of the hematology data be deferred and the present discussion concentrate on the carcinogenicity of benzene.

As a third principal reviewer, Dr. Davis also agreed with the conclusions and said the report provided a good analysis of the carcinogenicity data. She stated that a more detailed discussion of reproductive and chromosomal effects was needed, and the information on epidemiological studies, human exposure, and production patterns could be expanded. She said the lack of usable hematology data was not pivotal to the study.

As a fourth principal reviewer, Dr. Elashoff agreed with the conclusions. He stated that for some tumor sites specified in the conclusions statistical significance depended on the test used; the discussion section should contain

the rationale about why a particular test was used for a site. In view of the multiple sex/species/tumor sites, he recommended summary tables of response data in the discussion which would indicate significant findings. He discussed and attached a series of such tables to his review. Dr. Elashoff noted the patterns of late mortality with large numbers of animals dying after 90 weeks (rats) or 95 weeks (mice), emphasizing that survival was good to that time and the cause of death was likely associated with the neoplasms.

In discussion by the Panel, Dr. Scala stated there should be more balance in the discussion on metabolism of benzene, and said the indepth description of genetic toxicology information could be enhanced with a summary table. Dr. Davis noted that significant non-tumor toxic effects might be included in the abstract. Dr. Hook thought there was a consensus for inclusion of noteworthy non-tumor effects in the Abstract.

Dr. Hook said there appeared to be two issues to be discussed and resolved, one having to do with the interpretative conclusions and the other having to do with the report. Dr. Swenberg said there appeared to be no disagreement on the carcinogenicity conclusions. Dr. Beliczky moved that the conclusions be accepted as written. Dr. Swenberg seconded the motion and the conclusions were approved by seven affirmative votes with two abstentions (Dr. Holland and Dr. Scala).

The ensuing discussion focused around the format of what information could be released concerning the findings, and what subsequent action should be proposed with respect to the full technical report including the hematology data. There was some agreement that the conclusions could be released with a brief introduction and the data tables and text supporting the conclusions. The Abstract would not be included since it had not been voted on. With regard to the final technical report, the prevailing view of Panel members was to bring a revised report back for review at the next peer review meeting.

Dr. Friess moved that the carcinogenesis results and interpretations could be issued with the conclusions that were voted upon and accepted by the Panel. And finally, a full draft technical report on benzene will be reviewed at an upcoming meeting. The motion was seconded and approved by seven affirmative votes with two abstentions (Dr. Holland and Dr. Scala).

1,3-Butadiene. Dr. Van Ryzin, a principal reviewer for the technical report on the carcinogenesis studies of 1,3-butadiene, agreed with the conclusions, that: "Under the conditions of these studies, there was clear evidence of carcinogenicity for 1,3-butadiene in male and female B6C3F$_1$ mice, as shown by increased incidences and early induction of hemangiosarcomas of the heart, malignant lymphoma, alveolar/bronchiolar adenomas and carcinomas, and papillomas of the stomach in males and females; and mammary gland acinar cell carcinomas and granulosa cell tumors of the ovary in females. In addition, 1,3-butadiene was associated with nonneoplastic lesions in the respiratory epithelium and liver necrosis." He said there should be a clear statement in the abstract that this inhalation study was designed for 103-104 weeks exposure but was terminated at 60 and 61 weeks because of low survivability concomitant with neoplasms.

As a second principal reviewer, Dr. Beliczky said he agreed with the conclusions. He suggested that an immunotoxicology profile on butadiene might be worthwhile given the potential carcinogenicity in the Zymbal gland. In view of limited epidemiological evidence associating certain occupations with brain tumors in humans, perhaps more studies were needed to evaluate the significance of gliomas in male mice; likewise, the hemangiosarcomas of the heart are an unusual neoplastic response. Dr. Swenberg supported the need for highlighting the gliomas in view of their rarity in mice. Because of apparent differences between Sprague-Dawley rats and the B6C3F$_1$ mice in sensitivity to tumor induction by 1,3-butadiene, Dr. Beliczky recommended comparative pharmacokinetic studies in the two species. Dr. M. Powers, NTP Chemical Manager, responded that such pharmacokinetic studies were being designed.

As a third principal reviewer, Dr. Friess agreed with most of the major conclusions as to 'clear evidence of carcinogenicity'; however, he suggested that the strength of evidence for papillomas and carcinomas of the forestomach in male and female mice better fits in the category of 'some evidence of carcinogenicity' based on lack of dose-response. For both sexes, there were marked fall-offs in incidence rates from the low-dose groups to the high-dose groups. Conversely, Dr. Friess suggested adding liver adenomas and adenomas or carcinomas (combined) in females to the conclusions under the category of clear evidence. The dose trends and enhanced incidence rates at the high dose were clear and significant. Dr. J. Huff, NTP, indicated that a single category of evidence was generally selected for each sex/species and reflected the highest degree of evidence. In the discussion that followed, this concept was concurred with by several Panel members. Nonetheless, Dr. Friess asked that his minority position be put clearly on the record. Finally, Dr. Friess highlighted the increased incidence of nasal lesions in males, with no increase in neoplasia, and the almost complete lack of such lesions in female mice.

As a fourth principal reviewer, Dr. Harper also agreed with the conclusions. He asked for clarification regarding the major cause of early deaths; malignant lymphomas were stated as causative in one section while elsewhere hemangiosarcomas were given as causing the death of a number of animals. Dr. G. Boorman, NTP, replied that the lymphomas occurred principally in the thymus and likely caused suffocation of the animals, while in some cases the heart lesions were contributory.

In other discussion, Dr. Scala noted that 1,3-butadiene is highly explosive and the concentrations used in the 13-week studies seemed near this level. He asked for more information in the report on the safety procedures used at the

contract laboratory. He expressed concern that other inhalation studies with chemicals as potent as ethylene oxide and 1,2-epoxybutane were conducted in the same chamber room, and hoped this could be avoided in future studies. Dr. E. McConnell, NTP, agreed and said this would not be done routinely. He mentioned that the chambers used for each chemical were essentially closed systems and cross-contamination was unlikely. Dr. Scala stated that there was inadequate randomization of the animals by weight. Dr. Haseman agreed and said analysis shows that the initial weights in both sexes were significantly lower in the control groups than in the dose groups. He said the statistical evaluation would be included in the report. Dr. Swenberg commented that while results were given for the 14-week studies no pathology information was given although pathology was done. Statements should be made as to the findings or lack thereof.

In response to questions about the chronic inhalation study in Sprague-Dawley rats done at Hazleton Laboratories under the sponsorship of the International Institute of Synthetic Rubber Producers, Dr. Scala noted that the overall report had not been published but the findings of the two-year carcinogenicity study would be submitted to a toxicology journal. Dr. B. Schwetz, NTP, stated that these data were made available to the NTP and that an ongoing correspondence had been initiated.

Dr. Van Ryzin moved that the technical report on the carcinogenesis studies of 1,3-butadiene be accepted with the modifications discussed. To the conclusions would be added 'hepatocellular adenomas and adenomas or carcinomas' (combined) in female mice. Dr. Holland seconded the motion and the technical report was approved by seven affirmative votes with two abstentions (Dr. Holland and Dr. Scala).

Tris (2-ethylhexyl)Phosphate. Dr. Swenberg, a principal reviewer for the technical report on the carcinogenesis studies of tris(2-ethylhexyl)phosphate, agreed with the conclusions that: "Under the conditions of these studies, there was equivocal evidence of carcinogenicity in male F344/N rats receiving 2000 and 4000 mg/kg tris(2-ethylhexyl)phosphate, as evidenced by increased incidences of pheochromocytoma of the adrenal glands. There was no evidence of carcinogenicity in female F344/N rats or in male $B6C3F_1$ mice receiving tris(2-ethylhexyl) phosphate. There was some evidence of carcinogenicity in female $B6C3F_1$ mice that received 1,000 mg/kg tris(2-ethylhexyl)phosphate, as shown by an increased incidence of hepatocellular carcinoma. Tris(2-ethylhexyl)phosphate was associated with increased incidences of follicular cell hyperplasias of the thyroid gland in male and in female $B6C3F_1$ mice." Dr. Swenberg said that the lack of gastric irritation present in the two-year mouse studies was somewhat unusual since this effect was found in prechronic studies. He requested adding a statement that this chemical was not the same as the "Tris", found to be carcinogenic in rodents, that was used in childrens sleepware.

As a second principal reviewer, Dr. Scala said he agreed in principle with the conclusions although he questioned the bases for interpreting the occurrence of pheochromocytomas in male rats as equivocal evidence of carcinogenicity, and the occurrence of heptocellular carcinomas in female mice as some evidence of carcinogenicity. He said the equivocal designation apparently was based on a comparison with historical controls and not concurrent control animals. Dr. H. B. Matthews, NTP Chemical Manager, replied that the category of 'some evidence' of carcinogenicity was used for female mice because the evidence at the high dose was not overwhelming, the incidence of carcinomas was not significant at the low dose, and significant increases were not seen in male mice. He said that the 'equivocal' evidence of carcinogenicity designation was based in part on comparison with historical controls, especially from the other two studies at the same laboratory. There followed considerable discussion about how and when historical controls should be used in making comparisons. Dr. Van Ryzin expressed concern about the use being too ad hoc and not consistent or systematic. Dr. Scala stated that the rationale for the 'equivocal' and 'some evidence' designations should be included in the abstract. He commented that the subject of negative trends could be more fully discussed, especially with regard to the inverse relationship between malignant lymphomas and liver tumors in mice.

As a third principal reviewer, Dr. Holland also agreed with the conclusions. He inquired as to the basis for giving female rats half the doses of those given male rats. Dr. Matthews said he assumed that decreased weight gain in the 13-week study was the determining factor. Dr. Holland commented that there needed to be a more informative way to look at and summarize weight gain data. Dr. J. Haseman, NIEHS, replied that with the new Toxicology Data Management System, formal statistical analysis of weight gain and other parameters can be done more easily, as individual animal data are readily available.

As a fourth principal reviewer, Dr. Van Ryzin said he agreed in principle with the conclusions except that there should be a statement about the thyroid follicular cell tumors in male rats. Dr. Matthews said the thyroid tumor incidence was not statistically significant compared with controls. Dr. Van Ryzin also had reservations about the weight given to historical control values in designating the incidence of pheochromocytomas in male rats as equivocal evidence of carcinogenicity. Dr. Holland observed that the relative lack of non-neoplastic effects in the adrenal glands of male rats tended to diminish the

biological significance of the pheochromocytomas and support the equivocal designation. Dr. Davis requested that comment be made of the increased incidences of liver cytoplasmic vacuolization in treated female mice.

Dr. Swenberg moved that the technical report on the carcinogenesis studies of tris(2-ethylhexyl)phosphate be accepted with inclusion in the abstract of the rationales for assignment of 'equivocal evidence' and 'some evidence' as well as other additions and corrections. Dr. Scala seconded the motion and the technical report was approved unanimously by the Peer Review Panel.