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PEER REVIEW OF DRAFT TECHNICAL REPORT OF LONG-TERM TOXICOLOGY AND CARCINOGENESIS STUDIES BY THE TECHNICAL REPORTS REVIEW SUBCOMMITTEE AND PANEL OF EXPERTS

TRANSCRIPT OF PROCEEDINGS

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A P P E A R A N C E S

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DR. FREDERICA PERERA

DR. JAMES SWEMBERG

Panel Members

DR. JOHN J. CROWLEY

DR. KIM HOOPER

DR. THOMAS C. JONES

DR. RICHARD J. KOCIBA

DR. DAVID KOTELCHUCK

DR. FRANKLIN E. MIRER

DR. I.F.H. PURCHASE

DR. STEVEN R. TANNENBAUM

DR. BRUCE TURNBULL

C O N T E N T S

P R O C E E D I N G S (8:30 a.m.)

CHAIRMAN HOOK: Good morning. Welcome. I'd like to officially open the meeting of the Technical Reports Review Subcommittee and Panel of Experts who are assigned to be counselors to the National Toxicology Program. I'm Jerry Hook. Again, welcome to you all. We have a very busy day, and we shall try to get through the agenda that you have in the order of the chemicals to be reviewed today. Our goal is to end around 3:30. I'd like for us to get through both chlorinated paraffins by lunch, so you know roughly what our goal is.

I'd like to welcome the members of our panel. We have one new and old member, Dr. Frank Mirer, who is Direct of Health and Safety Department at the International Union of United Auto Workers. He's joined us again. I recall that he was going off as I came on the first time. We're glad to have you.

One other new member of the Board, Dr. Bob Scala, who is not with us today.

So as we begin, shall we introduce ourselves. First the panel and then members of the audience so we'll all know who we are. Dr. Purchase?

DR. PURCHASE: I'm Dr. Purchase from Imperial Chemical Industries in the United Kingdom.

DR. HOOPER: Dr. Hooper, the State Department of

Health in California.

DR. CROWLEY: John Crowley, from The Fred Hutchinson Cancer Research Center in Seattle.

DR. KOCIBA: Dick Kociba, Dow Chemical.

DR. MIRER: Frank Mirer, still from the UAW.

DR. SCHWETZ: Bernie Schwetz from NIEHS, NTP.

DR. RALL: Dave Rall, NIEHS, NTP.

DR. HART: Larry Hart, NIEHS, NTP.

CHAIRMAN HOOK: I'm Jerry Hook. I work at Smith Kline and French Labs.

DR. MCCONNELL: I'm Gene McConnell, NIEHS, NTP.

DR. MATTHEWS: Skip Matthews, NIEHS, NTP.

DR. HUFF: James Huff, NIEHS, NTP.

DR. PIEGORSCH: (Arthur) Piegorsch, NIEHS, NTP.

DR. BOORMAN: Dave Boorman, NTP.

DR. SWENBERG: Jim Swenberg, CIIT.

DR. TURNBULL: Bruce Turnbull, Cornell University.

DR. KOTELCHUCK: Dave Kotelchuck, Hunter Graduate School.

DR. JONES: Carl Jones, Harvard Medical School.

DR. PERERA: Frederica Perera, Columbia University.

(Appearances from the Audience.)

CHAIRMAN HOOK: Thank you very much. As we begin our discussion today, we'll ask Dr. McConnell if he has any

comments in general before we start.

DR. MCCONNEL: No.

CHAIRMAN HOOK: Dr. Huff?

DR. HUFF: No.

DR. HART: We just need to announce to our audience that we're taping this meeting. So please speak as clearly as you can. If you're speaking out here from the chairs on the side, please use the microphones on the side or the one by the podium and identify where you're from. Thank you.

DR. TANNENBAUM: I'm Steve Tannenbaum from MIT.

CHAIRMAN HOOK: I'm sorry, Steve, we missed you. Steve Tannenbaum.

Now we're ready to go. To remind you of our procedures, as we begin each chemical the chemical manager will be sitting at the head table and will review the study in general, provide the final summation of the conclusions. We then have from two to four reviewers on each compound. We will go through the reviewers as they're listed on the program, and then we will carry on our debate and discussions and reach a conclusion. On occasion members of the audience will be allowed to make comments. It's my understanding that on one of the compounds after lunch there is at least one individual with some prepared comments.

We are ready to begin then with the first chemical. It's decabromodiphenyl Oxide. Dr. Matthews is the chemical manager. Skip?

CHEMICAL MANAGER: Decabromodiphyenyl oxide is a completely (bromade) aromatic ether used as a flame redardant and plastic resin for synthetic fibers. Production figures are currently estimated at somewhere around or above ten million pounds per year. It has not been reported as an environmental contaminant. Human exposure is largely limited to production and use.

In a health survey of people involved in production and use there were some mild health symptoms reported, apparently nothing serious, and it wasn't sure whether these symptoms were due to decabromodiphyenyl oxide or to previous exposure to (polybromine) at the same plant.

This chemical has low animal toxicity. Doses up to two thousand milligrams per kilogram resulted in no acute toxicity. Repeat dose studies resulted in mild lesions in liver, kidney and thyroid. This chemical is not (ogenic), and it's not mutagenic in cell (

) not mutagenic in mouse lymphoma assay, and did not cause sister chromotid exchange or aberrations in Chinese mouse ovary cells.

It was not reported or found not to be

carcinogenic in a previous study using Sprague-Dawley rats in doses from .01 and 0.1 and 1.0 milligrams per kilogram per day. The chemical was nominated to the NTP and studied as part of the class study in flame retardants.

In this study, the fourteen-day and thirteen-week studies failed to detect any compound-related effect when administered at the maximum doses. Therefore, in the two-year study doses were set at fifty thousand parts per million as one-half, MTD and MTD, twenty-five thousand parts per million.

The results of these studies indicate that survival and body weight were unaffected in rats, except for low-dose males, and this became significantly different only in the last two weeks of the study. And it's probably not compound-related.

In mice survival of control males was decreased quite a bit. This is presumably due to fighting. Body weights in the males were unaffected.

Conclusions coming from this study were that some evidence of--that there was some evidence for carcinogenicity in male and female rat liver as evidenced by increased incidence of neoplastic nodules in the liver. And there was equivocal evidence of carcinogenicity in male mice as evidenced by increased incidences of hepatocellular adenoma and

A F T E R N O O N S E S S I O N (1:33 p.m.)

CHAIRMAN HOOK: Shall we reconvene?

For those of you who weren't here this morning, our procedure is to identify the compound and the chemical manager. The chemical manager will review the study for us. Then our principal reviewers will present their comments. And in the case of our first chemical we will have some comments from the audience. And then the chemical manager will respond, and then we will open the panel up for discussion.

The first chemical compound is Tetrachloroethylene, Perchloroethylene. Dr. Mennear?

TETRACHLOROETHYLENE

CHEMICAL MANAGER: Tetrachloroethylene, also commonly referred to as perchloroethylene, or PERC, is an industrial solvent used in a variety of processes such as metal degreasing and dry cleaning.

In 1983, two hundred and fifty kilograms of tetrachloroethylene were produced in the United States. It has been estimated that eighty-five percent of the tetrachloroethylene used annually is lost to the atmosphere.

PERC was nominated because of its widespread industrial use and because of the potential for human exposure. NIOSH estimated that approximately five hundred thousand

Americans are exposed to this chemical in the workplace.

Our studies of the toxicology and carcinogenicity of ninety-nine percent pure tetrachloroethylene were conducted by exposing Fisher 344 rats and B6C3F1 mice to air containing tetrachloroethylene which was stabilized with N-methyl-morpholine for six hours a day five days per week for 103 weeks. The exposure concentrations used in the chronic study were 0, 200 parts per million and 400 parts per million for rats and 0, 100 and 200 parts per million for mice. These doses were selected on the basis of the results of thirteen-week inhalation studies also conducted in rats and mice.

In these thirteen-week studies, 1600 parts per million of tetrachloroethylene was found to be lethal to a few of the male and female rats and mice. In rats, doses of 200 parts per million or more produced minimal to mile hepatic congestion. In mice, 200 parts per million or more caused minimal to mild liver and kidney changes. Liver changes included leukocytic infiltration, centrolobular necrosis, bile statis and mitotic alterations. Kidney changes were karomegaly of the tubular epithelial cells.

During the two-year studies, body weight gains for dosed rats and mice were essentially similar to those of the control animals. The survival of male rats dosed with 400 parts per million of tetrachloroethylene was significantly

reduced. This decreased survival may have been related to an increased incidence of mononuclear cell leukemia in dosed males.

There was a compound-related increase in the number of early deaths that were attributed to mononuclear cell leukemia while deaths among the male rats from other causes remained unchanged. The survival of dosed female rats was not affected by tetrachloroethylene.

The survival of both groups of dosed male mice was reduced relative to controls; however, untreated control survival in this study was unusually high with ninety-two percent of the animals living until the scheduled termination of the study. Exposure to 200 parts per million significantly reduced the survival of female mice. The early deaths among both male and female mice may have been influenced by the development of hepatocellular carcinoma.

Both exposure concentrations of tetrachloroethylene were associated with increased incidences of mononuclear cell leukemia in male and female rats. In females both concentrations reduced the time to the diagnosis of the tumor.

Tetrachloroethylene caused renal tubular cell karyomegaly in both male and female rats and renal tubular cell hyperplasia in males. There was also an increase in renal tubular cell adenomas and adenocarcinomas, combined,

in dosed male rats. The renal changes have been consistently found in our earlier chronic studies of chlorinated ethanes and tehylenes.

Four high-dose male and two high-dose female rats had gliomas of the brain, but one control male and one control female also had the tumor.

In both male and female mice, exposure to tetrachloroethylene caused increases in hepatocellular neoplasms, with increases in the incidences of both adenomas and carcinomas being detected in males and increases in carcinomas being detected in females.

Tetrachloroethylene also caused renal tumbular cell karyomegaly in both sexes of mice, and one low-dose male also had a renal tubular cell adenoma.

There were no neoplastic changes in the respiratory tracts of either rats or mice, but there was an increased incidence of squamous metaplasia in the nasal cavities of dosed male rats.

There was no evidence of mutagenic activity for tetrachloroethylene when it was tested with or without activation in four strains of salmonella. Tetrachloroethylene was also inactive in the mouse lymphoma, Chinese hamster ovaries, sister chromatid exchange and drosophila tests.

During the audit of the data of this study, a

few untrimmed lesions were found. All were adequately resolved and had no impact on the interpretation of the results.

Under the conditions of these inhalation studies, there was some evidence of carcinogenicity of tetrachloroethylene in F344 rats as shown by increased incidences of mononuclear cell leukemia in males and female and renal tubular cell neoplasms in males. There was clear evidence of carcinogenicity in B6C3F1 mice as shown by increased incidences of both hepatocellular adenomas and carcinomas in males and of hepatocellular carcinomas in females.

CHAIRMAN HOOK: We'll begin with our reviews. Dr. Swenberg?

DR. SWENBERG: I thought this was a well-written Report of a well-conducted study on tetrachloroethylene. It's encouraging to see this one compared to the gavage studies.

Items that should be discussed prior to the acceptance of the Report include the following:

On page 16 there should probably be some mention of tetrachloroethylene as a ground water contaminant. That's one of the most common routes of human exposure, albeit low doses.

Pages 53 and 84 refer to renal lesions and comment on their likeness to the hydrocarbon-induced nephropathy.

I think that in order to make this correlation some additional work will be necessary. This will be the staining of sections for hyaline or protein droplets that are characteristic of hydrocarbon nephropathy. It's my understanding this is underway. If they are present, then their conclusion is supported. If they are not present, I'm afraid it's rejectable.

Page 54 and the Abstract should clearly point out that the renal tumors present in the rats were not statistically significant, but that similar lesions have been noted in the prior gavage studies.

Page 57. I questioned the statistics that were present on interstitial cell tumors of the testicles. It seemed highly unlikely to have values of .001 for such small differences, but maybe I'm wrong.

Page 58. The thromboses that were observed in the nasal cavity are most likely secondary to mononuclear cell leukemia. We've noted a very high incidence of this lesion in nasal cavities of leukemic rats and have never seen it in non-leukemic rats. So I'd like to see the records of the gross or the other lesions reviewed in that regard.

To get into two of the more substantial aspects of this, I think the Report should very clearly state that the interpretation of mononuclear cell leukemia is really based on the standard method of data evaluation and that it's

additionally supported by the dose-response effect on tumor latency and the staging evaluation. The tumor latency data should include the mean latency by dose group for animals dying prior to the terminal sacrifice.

And I think it should also point out that this staging effort is really a preliminary effort and may require additional refinements. We have to find out if it really is giving us meaningful data before we hang our hat on it very strongly.

The Stromberg and Vogtsberger paper was cited in this Report saying that mononuclear cell leukemia has a course of two to six weeks. And I think if that in fact is the case, then staging is really irrelevant. That citation, by the way, was incomplete and could not be referred to by anyone the way the report was. You need to get that citation in there.

The discussion of this Technical Report should be expanded to discuss possible mechanisms of carcinogenesis of tetrachloroethylene. In particular, it should point out that the mutagenicity studies were negative and that it caused tissue toxicity at the same site as neoplasia in at least two of the three tissues, i.e, the mouse liver and the rat kidney.

And I don't think we really have a good handle

on whether it's caused a similar type of lesion in the immune system of the rat. This is somewhat of a difficult tissue to evaluate from the pathology standpoint for toxicity. But I know the NTP has an immunotox program, and I would strongly recommend that this compound be examined by them for its effect on immunotoxicity.

The particular endpoint we're talking about here--that being mononuclear cell leukemia--has one of the sharpest age response incidences for tumor induction of any endpoint I know of. And if we were affecting the age of the tissue through toxic responses, we might see this kind of response. So I think it is a very important project for future work of the NTP.

I think those are the only comments I have. I basically agree with the conclusions of the draft Technical Report.

CHAIRMAN HOOK: Thank you. Dr. Mirer?

DR. MIRER: I also thought this was a well-conducted, very important study. I will want to talk about the conclusions at the end.

Under Editorial Comments:

The OSHA limit for perchloroethylene is incorrectly stated on page 16, it's a hundred parts per million, which is equal to 680 milligrams per cubic meter, not fifty parts

per million as stated in the introduction. That latter number, the fifty parts per million number, is a NIOSH-recommended limit. And I guess I should say that although it is generally inapproprite to discuss considerations for human risk in these Reports, the fact of testing a substance at the existing exposure limit and finding a substantial effect at that level ought to be noted.

Throughout the introduction, doses are quoted in a variety of units. For example, dry cleaning exposures are quoted in mg/M^3m all other inhalation exposures in parts per million. And I think it would be helpful to convert these. And also to attempt to estimate the dose perchloroethylene in rats and mice in milligrams per kilogram per day. This would permit some quantitative comparisons of the results of the gavage and inhalation studies and eventually comparisons to the human exposure levels.

Under non-tumor pathology, to repeat my previous theme, the summary should place greater emphasis on the doses associated with the appearance of non-tumor pathology. Kidney lesions at two hundred parts per million exposure in male and female rats and other lesions of male and female mice at a hundred parts per million, together with the failure to demonstrate a no-observed-effect level for these pathlogical changes should be pointed out.

In addition, the text now associates these effects with a range of doses, especially in the thirteen-week studies, without making a call at what level of exposure they first appear.

Now, back to semantics. Under conclusions, I agree with the conclusions of "clear evidence of carcinogenicity in both sexes of mice." I also think the conclusion should indicate "clear evidence" of carcinogenicity in male and female rats to be consistent with the definitions of these categories. The neoplasm is malignant. It is present in increased incidence, and the increase in this study appears to be chemically related by all the usual criteria.

The use of the life table test for statistical analysis is well justified by the text and is, therefore, judged appropriate. In the alternative, should the previous method of non-time dependent pairwise comparison be used, elevations were significant by trend test and marginally significant in pairwise comparisons; for female rats elevations were statistically significant by trend test and in pairwise comparisons using the late, lamented Benferroni inequality criterion.

The finding is apparently viewed as less convincing because control animals in this group showed a high rate of mononuclear cell leukemia compared to historic

controls. Despite this, rates of tumors in the treatment groups were significantly elevated over the concurrent control and presumably markedly higher than historical controls. There is no biological or other explanation advanced for using a high incidence in controls to discount the significance of elevations in the treated animals. Indeed, the historical/concurrent control argument is usually stated in the other direction.

The increased incidence which appears in the crude analysis persists when the exercise is extended by staging and comparing groups by time of death. This analysis should lend additional weight to the finding. And I add that it would be desirable to add statistical tests to the data on Table 25, page 77.

The leukemia evidence is bolstered by the presence of renal tubular cell adenomas and carcinomas in male rats. These rare tumors would alone constitute some evidence of carcinogenicity in male rats.

Therefore, I do not agree with the conclusion regarding the level of evidence in rats. Although I should say that this "clear" versus "some" debate, which is new to me this term--I'm quite clear where we're going with that today.

(Spontaneous discussion.)

CHAIRMAN HOOK: I think we're going in circles. Thank you, Dr. Mirer. Dr. Turnbull?

DR. TURNBULL: Well, my first comment was just to remark that there is no sentinel animal in this study.

My next remark concerns historical rates. And this has come up with leukemias. And the first point about that is a generic one that, for instance, in the analysis on page 52, leukemias for the rats, it states, "Historical incidence of, say, twenty-seven percent plus or minus nine percent." It wasn't clear to me whether that was one standard deviations, two standard deviations or extremes experienced in the historical studies.

It turns out from Appendix F that it is in fact one standard deviation. So the range could even be bigger than what's seemingly suggested by twenty-seven plus or minus nine.

Also, with these historical controls, I'm not sure what the fair comparison is, whether, for instance, we should have--one historical comparison might be just with control groups in inhalation studies. Just as perhaps with a dermal study, one historical control group for comparison should be other skin painting studies rather than just all control groups.

That leads on to my next point. Apparently, there

was another concurrent control group going on at the same time in the same lab, also an inhalation study. That was the one we reviewed last time, methylene chloride. These are very insensitive screens that we're doing. And I'm always looking for more power.

One way to do that would be to include this second concurrent control group if that's fair. It s emed that conditions were right. This is the same time and the same lab. But apparently it might have been a different pathologist. I don't know what that means when it's a different pathologist. I had hoped that with the reviews and the coded readings and the PWG and everything, that that would somehow control the interpathologist variability, but maybe someone can put me right on that.

Anyway, if you do, then I asked NTP to do the analysis. It was just given to me five minutes ago, and I'm not sure whether there are any differences or not. I haven't been able to assimilate this work using the larger--the enlarged--data.

Anyway, even if you don't agree with including it as a concurrent control group, then perhaps it should have been included in the historical data base. Because it is history, it has been approved, and we only have two inhalation historical groups. And all the rest of the thousands of

animals were not inhalation studies from what I understand. I don't know whether that makes a difference or not. Certainly, I would think it would make a difference in skin painting vehicle controls.

Okay. The high incidence of leukemia in control rats--now this is very similar to that first chemical that we reviewed today, the decabromodiphenyl oxide, which also had a high incidence of leukemia in the rats if I recall right. There, in the study, NTP tried to play down the statistical significance because it was an abnormally high rate. No such attempt to downplay the significance was don in this Report, although it seems to me that there was randomization going on here. There was uniformity of pathology. So it should be fair enough just to compare the groups here.

I would like to know what the incidence was of leukemia in the methylene chloride, control group. I don't have that with me. Is that available?

CHEMICAL MANAGER: I don't remember the exact number, but it was within two percentage points.

DR. TURNBULL: So it's very close to this?

DR. HUFF: Yes, it is.

CHEMICAL MANAGER: Yes.

DR. TURNBULL: So I'm almost prepared to forget

the low--I don't think this is a point, the high rate of control leukemias.

(COMMENT): The control was sixty-eight percent.

DR. TURNBULL: Sixty-eight percent in the methylene chloride?

So I think this is a fair analysis here. I agree with Dr. Mirer on the point he was making about the leukemia in rats.

This special analysis on staging of leukemia, I'm a little skeptical about it. It does look a little bit like data dredging to me, so I would downplay those P-values some for that special analysis.

CHAIRMAN HOOK: Thank you. Dr. Jones?

DR. JONES: I think almost everything I was going to say has been said. Essentially, I agree with the conclusions of the Report. There are a couple of editorial and other comments I would like to make.

The first thing I noticed, as I had noticed in quite a few of the Reports, there seems to be no clear correlation between the results of the mutagenicity tests and the bioassay tests. And I missed a meeting, and I wonder if such a study of these correlations have been made, and has it been presented to this group? If so, I missed it.

CHAIRMAN HOOK: It wasn't presented here; I believe

it was presented to the Board.

DR. JONES: Has that been published? Can we get anything on it?

DR. MCCONNELL: We will take care of getting you some of the publications on that.

DR. JONES: I'd be very much interested in that. I think that we have sort of passed over that as we've gone along, but it seems to me it's something we probably should notice.

From an editorial standpoint, I noticed that many of the reference citations are incomplete, as they were in many of the Reports today. I don't know whether that's a code, you have some way of picking these up later on or what happened. And I know that you couldn't find those references when they just had the year and the name of the person. Is it intended that they're going to be completed later?

CHEMICAL MANAGER: Oh, yes. They will be completed later. The material is all sent out of town for typing and putting into this final form. And the contractor who does it has been instructed not to try to complete a reference until they have the hard copy in their hands. Sometimes it will be the chemical manager who is negligent and doesn't get the hard copy to the people who are typing these reports. That's why some of them are incomplete.

DR. JONES: It's been my experience that if you dump a lot of sand in the stream, it's very hard to get it all out downstream a mile or so. I hope it doesn't come to that.

(Spontaneous discussion.)

DR. JONES: Concerning the conclusions, I point out that the first sentence--and I'll read it: "Under the conditions of these inhalation studies, there was some evidence of carcinogenicity of tetrachloroethylene in F344/N rats as shown by an increased incidence of mononuclear cell leukemia in males and female and rare renal tubular cell neoplasms in males."

Now I assume that that sentence means that the--there's two bits of information. The renal cell tumors and the mononuclear cell leukemia. Those two factors were the reason for saying that there was "some evidence." It wasn't one or the other, it was both of them. That's the way I read it. I assume that's the way it was intended.

And I have no more comments to make on this very excellent Report.

CHAIRMAN HOOK: Thank you.

The Halogenated Solvents Industry wants to respond to the study. You all have copies of their written comments. And I believe that we have a representative who

wants to make some comments to us now.

DR. ROBINSON: Good afternoon. My name is Thomas Robinson of Vulcan Chemicals, and I am here to speak on behalf of the Halogenated Solvents Industry Alliance regarding the recent draft Technical Report for perchloroethylene inhalation bioassay. You have already received a copy of our written comments and consequently I will keep my remarks brief.

I do wish to point out that on Table 3 of the written comments, the last entry under female rats should read eighteen out of fifty, not fifteen out of fifty.

Of major concern is the NTP's finding of "some evidence of carcinogenicity of tetrachloroethylene in Fischer 344/N rats as shown by increased incidences of mononuclear cell leukemia in males and females..."

We do not believe that such a finding is warranted based on our brief review of the Technical Report and its underlying data. Although there is an equally increased incidence of leukemia in dosed female rats, the incidence of mortality at both dose levels is the same as in untreated controls. Further, the number of animals and the number of leukemias for all dose levels at terminal sacrifice were essentially the same.

A similar lack of correlation of mortality with

with the incidence of leukemia is also seen in the male rat. Even though the high dose male rat had a higher early mortality than the low-dose animals, the incidence of leukemia was essentially the same for both dose levels. In contrast to this observation was an equivalent early mortality in both untreated and low-dose animals.

There are two additional issues which must be considered regarding the association of mononuclear cell leukemia with exposure to perchloroethylene. There was an unusually high incidence of leukemia in control animals in the perchloroethylene bioassay. In both male and female rats, the rate of leukemia is more than twice that seen in historical controls for the NTP program.

In fact, the incidence of leukemia in male rats reached a record high fifty-six percent, which was ten percentage points above the NTP's previous upper bound as contained in the draft Technical Report.

It is important to note that in the methylene chloride study, the incidence of leukemia in male control rats was sixty-eight percent. Moreover, the Battelle propylene and propylene oxide studies had a leukemia incidence in male rat controls of thirty-two percent and forty percent respectively. This high and apparently increasing incidence in Battelle controls raises questions as to the ability to

draw any conclusions from the perchloroethylene study relative to the incidences of leukemia as evidence of carcinogenicity.

The second issue is of even greater significance since it may have long-term implications for the NTP bioassay program. This study is the first to base a conclusions, at least in part, on the staging of mononuclear cell leukemia in Fischer rats. The staging of leukemia and other cancer is for the assessment of the degree of neoplastic progresssion. Staging requires knowledge of the biological course of the cancer and a correlation of the morphological findings with duration of the disease and the severity of the effects.

The biological course of the Fischer rat leukemia is not well understood and it is questionable whether the current staging method used by NTP actually reflects this correlation. Some examples of the perceived deficiencies in the current staging method were given in our written comments and need not be addressed here other than to say they exist.

To insure the proper interpretation of the significance of mononuclear cell leukemia in the perchloroethylene study and in future bioassays, staging criteria must be established which will reflect as closely as possible disease duration and the extent of progression. Therefore, we propose the co-sponsoring with NTP of a panel of experts

in Fishcer rat leukemia to develop an appropriate staging scheme and to evaluate the rat leukemia pathology in the perchloroethylene study.

Unless the significance of the mononuclear cell leukemia is established by a valid staging scheme, we are concerned that the current procedure may set a precedent that will undermine the objective evaluation of this and of future bioassays.

The NTP ocncluded that there was "some evidence of carcinogenicity" in male rats based on a slight increase in kidney tumor pathology; however, the incidence of combined adenomas and adenocarcinomas was not statistically significant. Perhaps this finding has some other explanation related to the in-life phase of the study. Moreover, due to the brief interval between the issuance of the draft Technical Report and today's meeting, we were unable to examine in any detail the mouse portion of this study.

Althoug we're aware that an audit of the perchloroethylene inhalation bioassay has been conducted by Dynamic Corporation, HSIA will also conduct its own audit of this study.

Regarding the issue of mononuclear cell leukemia in the rat, we urge the development of a suitable staging scheme for this lesion by an expert panel. Further, based

on the lack of correlation of the incidence of leukemia with mortality, and more importantly, the extremely high incidence of leukemia in Battelle controls, we believe that a finding of "some evidence of carcinogenicity" is NOT warranted. The present data is inadequate to establish the significance of the incidence of leukemia relative to perchloroethylene exposure.

We appreciate the opportunity to make this presentation to the Board of Scientific Counselors, and I will attempt to answer any questions. Thank you. I also have additional copies of our written comments should someone like to have one.

CHAIRMAN HOOK: Thank you.

At this time I should say that this is the only request from someone to make comments from the audience. We will take other contributions from the audience at this time.

(No response.)

CHAIRMAN HOOK: There being none, then Dr. Mennear, respond, if you would, to the Panel.

CHEMICAL MANAGER: All right. I think many of the concerns voiced by the reviewers as well as by Dr. Robinson overlap, so I'll not try to differentiate who brought what up, but just comment on these things as we go along.

First of all, I want to point out or agree that

we should not make a conclusion of presence or absence of carcinogenicity based upon staging of the leukemia in and of itself. As Dr. Robinson noted, we are simply at the beginning stages of this kind of diagnosing.

But I also want to point out that if you look at the statistical tables, our routine statistical tests do indeed show statistically significant increases in the incidences of mononuclear cell leukemia in these animals.

One of the things that we thought we wanted to do was go in and look more closely at this. So we're using the staging, the special Kaplan Meirer curves, special Kaplan-Meirer curves, that we put in the discussion section. We're using all of this as a way of trying to understand better what the data is trying to tell us.

And as we have gone through, one of the things that the data seems to be telling us is that, indeed, in the female rat exposure to tetrachloroethylene the animal develops mononuclear cell leukemia earlier. Now, it's also been pointed out that it seems strange that if we've got more mononuclear cell leukemia in the female rats, we should also have a greater incidence or effect on survival. And in the case of female rats, survival was the same in the dosed groups as it was in the control groups.

We went back and began looking at what the

pathologists diagnosed as the cause of death of these animals. And we found some rather interesting data.

Considering only the early-death animals--by the way, I'm trying to articulate this without going back to my old trick of using the overhead projector. If I can't get it out in words, I've got the data on an overhead, and you can look at the numbers.

But what was found was when the mononuclear cell leukemia was considered to be the primary cause of death in these female rats, we got a dose-related increase in the incidence of death considered to be due to mononuclear cel leukemia. The incidence was 8, 18 and 20 in animals dying primarily due to mononuclear cell leukemia.

And we started wondering what happened to the other deaths. And as we looked at what some of these other deaths in the control animals--what were the primary causes of other deaths in the control animals--we found two categories that particularly stuck out.

One was animals that died from pituitary adenomas or pituitary carcinomas. And we found that in our control group six animals died in this category. In our low-dose group we had only two animals die in this category. In our high-dose group we had only one animal die in the category.

Another cause of death that stuck out was anima

that had to be killed for humanitarian reasons because of large mammary gland or (clitorol) gland tumors. Again, we found six animals in the control group that were killed early. And these deaths, these early terminations, were between weeks 84 and 98 of the study. So they were rather late in the study.

In the low-dose animals we found only four animals that had to be killed early, and in the high-dose group we found three animals that had to be killed early.

Now, this raises an obvious question. Does tetrachloroethylene protect against pituitary tumors and mammary tumors and that sort of thing? And the answer is no. If we go through and irregardless of what the primary cause of death in these animals might have been, if we just total up the number of animals that had pituitary tumors and total up the number of animals that had the mammary or the (clitorol) gland tumors, we find that across the board all animals--that the animals had the same rate.

So the conclusion that we've come to here is that the mononuclear cell leukemia in the female rats is causing the death of these animals before these other tumors can develop to a stage sufficient to kill the animals.

If you look at the median time to death of the early-death animals and the diagnosis of mononuclear cell

leukemia, we find that the median time to the diagnosis in the controls was 98 weeks, the median time to the diagnosis in the low-dose animals was 87 weeks, and the median time in the high-dose animals was 89 weeks.

I think that an important question that came up is this business about the historical incidence of mononuclear cell leukemia. And we struggled with this also. And Dr. Rao of the NTP has got some information that we would like for him to share with you on the historical rates.

CHAIRMAN HOOK: Well, very quickly. We've gone through it once today. Very quickly.

DR. RAO: Well, I would rather use an overhead projector so it will be clear and can be done faster than rattling off some numbers.

Let me pass out some of the tables quickly so you will have them also.

Specifically, I wanted to show you the incidence of mononuclear cell leukemia in inhalation studies. Some months ago when we started seeing (the increase in) incidence, we were puzzled too and wondered what is happening.

So to really get a handle on this, we took mainly the inhalation study to address that, the present Report. You can see we have a 1981 Report (indicating an average of thirty-five percent). But if we take the 1983 necropsies--

we're going by the year of necropsy. The incidence was 52, and then in 1984 we have some studies. Fifty-nine in the male. And we do not have any studies available that were necropsied in 1982. And that really indicates a dramatic jump or increase in 1983. But the number of studies are so few we cannot really draw a conclusion on what has happened.

Is there a change in environment, animals, procedures, whatnot? So we had to take some other data base, proceed on that and try to get a different handle on that one.

Here we have a series of studies, dose feed, 1980 necropsies of the male rats, getting a twenty-seven percent average. In 1981, twenty-six percent average. And then in 1982 necropsies we see a quick change happening right here, and I included two arrows. I will explain what that is. And then 1983, forty-five and forty-nine percent.

Look back at the source of the animals. The animals were not (redivided). And for most of these studies the sources are the same, Charles River or Kingston, where the foundation animals are the same.

And also this arrow indicates there is a change here. The commercial diet, we started the NIH07 diet here. To resolve this question of why, even with the NIH07 diet, the two studies, all of them with less than forty percent

incidence whereas these are more than forty percent, we selected this study and this study and restaged them. And we staged--looked at two pathologists together.

We found that this represented a truly stage three or advanced case of leukemia. But our (presents) showed up as twenty-two and the ( ) pathologist was twenty-four.

When we looked at this one, stage three or advanced case, was studied, six percent. And then stage two or the clear evidence of the lesion was the other thirty percent.

So this represented more an addition about at stage two and stage three. And this change happened--in October of 1982 we had a conference on hematopoietic tumors. And in that there was considerable presentations, discussions on mononuclear cell leukemia.

These are necropsies of the June-July time period, probably right after the conference. From then on we have a change in the incidence reported. Not really, actual change in the animal or the actual incidence of the lesion.

So that gave us an adequate indication at this point that there is no real change in the animal or the environmental conditions. Except because of the increased awareness of the pathologists as well as some criteria set

forth for diagnosing the lesion, they are including additional cases.

CHAIRMAN HOOK: Thank you very much. Are there any questions specifically for Dr. Rao? Dr. Purchase?

DR. PURCHASE: I didn't quite understand.

You said that you compared two studies? One with a twenty-four percent incidence, one with a sixty percent. Did the pathologists go back and reread the twenty-four percent study?

DR. RAO: We have the slides available. Two pathologists--we went back, looked at all the slides. We have restaged them, stage two, stage three. Stage three, that's the ( ) case. It agreed with the original pathologist. Plus, we have forty other percentile, forty more of twenty other animals with stage two which are not included in the original.

DR. PURCHASE: So the percentage went up sixty percent?

DR. RAO: Went up to the sixty-six area. It's approximately the same as others.

DR. JONES: Do I understand what you say here that after this conference you went back and looked at a study that had been completed before?

DR. RAO: Yes.

DR. JONES: This one that's at twenty-four percent?

DR. RAO: You see, the second arrow represents June necropsy, June of 1982, which usually comes for reading around October of 1982. And time of the conference. Right after the conference all the incidences shifted.

DR. JONES: In connection with this twenty-four percent under 1982---

DR. RAO: Yes?

DR. JONES: ---you said that you looked at those slides and they were all stage three?

DR. RAO: That's right.

DR. JONES: So that means that stage one and two were not recognized?

DR. RAO: Weren't called at the time.

DR. JONES: And that would explain---

DR. RAO: Stage one and two are present, but our reading indicates we had actually--if we included stage two and three, it would be more like sixty-six percent.

DR. JONES: Okay.

CHAIRMAN HOOK: Is there another hand over here?

(No response.)

CHAIRMAN HOOK: Thank you, Dr. Rao.

Back to our discussions on the study. Do we have other comments?

CHEMICAL MANAGER: I would comment regarding the actual staging of leukemias, I don't know whether Dr. Boorman would care to make any comments about this now with respect to what Dr. Rao said.

DR. BOORMAN: I think it's appropriate. You know, I think it's been treated with a little bit more mystique than it deserved. It was simply a matter of trying to look at whether we were dealing with advanced stages or very early cases or somewhere in between, so we simply broke it into three stages. And I think it's added a lot of clarity to know whether our calls and our judgments are being based on early cases that are difficult to (tell from back count) or whether they are such obvious cases that anybody would agree that it's clearly an advanced case of mononuclear cell leukemia.

So that's simply what we've been doing. Once we started doing it, there's more and more people interested in it. I don't want to take too much time.

There's sort of an implication that so little is known about mononuclear cell leukemia that we should not be staging it. That's simply not the case. I think (Malone) was one of the first ones to report the disease in 1970 in the Fischer rat. And there's literally dozens of papers. There's been real good work that's come out of Battelle-

Columbus with Stromberg, for example.

We also had an in-house group here with Jeff Branch, Mike (Peters), Bob (Marigold) (where we're maintaining it transplantation). And we know how long it takes from the time you inject the cell until they die and how long it takes from the time you see cells in (blood) until they die and so forth. I think that's more detail than you want it right now. But it's not as mysterious as you might be led to believe.

I think in the interest of time I'll stop there.

CHAIRMAN HOOK: Fine. Thank you. Other comments or discussion around the study? Dr. Purchase?

DR. PURCHASE: I have a couple of comments on this. Firstly, I think the summary omits the dose levels that were used in the long-term study. I may be wrong. I think it actually misses them out. And I think that should be put in there.

On page 56 there is a discussion of gliomas. And again, I wonder whether this has taken into account whether the glioma is fatal or not. Maybe a pathologist could help. I think that they are usually non-fatal tumors. And therefore, there is no significant increase if that is correct. And the discussion in regard to the gliomas looks to me as if it could be tightened up some.

And on page 83 there appears to be reference here and elsewhere in the report to kidney effects with both chlorinated alkanes and alkenes, some of which refer to Reports which are still under preparation within the NTP. Therefore, by definition, they are not available to the Peer Review Panel. And it would seem to me to be unwise to use that as a means of confirming a conclusion from this statement.

It's not entirely clear which of those studies are in the pipeline and which are the ones that have already ben published. I'm sure that when those studies come out, they will refer to this one as supporting evidence. We can't have it both ways, particularly when they are not yet published.

CHEMICAL MANAGER: With the exception of the study that I referred to of four strains of rats, they've all been peer-reviewed, and the four rat strain studies will be coming through, we hope, in the next peer review. But we won't use that one as a reference here.

DR. PURCHASE: The only other comment I have to make is that the leukemia incidence and the way in which it has been analyzed in this report, I would support Dr. Swenberg's statement that one must recognize that the primary conclusions are drawn on the straight statistics and the staging is a secondary sort of elaboration of that. If

you look at it that way, I think that it will be unwise to move this one up to "clear evidence." I think it should remain "some."

CHAIRMAN HOOK: Thank you. Other comments? Dr. Kociba?

DR. KOCIBA: Is this going to be the standard approach that NTP uses in "staging" the mononuclear cell leukemias in all future reports being this is identified as a problem area?

DR. BOORMAN: No. We're not routinely staging leukemias. If there is an increase in mononuclear cell leukemia and it becomes especially a question of whether it's related to mortality, then it's important to know whether or not there are advanced cases or not advanced cases.

We recognize that it needs some additional work, and we're working on a publication right now that we will be submitting for peer review and getting input and stuff like that. So, this is just a crude step, but we hope to make it a little bit more elaborate.

DR. KOCIBA: So is it your plan to delete it from this Report here until your work is done?

DR. BOORMAN: No. I think there's nothing wrong--I think the statistics and all the conclusions are based on the raw incidences, as Dr. Swenberg has said. All we've done

is we've also added for your information--these are the cases where it's advanced, these are the cases where it's very early, and these are the cases of stage two where it's in between.

And I think to delete that data would be deleting useful data. We're not making our call on the stages.

CHAIRMAN HOOK: Other comments? Dr. Hooper?

DR. HOOPER: Yeah. I wanted to comment on what Dr. Mirer said. I think what I would encourage doing is separating the characterizations of male and female rats. And I would call the effect in males with mononuclear cell leukemia as clear. If you look on page 52 at Table 12, the statistics of using a life table test for the leukemia is significant trend, highly significant trend, and highly significant high-dose, and a significant low-dose.

And also, as I think he commented on, the fact that the high control incidence in a sense would mask or make more difficult to (attach) to the fact in the low-dose and high-dose groups.

The mention that the highest reported incidence of leukemia in these animals is forty-six percent, and we're considerably above that in the high-dose and the low-dose groups. This finding in males is supported by the finding in females, which is significant to the low-dose group. So,

in a sense, then, I think you have "clear evidence" because you have the malignancy being clear in statistical effects, and then support from the opposite sex.

In addition, I'm curious about the findings in the brain tumors. On page 56, Table 15, we looked at gliomas, and the controls were one of fifty in low-dose, zero of fifty and high-dose four of fifty in male rats. And here, at least my understanding is that the historical control incidence, the mean is 0.8 percent.

So you have a control incidence which obviously is higher than historicals. Just one animal. But your high-dose animals is quite higher than historical. And that's not statistically significant, but in the past we've talked about historical control incidences. And if we're going to use them to diminish the significance of the tumor, I wonder if you oughtn't to be flexible and in a sense look at this tumor of increased significance. Because you have some effect that's much higher than historical control incidence.

So I would say that this tends again to augment the fact that something is going on in males. I'd argue that you have "clear" in males and "some" in females.

CHAIRMAN HOOK: Dr. Swenberg wanted to respond.

DR. SWENBERG: Yes. Let me address the brain tumor issue before I go on to the point I was going to make.

For some reason in the last few years we're starting to see a lot more brain tumors, and they seem to cluster. And the statisticians don't have any idea why this is happening. But I think--maybe Dick could comment on it, but you recently had a study in Fischer rats where I think the control incidence had fourteen percent or sixteen percent in the controls.

And we've had the blue dye studies and this whole food color series, and brain tumors have varied from one to fourteen percent or zero to fourteen percent in the controls. And therefore, I no longer accept that brain tumors are as rare as we used to previously think they were. And we don understand why they tend to cluster, but there sure as hell is evidence that they are.

As to the males being "clear evidence," I'd like to argue strongly against that. We've already heard that the methylene chloride controls run in the same lab at approximately the same time had sixty-eight percent mono-nuclear cell leukemias. So you're going to say sixty-eight, seventy-four, seventy-four is a clear a clear effect of carcinogenesis? I just can't buy that. It just doesn't make good sense.

DR. RALL: It wasn't the experiment---

DR. BOORMAN: This could be a relevant comment

on the brain tumors. And this is something that Dr. Swenberg just pointed out, but I would like to reiterate it. Dr. (Solfeld) went back and looked at the aging study where we had done several different studies at Hazelton, lifespan.

And he found in some groups zero brain tumors and others I think it was four or five. But it was clear there was statistically significant difference between some control groups and the other control groups in brain tumors.

We pulled these brain tumors out and looked at them. And they are not just a few clusters of gliol cells. They are real tumors that are fairly large lesions.

Yet just for the exact reason that Swenberg stated, we felt the best way to handle it was to put it out here in the Report where everybody could see it, but we didn't call it.

DR. HUFF: But those were lifespan studies and to two years.

DR. BOORMAN: Yes.

CHAIRMAN HOOK: Dr. Kotelchuck and then Dr. Kociba.

DR. KOTELCHUCK: In reference to what Dr. Swenberg said, it is the concurrent controls that are the ones that are the key and should be the main ones in making the determination. They are fifty-six percent. It's not

sixty-eight versus seventy-four. And I think that comes up in a number of different cases. I think that the material on page 52, in fact, argues for "clear," not for "some."

CHAIRMAN HOOK: Dr. Kociba and Dr. Perera.

DR. KOCIBA: Just one comment on the brain tumors. I concur with what Dr. Boorman and Dr. Swenberg said about the variability for whatever reason, we have on our own observed these extreme variations in control groups. So I really don't attach any significance to the observation of brain tumors in this particular study. I wouldn't want to see that be given more significance than what it really indicates.

CHAIRMAN HOOK: Thank you. Dr. Perera?

DR. PERERA: Yes. I think Dr. Mirer's conclusions about the mononuclear cell leukemias constituting "clear evidence" are reasonable. But I also wonder why the increase in the male rats of testicular interstitial cell lesions were not included in our consideration that's found on page 57, Table 16. And there is a positive trend by both tests and a significant increase in both dose groups by the incidental tumor tests. And I don't believe that's even mentioned in the Abstract or the Conclusions. Why is that not also factored into consideration of the positive evidence here?

CHAIRMAN HOOK: Dr. Mennear?

CHEMICAL MANAGER: This is a very common tumor, of course, that develops in these animals. And although it was statistically significant, usually we find ninety-nine to a hundred percent of the animals have the tumor. So consequently based on our past experience, we're just not enthusiastic about making that a--making a call on that tumor.

Dr. Swenberg had mentioned that, and the statistics were--however, the life table analysis data were correct. But the incidental tumor test, it comes down to .05, but it's not a life-threatening tumor, so you don't want to use it. You wouldn't use your life table analysis.

CHAIRMAN HOOK: I think it's time we brought this thing to a close.

We are at a point now where some of you are going with "some," and maybe some are going with "clear." But I'm ready to accept a Motion to allow us to vote.

DR. SWENBERG: All right. I move that we accept the conclusion as written in the Draft Technical Report of "some evidence" in the rats and "clear evidence" in mice.

CHAIRMAN HOOK: Thank you. Is there a second?

DR. HOOPER: Second.

CHAIRMAN HOOK: We have a second.

All right. We have a Motion on the floor that we accept the Report. Are there substantive contributions

to this debate? Or should we bring it to a vote?

(No response.)

CHAIRMAN HOOK: Hearing none, I would bring it to a vote. If you vote "Aye," then you're supporting "some evidence" in rats based on the discussion we just had. If you vote "Nay," we'll try again.

Those in favor of the Motion, which is to accept the summary as written, indicate by raising your hand, please. I see four positive votes. Those opposed? Five. And those abstaining? Dr. Kociba and Dr. Purchase are abstaining. Thank you. There seemed in my mind no real debate around the mice. May I have a Motion about accepting the Report for mice as "clear evidence" of carcinogenicity?

DR. HOOPER: So move.

DR. PERERA: Second.

CHAIRMAN HOOK: Moved and seconded. Those in favor of accepting the Report regarding mice indicate by a positive response. Nine. Abstentions? Dr. Purchase and Dr. Kociba. Thank you.

Female rats, "some evidence" of carcinogenicity in female rats. Do I have a Motion?

DR. HOOPER: So move.

CHAIRMAN HOOK: Second?

DR. CROWLEY: Second.

CHAIRMAN HOOK: Okay. We're voting for "some evidence" of carcinogenicity in female rats. Those in favor, please so indicate. Eight. Opposed? One. And two abstentions.

Therefore, our alternative with male rats is someone wants us to say "clear evidence." A Motion for "clear evidence"?

DR. MIRER: So move.

CHAIRMAN HOOK: And a second?

DR. PERERA: Second.

CHAIRMAN HOOK: Okay. An "Aye" vote means you will accept "clear evidence" of carcinogenicity in male rats. Those in favor, indicate by raising your hand. Five. Opposed? Four. And our two abstentions.

Well, we didn't change a thing, we confirmed our decision. Thank you. So, finally, we accepted everything other than we strengthened the position in male rats and made it "clear." Agree? Thank you very much.

(Spontaneous discussion.)

CHAIRMAN HOOK: Okay. Let's move on to the next chemical, which is chlorendic acid. Dr. French, you did the study.

CHLORENDIC ACID

CHEMICAL MANAGER: Chlorendic acid is a chemical

intermediate used in the preparation of flame-retardant polyester resins and plasticizers. In 1981, the manufacture of chlorendic acid was estimated at approximately seven million pounds and imports at a hundred and forty thousand pounds.

Chlorendic acid is manufactured in an essentially closed system and as a chemical intermediate becomes an integral component of the final product. This chemical was nominated for studying after a class review of flame retardants because of its large production, structure-activity considerations, and the potential for human exposure. Presumably, the potential for human exposure is oral, primarily through the environmental degradation of pesticides and polyesters.

Toxicology and carcinogenesis studies were conducted by administering chlorendic acid in feed to groups of fifty male and fifty female F344/N rats and B6C3F1 mice at concentrations of 0, 620, or 1,250 parts per million for 103 weeks.

These concentrations were selected because at higher concentrations in the fourteen-day and thirteen-week studies in rats and mice, dosed groups had decreased mean body weights, more deaths, and an increased incidence of liver lesions. For example, centrilobular cytomegaly and mitotic