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

June 22, 1993

Research Triangle Park, NC

The review meeting began at 9:00 a.m. on June 22 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, Kowetha Davidson, Harold Davis, Daniel Longnecker, Louise Ryan, Ellen Silbergeld, Robert Taylor, Matthew van Zwieten, Jerrold Ward, Lauren Zeise, and Mr. Louis Beliczky. Drs. Longnecker and Silbergeld were 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, NC, 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 November 16 and 17, 1993, in Research Triangle Park, North Carolina. For information, contact Dr. Hart, 919/541-3971.

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NTP TECHNICAL REPORTS REVIEW SUBCOMMITTEE MEETING
June 22, 1993

Barium Chloride Dihydrate. Dr. K.M. Abdo, NIEHS, introduced the toxicology and carcinogenesis studies of barium chloride dihydrate by discussing the uses of the chemical, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related non-neoplastic lesions in male and female mice. The conclusions for the studies were that:

> Under the conditions of these 2-year drinking water studies, there was **no evidence of carcinogenic activity** of barium chloride dihydrate in male or female F344 rats that received 500, 1,250, or 2,500 ppm. There was **no evidence of carcinogenic activity** of barium chloride dihydrate in male or female B6C3F$_1$ mice that received 500, 1,250, or 2,500 ppm.
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> There was a chemically-related increased incidence of nephropathy in male or female mice.

Dr. Davis, a principal reviewer, agreed with the conclusions. He suggested that plasma concentrations are a better measure of exposure than dose per unit surface area or body weight, noting that compounds excreted by the kidney and having the ability to cause nephropathy may have their plasma concentrations significantly raised, thereby skewing the relationship between administered dose and actual exposure. Dr. Abdo agreed that measurements of area under the plasma concentration curve would have been the best way to determine actual exposure. Dr. Davis thought the decrease in water consumption by male rats was sufficient justification for the dose being high enough. Dr. Abdo said he would add a sentence to the discussion about the decrease in water consumption being a consideration in dose setting for the 2-year studies in rats.

Dr. Bailey, the second principal reviewer, agreed with the conclusions. He asked why plasma barium levels were measured in the 2-year studies while serum levels were determined in the subchronic studies. Dr. Abdo said that the use of plasma was more for convenience as the volume available for analysis was greater.

Mr. Beliczky, the third principal reviewer, also agreed with the conclusions. He commented that for future industrial chemical studies, when available, Material Safety Data Sheets should be provided to reviewers. As another example of useful information, he provided a full review of barium and its soluble compounds prepared by the American Council of Government and Industrial Hygienists. Dr. D. Walters, NIEHS, reported that the NTP Laboratory Health and Safety Office requires contractors to request and obtain Material Safety Data Sheets whenever they order a chemical, and additionally, they are required to search the hazardous substances data base or equivalent for information about the material.

Dr. Brown inquired as to the rationale for the study. Dr. Abdo responded that the International Agency for Research on Cancer had found there was sufficient evidence that barium chromate was a human carcinogen. Since there was sufficient evidence that all the hexavalent chromium compounds were carcinogenic, it was hoped the current study would shed light on the potential carcinogenicity of barium itself. There was some discussion as to the chemical forms available in the body, whether elemental barium or the chloride

dihydrate. Dr. J. Haartz, NIOSH, suggested including the èxposure concentrations for barium.

Dr. Davis moved that the Technical Report on barium chloride dihydrate be accepted with the revisions discussed and with the conclusions as written for male and female rats and mice, **no evidence of carcinogenic activity.** Dr. Bailey seconded the motion, which was accepted unanimously with ten votes.

Hexachlorocyclopentadiene. Dr. K.M. Abdo, NIEHS, introduced the toxicology and carcinogenesis studies of hexachlorocyclopentadiene by discussing the uses, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related non-neoplastic lesions in rats and mice. He said a stop-exposure study also was done in male mice to determine whether there was regression or progression of metaplastic lesions in the respiratory tract. The conclusions for the studies were that:

> Under the conditions of these 2-year inhalation studies, there was **no evidence of carcinogenic activity** of hexachlorocyclopentadiene in male or female F344 rats or B6C3F$_1$ mice exposed to 0.01, 0.05, or 0.2 ppm.
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> Exposure of rats to hexachlorocyclopentadiene produced pigmentation of the respiratory epithelium of the nose, trachea, and bronchi and bronchioles and squamous metaplasia of the laryngeal epithelium (females). Suppurative inflammation of the nose as well as pigmentation of the respiratory mucosal epithelium occurred in mice exposed to hexachlorocyclopentadiene.

Dr. Zeise, a principal reviewer, agreed in principle with the conclusions. She thought that rats may have been able to tolerate higher doses, as seen by the survival, mean body weights, and clinical findings in the 2-year study. This should be noted in the Abstract and elsewhere. Dr. Zeise said there needed to be more discussion on the significance of the alveolar epithelial hyperplasia seen in the male mice stop-exposure study. Dr. Abdo agreed.

Dr. Ward, the second principal reviewer, also agreed in principle with the conclusions, stating that rats may have tolerated a higher top dose because no effects on body weight gain or survival were seen and toxic lesions were limited to pigmentation of the respiratory tract epithelium and mild squamous metaplasia in the larynx of females. In response to Dr. Zeise and Dr. Ward, Dr. Abdo said that the sharp increase in mortality in rats between 0.4 and 1.0 ppm along with the decrease in body weight gain at 0.4 ppm in male rats in 13-week studies justified the top dose chosen for 2-year studies. Dr. Ward criticized the use of less than 50 animals for complete histopathology in low and mid dose groups, wondering if the reduced statistical power might have affected interpretation in organs where there were equivocal effects. Dr. S. Eustis, NIEHS, noted that the NTP has used the reduced protocol for many years, and in this study, the only case where use of a full protocol might have resolved uncertainty was with the pituitary gland tumors in male rats.

Dr. Davidson, the third principal reviewer, agreed with the conclusions. She said information should be added to the Abstract to describe the severity of the respiratory lesions, and to explain how the exposure concentrations and durations were selected for the stop-study.

Mr. Beliczky asked that a comment be included in the report as to whether the eyes were examined and whether any effects were seen. Dr. G.N. Rao, NIEHS, said that when rodents are exposed to an irritant chemical they close their eyes which might explain why no ocular lesions were seen. Dr. van Zwieten observed that in female rats, there were significantly increased incidences of squamous metaplasia of the larynx in low and high dose groups, yet there was uncertainty expressed as to the relevance of these findings. Dr. Eustis said that because there is a transition point in the larynx from squamous to respiratory type epithelium coupled with the difficulty of getting sections from precisely the same spot, uncertainty in interpretation was introduced. Dr. J. Haartz, NIOSH, stated that there is an OSHA permissible exposure level for hexachlorocyclopentadiene, a regulatory limit as opposed to a voluntary limit, that should be cited in the report.

Dr. Davidson moved that the Technical Report on hexachlorocyclopentadiene be accepted with the revisions discussed and with the conclusions as written for male and female rats and mice, **no evidence of carcinogenic activity**. Dr. Bailey seconded the motion. Dr. Zeise offered an amendment that a sentence be added to the conclusions stating that rats might have been able to tolerate higher doses. Dr. Ward seconded the amendment, which was defeated by two yes (Ward, Zeise) to eight no votes. The original motion by Dr. Davidson was then accepted unanimously with ten votes.

Methylphenidate Hydrochloride. Dr. J.K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of methylphenidate hydrochloride by discussing the use and rationale for study, describing the experimental design, reporting on survival and body and liver weight effects, and commenting on compound-related neoplastic and non-neoplastic lesions in mice. The conclusions for the studies were that:

> Under the conditions of these 2-year feed studies, there was **no evidence of carcinogenic activity** of methylphenidate hydrochloride in male or female F344 rats receiving 100, 500, or 1,000 ppm. There was **some evidence of carcinogenic activity** of methylphenidate hydrochloride in male and female B6C3F$_1$ mice based on the occurrence of hepatocellular adenomas.
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> Treatment of female rats with methylphenidate hydrochloride was associated with a decrease in the incidence of mammary gland fibroadenomas.

Dr. Taylor, a principal reviewer, agreed with the conclusions. He thought the discussion of the metabolism and certain selective aspects of the sterochemistry related to the metabolism to be quite good. He asked that more explanation of the genetic toxicology data be given.

Dr. Ryan, the second principal reviewer, agreed in principle with the conclusions. She asked that the trends for increased thyroid tumors be addressed in more detail, especially in light of perceived hormonal effects of the chemical. Dr. Dunnick said the numbers didn't support this being a treatment-related effect. Dr. Ryan thought there needed to be more discussion on whether the level of evidence in mice based on hepatocellular neoplasms should be raised. Dr. Ryan said that since this is a drug taken by young children she had concerns as to whether the study animals were too old at the start and whether measurements of bone density might have been useful. Dr. Dunnick responded that the animals are six or seven weeks old at the beginning of the 13-week and 2-year studies, and bone measurements were taken during the 13-week studies. Methylphenidate had no effect on bone density or bone length in these studies. Dr. Dunnick noted that our charge was to evaluate the carcinogenic potential and that there are ongoing studies at other NIH institutes on other potential adverse effects of methylphenidate.

Dr. Davis, the third principal reviewer, did not agree with the conclusions in mice. He said a conclusion of **clear evidence of carcinogenic activity** is supported by dose-related increases in the combination of hepatocellular carcinomas and adenomas, and increase in the incidence of hepatoblastomas, a very rare and malignant neoplasm. Dr. Davis suggested that the report should include a unifying conclusion as to whether or not methylphenidate was a genetic toxicant. Dr. E. Zeiger, NIEHS, said there isn't generally accepted agreement on what defines the genotoxicity of a chemical. He said that a revised genetic toxicology section would be added to explain the results.

In response to the reviewer's concerns about the level of evidence in mice, Dr. R. Hailey, NIEHS, led a discussion about the nature of the hepatoblastomas. He said that although not much is known about this tumor, the NTP is seeing a few hepatoblastomas in control mice in more recent studies that do not yet appear in the historical control database. They are late appearing tumors in mice that generally are observed within other hepatocellular neoplasms, more often carcinomas, and most likely may be considered a more primitive variant of carcinoma. He said the most appropriate treatment for statistical analysis of the hepatoblastomas should be to combine them with adenomas and carcinomas. Dr. Davidson asked that some of this discussion be summarized in the report. Dr. Ward opined that the

high incidence of neoplasms in high dose female mice and the occurrence of the rare tumors in males lent support to a conclusion of **clear evidence of carcinogenic activity** in mice. Dr. J. Haseman, NIEHS, defended the conclusion of **some evidence** based on the fact that most of the increases in treated animals were in benign tumors, other than the hepatoblastomas, and the latter occurred in animals that already had other hepatocellular neoplasms.

Dr. Brown moved that the Technical Report on methylphenidate be accepted with the revisions discussed and with the conclusions as written for male and female rats, **no evidence of carcinogenic activity**, and for male and female mice, **some evidence of carcinogenic activity**. Dr. Taylor seconded the motion, noting that the wording at the end of the first paragraph of the conclusions should be changed from "hepatocellular adenomas" to "hepatocellular neoplasms." Dr. Zeise offered an amendment that the level of evidence for male mice be changed to **clear evidence of carcinogenic activity.** Dr. Ward seconded the amendment, which was defeated by four no votes (Bailey, Brown, Davidson, Taylor) to three yes votes (Davis, Ward, Zeise) with two abstentions (Ryan, van Zwieten). Dr. Ryan abstained because she was undecided and Dr. van Zwieten abstained for reasons of company affiliation. The original motion by Dr. Brown, including the wording change, was then accepted by eight yes votes with one abstention (van Zwieten).

p-Nitrobenzoic Acid. Dr. J.K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of p-nitrobenzoic acid 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 lesions in female rats and non-neoplastic lesions in male rats (nephropathy) and in female rats (hematologic toxicity). Additional step-sections of the kidney were performed in male rats. The conclusions for the studies were that:

> Under the conditions of these 2-year feed studies, there was **no evidence of carcinogenic activity** of p-nitrobenzoic acid in male F344 rats exposed to 1,250, 2,500, or 5,000 ppm. There was **some evidence of carcinogenic activity** of p-nitrobenzoic acid in female F344 rats based on increases in the incidences of clitoral gland adenoma and of clitoral gland adenoma or carcinoma (combined). There was **no evidence of carcinogenic activity** of p-nitrobenzoic acid in male or female $B6C3F_1$ mice exposed to 1,250, 2,500, or 5,000 ppm.
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> There were chemical-related decreases in the incidences of mononuclear cell leukemia in exposed male and female rats. p-Nitrobenzoic acid caused hematologic toxicity in female rats.

Dr. Brown, a principal reviewer, agreed with the conclusions. He noted the seemingly paradoxical decrease in the incidence of mononuclear cell leukemia in exposed rats and asked for comment on this, the hyperplasia or the increased weight of the spleen and the decreased incidence of the leukemia. Dr. Ward noted that there was hematopoietic toxicity associated with the chemical and speculated that the stem cell in the bone marrow or spleen from which the leukemia derives may be one of the targets of the chemical resulting in an inhibition of leukemogenesis.

Dr. van Zwieten, the second principal reviewer, agreed with the conclusions. He asked for discussion to substantiate why the preputial gland tumors, as well as presumably the clitoral gland tumors, in rats were regarded as potentially being lethal tumors, since in his experience these tumors tended to be quite small and well circumscribed. Dr. S. Eustis, NIEHS, responded that the preputial gland tumors are not lethal in the sense of causing the animals death, but as they get quite large with some becoming ulcerated, the animals are humanely sacrificed. Dr. J. Haseman, NIEHS, added that if a tumor were incidental, one would expect it to be more or less evenly distributed among the animals that died naturally and those that survived. In this case, the likelihood of an animal that died early having a preputial gland tumor was almost three times as high as in those that survived.

Dr. Ryan, the third principal reviewer, deferred her agreement or disagreement with the conclusions until there was further discussion of dose effects on clitoral gland lesions in female rats, and related effects in preputial glands in male rats. She said there were inconsistencies on how effects on body weight were discussed, e.g., decreased body weight is offered as a possible explanation for the dose-related decrease in leukemia incidence in rats. On the other hand, lack of dose-response with regard to the clitoral gland tumors was the main reason for **some evidence** rather than **clear evidence** in female rats, and likely due, in her opinion, also to decreased body weight. Dr. Dunnick said the conclusion in female rats was based primarily on there being approximately the same increases in tumors at all three dose levels, these being mostly adenomas. Body weight can affect the incidence of neoplasms but at least with the decrease in leukemia, it was believed to be more of a chemical effect rather than a body weight effect. With regard to the preputial gland tumors, Dr. Haseman said it was a close call between **no evidence** and **equivocal evidence of carcinogenic**

activity. Dr. Eustis noted that the incidence of carcinomas in the high-dose group was well within the historical control range.

Mr. Beliczky asked for inclusion of comment as to whether *in vivo* transnitrosation to a N-nitrosamine could have occurred. Dr. Ward asked for comment on the presence of hyaline droplets in the kidneys of rats in subchronic studies and whether they were associated with alpha-2μ-globulin accumulation. Dr. Eustis said there was no evidence for accumulation of alpha-2μ-globulin in this study.

Dr. Brown moved that the Technical Report on p-nitrobenzoic acid be accepted with the revisions discussed and with the conclusions as written for male rats and male and female mice, **no evidence of carcinogenic activity**, and for female rats, **some evidence of carcinogenic activity**. Dr. Taylor seconded the motion, which was accepted unanimously with ten votes.

4,4'-Thiobis (6-Tert-Butyl-m-Cresol). Mr. J.D. Cirvello, NIEHS, introduced the toxicology and carcinogenesis studies of 4,4'-thiobis (6-tert-butyl-m-cresol) (TBBC) by discussing the uses and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related non-neoplastic lesions in rats and mice. The conclusions for the studies were that:

> Under the conditions of these 2-year feed studies, there was **no evidence of carcinogenic activity** of 4,4'-thiobis (6-*t*-butyl-*m*-cresol) in male or female F344 rats administered 500, 1,000, or 2,500 ppm or in male or female B6C3F$_1$ mice administered 250, 500, or 1,000 ppm.
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> The administration of TBBC in feed for 2 years was associated with the occurrence of Kupffer cell hypertrophy, cytoplasmic vacuolization, and mixed cell foci in the liver of male and female rats, and of fatty change in the liver of female rats.

Mr. Beliczky, a principal reviewer, agreed with the conclusions. He asked whether the literature had been recently reviewed as most of the references were from the 1950s. Mr. Cirvello said a literature search had been done last year. Mr. Beliczky questioned the reference to the NIOSH Permissible Exposure Limit (PEL) because the levels that were mentioned as either total dust or respirable dust generally referred to nuisance dust, those dusts which are physiologically inactive or inert, and he didn't think you could call TBBC inert or physiologically inactive. He commented that the nomination for review by the NTP was referenced to a 1978 study at Harvard, and wanted to note that this epidemiological study had been funded by the United Rubber Worker's Joint Occupational Health Program.

Dr. Zeise, the second principal reviewer, agreed in principle with the conclusions. She pointed out that while the liver in male rats is clearly a target organ for toxicity, the data are unclear as to whether or not the liver is a target organ for carcinogenicity. She said the incidence of hepatocellular carcinomas/adenomas would be statistically significant if the historical control incidence at the study laboratory were used instead of the concurrent controls. She said there should be consideration given to changing the conclusion in male rats to **equivocal evidence of carcinogenic activity**. Mr. Cirvello commented that if one looks at the overall historical control database, there were three other studies that had the same top range as that at the study laboratory.

Dr. Ward, the third principal reviewer, agreed in principle with the conclusions. He said it should be noted that the degree of nephropathy was increased in female rats and there should be a statement that male rats may have tolerated a slightly higher dose. Mr. Cirvello said a statement about the nephropathy should have been included. He said that toxicity and reduction in body weight gain in the prechronic and 2-year studies indicated that the top dose was correct in male rats. Dr. Ward agreed with Dr. Zeise as to the uncertain significance of the liver tumors in male rats. Since mixed cell foci were increased more in treated animals, he said it would be useful to have a morphologic description and an assessment as to whether they are preneoplastic lesions. Dr. Eustis said a description would be added to the report but it was difficult to say whether the foci were preneoplastic. There was no atypia reported, a finding often found in foci induced by hepatocarcinogens.

Mr. Beliczky moved that the Technical Report on 4,4'-thiobis (6-*t*-butyl-*m*-cresol) be accepted with the revisions discussed and with the conclusions as written for male and female rats and mice, **no evidence of carcinogenic activity**. Dr. Bailey seconded the motion, which was accepted unanimously with ten votes.

Tricresyl Phosphate. Dr. R.D. Irwin, NIEHS, introduced the toxicology and carcinogenesis studies of tricresyl phosphate by discussing the uses, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related non-neoplastic lesions in rats and mice. The conclusions for the studies were that:

> Under the conditions of these 2-year feed studies, there was **no evidence of carcinogenic activity** of tricresyl phosphate in male or female F344 rats that received 75, 150, or 300 ppm. There was **no evidence of carcinogenic activity** of tricresyl phosphate in male or female B6C3F$_1$ mice that received 60, 125, or 250 ppm.
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> Nonneoplastic lesions associated with exposure to tricresyl phosphate included cytoplasmic vacuolization of the adrenal cortex and ovarian interstitial cell hyperplasia in female rats, increased incidences of clear cell focus, fatty change, and ceroid pigmentation of the liver in male mice, and increased severity of ceroid pigmentation of the adrenal cortex in male and female mice.

Dr. van Zwieten, a principal reviewer, agreed with the conclusions. He thought the description of the rationale for the maximum tolerated dose (MTD) to be extremely well done.

Dr. Davidson, the second principal reviewer, agreed with the conclusions. She asked for an explanation as to why there was high mortality in male and female rats in 16-day gavage studies at 2,905 mg/kg while at double that dose there was no mortality, and with similar results in mice. Dr. Irwin said the higher dose was pure tricresyl phosphate, which is a liquid, while the lower dose was the chemical diluted half-and-half with corn oil. He speculated that the corn oil may have enhanced the absorption but since the decision was to use dosed feed in the 2-year studies, this interesting observation was not pursued further.

Dr. Bailey, the third principal reviewer, also agreed with the conclusions. He said that the introductory toxicity section should mention that tricresyl phosphate esters with only one *ortho*-cresyl substituent are much more potent neurotoxicants than the tri-*ortho*-cresyl ester. He provided a reference.

Dr. Ryan inquired as to why extensive neurotoxicology testing was reported in an appendix but there was little discussion of the results. Dr. Irwin replied that neurotoxicity was considered to be a possible complicating factor that might interfere with evaluation of carcinogenic potential. Tests such as measurement of grip strength and responses to acoustic and thermal stimuli were intended to determine whether there was neurotoxicity present. In public comments, Dr. Mary Barth, Mobil Oil Corporation, reported that there are several unpublished studies that indicate tricresyl phosphate is somewhat more toxic with corn oil as a vehicle than with mineral oil as a vehicle.

Dr. van Zwieten moved that the Technical Report on tricresyl phosphate be accepted with the revisions discussed and with the conclusions as written for male and female rats and mice, **no evidence of carcinogenic activity**. Dr. Bailey seconded the motion, which was accepted unanimously with nine votes.