The review meeting began at 9 a.m. in the First Floor Auditorium, Hubert Humphrey Building, Department of Health and Human Services, 200 Independence Avenue, S.W., Washington, D.C. Members of the Subcommittee are: Drs. Jerry Hook (Chairperson), Curtis Harper and James Swenberg. Members of the Panel are: Drs. Louis Beliczy, Devra Davis, Seymour Friess, Thomas Jones, Richard Kociba, David Kotelchuck, Tom Slaga, Steven Tannenbaum, Bruce Turnbull, and John Van Ryzin. Dr. Friess was unable to attend the meeting.

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The next NTP technical reports peer review meeting will be held July 27, 1984, in Research Triangle Park, North Carolina. For information contact Dr. Larry G. Hart, (919) 541-3971; FTS 629-3971.
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Chlorodibromomethane. Dr. Swenberg, a principal reviewer for the technical report on the carcinogenesis studies of chlorodibromomethane, agreed with the conclusions that: "Under the conditions of these studies, there was no evidence of carcinogenicity in male or female F344/N rats receiving chlorodibromomethane. Fatty metamorphosis and ground-glass cytoplasmic changes of the liver in male and female F344/N rats were related to administration of chlorodibromomethane. There was equivocal evidence of carcinogenicity for male B6C3F1 mice; chlorodibromomethane caused an increased incidence of hepatocellular carcinomas, but the combined incidence of hepatocellular adenomas or carcinomas was only marginally increased. Some evidence of carcinogenicity was observed for female B6C3F1 mice, since chlorodibromomethane caused an increased incidence of hepatocellular adenomas and an increased combined incidence of hepatocellular adenomas or carcinomas."

Dr. Swenberg suggested that the abstract and the discussion indicate that the high dose male mice exceeded what is normally recognized as the maximum tolerated dose, however, the females did not appear to exceed the MTD. He asked for clarification on the lack of lesions in the livers and kidneys of rats exposed to 60 and 125 mg/kg in the 13-week studies, as well an expanded discussion of salivary gland lesions since there was a clear dose-response relationship.

As a second principal reviewer, Dr. Davis agreed with the conclusions. In view of the viral infections in male and female sentinel rats that were killed at 6, 12, and 24 months, she suggested that in future studies it might be useful to examine antibody titers in living cohorts to determine whether chronic antigenic stimulation may be having a role in mortality. She said the observed negative trends for several common rodent tumors should be compared with historical control data.

As a third principal reviewer, Dr. Tannenbaum said the more appropriate route of administration would have been drinking water since the predominant route of human exposure is in drinking water. Further, the vapor pressure and other physical/chemical properties of the compound in oil or in water will differ. A clarifying statement could be added to the rationale for selecting the gavage route of exposure. Dr. J. Dunnick, NTP Chemical Manager, reported there was a Japanese study in progress where chlorodibromomethane was being given as a microencapsulated form in the feed. Dr. Tannenbaum noted there was decreased body weight gain in both male and female mice, which was likely indicative of toxicity.

Therefore, although he agreed with most of the conclusions of the studies in rats and male mice, Dr. Tannenbaum thought the conclusion for female mice should be equivocal because of toxicity at the high dose and no effect at the low dose. Dr. E. McConnell, NTP, said the high dose in NTP chronic studies was intended to cause some sort of toxic but non-lethal effect; a 10% to 15% reduction in weight gain compared to controls being an example. He further stated that rarely does one reach the "optimal dose". Survival in high dose female mice was greater than in the concurrent control group. Dr. Swenberg said a moderate exceeding of the maximal tolerated dose (MTD), as seen here, does not invalidate tumor findings. In view of the relatively low and nonvariable historical rate of liver tumors in female B6C3F1 mice, the dose-response, and the statistical significance, he thought the interpretation of some evidence was correct.
There was considerable discussion about implications of an overdosing error in low dose male and female mice groups at 58 weeks, as well as the deaths of eleven high dose mice at 82 weeks for which a cause of death was unknown. Dr. Haseman said that this clustering of mortality in male mice in the high dose group at a single point in time was strongly suggestive of deaths due to accidental causes. Dr. Kociba said histopathology might indicate whether the deaths at week 82 were due to overdosing or some other reason. Dr. Dunnick stated that the dosing accidents would be footnoted in the tables of data for male and female mice and highlighted in the abstract.

Dr. Swenberg moved that the technical report on the toxicology and carcinogenesis studies of chlorodibromomethane be accepted with the conclusions as written and with the modifications discussed. Dr. Tannenbaum seconded the motion and the report was approved by ten affirmative votes. There was one negative vote (Dr. Beliczky).
Di allylphthalate. Dr. Beliczky was a principal reviewer for the technical report on the carcinogenesis studies of diallylphthalate. The conclusions of the study were: "Under the conditions of this study, the administration of diallylphthalate to male and female F344/N rats caused chronic liver disease characterized by periportal fibrosis and pigment accumulation, and an increased severity of bile duct hyperplasia. The incidence of mononuclear cell leukemia was significantly increased (P<0.05) in female rats receiving 100 mg/kg, providing some evidence of carcinogenicity of diallylphthalate in female rats. There was no evidence of carcinogenicity in male rats." Based on definitions for strength of experimental evidence of carcinogenicity currently used by the NTP (beginning with the review meeting of June 1983), he stated that the data supported a finding of clear evidence for female rats. Supporting his assessment were the current data as well as the increased incidences of hematopoietic system tumors in rodents from other NTP studies of "allyl" compounds. Dr. Beliczky also said the study of diallylphthalate in mice (NTP TR #242) should have been reviewed concurrently with the study in rats, and therefore the study should be reevaluated with the currently used criteria for strength of evidence. Further, noting that the Panel had received summary information from a recently completed comparative disposition and metabolism study in rats and mice, he asked that the study results be fully incorporated into any final report. Both Dr. Slaga and Dr. Kociba, the other principal reviewers, said the rat study should stand on its own.

In response, Dr. W. Kluwe, NTP Chemical Manager, explained that the mouse and rat studies were started concurrently but the study in rats had to be restarted because of an error in dosing. He said the data from the comparative disposition studies suggest a species difference in hepatotoxic effects but do not explain the differences in tumor incidence. Dr. Kluwe said a program decision had been made not to apply the current categories for strength of evidence retrospectively. Dr. D. Rall, NTP, proposed that an appendix describing the mouse study design, results, and conclusions be added to the report of the rat studies.

As a second principal reviewer, Dr. Slaga stated that the increased incidence of mononuclear cell leukemia in high dose female rats should be considered equivocal rather than some evidence of carcinogenicity because an increased incidence was not observed in male rats and this neoplasm occurs at a moderate rate in control rats.

As a third principal reviewer, Dr. Kociba also believed that the data supported a designation of 'equivocal' in female rats since this leukemia occurs at a rather high rate in control rats, more so in males, and there is a variable historical control rate; the high dose female rat group did have an incidence higher than seen in female vehicle control rats. Dr. Beliczky retorted that the definition of equivocal was different now than when the mouse study was reviewed. Dr. Swenberg opined that the mouse study would still be considered equivocal. Additionally, Dr. Kociba commented that the prechronic (13-week) study might have included liver histopathology on the lowest dose group and a more complete toxicologic evaluation to aid in setting doses for the two year studies, while the two-year studies could have included more hematology to aid in interpretation of the leukemias.
The key issue was whether the increased incidence of mononuclear cell leukemia in female rats best supported an interpretation of some or equivocal evidence of carcinogenicity. Dr. Boorman, pointed out that there were some borderline diagnoses, more in the high dose group than in the controls. Dr. Jones commented that the greater numbers of borderline diagnoses in treated animals supported a more conservative interpretation. Dr. Kluwe listed valid reasons for each interpretation, and said the NTP staff could support either choice. Dr. Swenberg suggested that Dr. Kluwe adjust the report discussion to reflect the difficulty in coming to a decision. In response to a request by Dr. Beliczky, Dr. Boorman said a table with a grading of the toxic liver lesions would be added to the report.

Dr. Jones moved that the technical report be accepted with the conclusions as written (some evidence in female rats). Dr. Swenberg seconded the motion. The motion was rejected by five yes to six no votes. Dr. Jones then moved that the technical report on the toxicology and carcinogenesis studies of diallylphthalate in rats be accepted with the conclusion of equivocal evidence of carcinogenicity in female rats and with other modifications as discussed. Dr. Slaga seconded the motion and the report was approved by eight affirmative votes. There were two negative votes (Dr. Beliczky and Dr. Davis), and one abstention (Dr. Kotelchuck).
Hamamelis Water (Witch Hazel). Dr. Slaga, a principal reviewer for the technical report on the carcinogenesis studies of Hamamelis water, agreed with the conclusion that: "Under the conditions of these studies, there was no evidence of carcinogenicity in male or female F344/N rats or in male or female B6C3F1 mice that received dermal application of Hamamelis water." However, he noted two positive trends for tumors, fibromas or fibrosarcomas in male rats and alveolar/bronchiolar adenomas or carcinomas in female mice; and he said that even though the trends were not statistically significant by the appropriate tests, the numerical increases should not be ignored. Dr. Slaga was concerned with how much of the applied dose was lost by runoff of the aqueous solution, and with whether the control vehicle should have contained ethanol equivalent to the dose vehicle (14-15% volume). Even though the route chosen was the correct one to mimic human exposure, he said that a study with Hamamelis Water in drinking water might have indicated more about the potential for carcinogenic effects.

As a second principal reviewer, Dr. Jones agreed with the conclusions. In comment on the fibromas and fibrosarcomas in male rats, he disagreed with the statements in the abstract and discussion that fibrosarcomas are not life threatening lesions. Dr. E. McConnell, NTP, said the statement referred to both lesions in this study, and of 13 lesions in the dose groups and control only two were fibrosarcomas; however, the sentences would be changed.

As a third principal reviewer, Dr. Van Ryzin also agreed with the conclusions. He questioned basing statistical significance for trend or pairwise comparisons on a one-tailed P value of 0.05, rather than on a P value of 0.025. He stated that use of a 5% one-tailed criterion resulted in too many marginal effects that were discussed in detail and later dismissed as not biologically meaningful. Dr. Haseman said that in the results section all P is < 0.05 effects are noted, but detailed statistical analyses are given only for the more biologically meaningful effects. He conceded that in this report there may have been too many marginal effects with detailed statistical analyses provided in the text, and that this concern would be taken into account in the preparation of the final report.

In other discussion, Dr. Kotelchuck stated that whether a lesion is life threatening should not be the controlling variable; the key is whether the lesion is considered to be compound related. Dr. Turnbull said the lesion could be life threatening and yet not cause death, in which case the incidental tumor test would be appropriate. Dr. Boorman mentioned that Program and contract laboratory pathologists were being asked to state whether or not a particular lesion was the probable cause of death which will aid in choosing the most appropriate statistical test.

Dr. Slaga moved that the technical report on the toxicology and carcinogenesis studies of Hamamelis water be accepted. Dr. Jones seconded the motion and the report was approved unanimously by the Peer Review Panel.
HC Blue No. 1. Dr. Friess, who was unable to attend the meeting, had submitted written comments in advance; these were read by Dr. Hook, Panel Chairperson. As a principal reviewer for the technical report on the carcinogenesis studies of HC Blue No. 1, Dr. Friess agreed with the conclusions that: "Under the conditions of these studies, there was equivocal evidence of carcinogenicity in male F344/N rats, since HC Blue No. 1 caused a marginal increase in the incidence of hepatocellular neoplastic nodules/carcinomas. For female F344/N rats, there was some evidence of carcinogenicity in that HC Blue No. 1 induced increases in alveolar/bronchiolar adenomas or carcinomas. There was clear evidence of carcinogenicity of HC Blue No. 1 in male B6C3F1 mice as shown by increased incidences of hepatocellular carcinomas and follicular cell adenomas and hyperplasia of the thyroid gland. There was clear evidence of carcinogenicity of HC Blue No. 1 in female B6C3F1 mice, shown by increased incidences of hepatocellular carcinomas." Dr. Friess noted that cytoplasmic pigmentation of the follicular epithelial cells of the thyroid occurred at increased incidences in dosed animals of both species and sexes, and wondered whether or not the unidentified pigment may have had some causative role in enhanced incidences of follicular cell adenomas. Further, he inquired as to whether the positive Sendai virus titers at as early as six months in rats and mice could have had any impact on development of proliferative lesions in the lungs, or in other target tissues. Dr. J. Mennear, NTP Chemical Manager, responded that a relationship between pigment deposition and proliferative changes in follicular cells could not be ruled out but in other studies with dyes by the NTP, or by other laboratories, a consistent relationship was not observed. With regard to the possible role of Sendai, the available data do not allow a definitive conclusion about any interaction between virus and chemical in tumor causation. NTP data (unpublished) show no difference in incidence of pulmonary tumors in controls that have had positive Sendai titers and those that did not.

As a second principal reviewer, Dr. Harper agreed with the conclusions. He thought the doses selected for the two-year study in female mice to be rather high perhaps contributing to low survival. In retrospect, the doses selected for male and female mice could have been the same. Nonetheless, he did not think the low survival would invalidate interpretation of the findings in female mice. Dr. Mennear observed that the early deaths were likely due to hepatocellular carcinomas. Dr. Friess's report was in agreement. Dr. Harper asked for a statement in the discussion explaining why the feed route was used rather than dermal application.

As a third principal reviewer, Dr. Turnbull said he agreed with the conclusions in mice but was not totally convinced about those for rats. He questioned the use of a one-sided P value of 5% (0.05) as the nominal limit value for significance, and he expressed concern about a high probability of false positives. He proposed that a 1% criterion (p<0.01) would be preferable. As further reasons that lower evidence for a positive finding, he mentioned different primary tumors in male versus female rats and the marginally significant increase of lung tumors in female rats. Dr. Turnbull supported a designation of equivocal evidence for both sexes of rats. Dr. J. Haseman, NIEHS, said that the NTP does not employ a rigid decision rule (5% or 1%) in the final interpretation of carcinogenicity data. He stated that some evidence of carcinogenicity in female rats was
supported by: (1) the seven lung tumors (14%) in the high dose group exceeded the highest control incidence seen in the Program, and (2) the increase in neoplasms is paralleled by an increase in adenomatus hyperplasias.

Dr. Tannenbaum and two of the principal reviewers expressed concern about the incomplete chemical identification of the 3% impurities. He said that during the synthesis of nitroaromatic chemicals nitrosamines are often formed as contaminants; in this case probably derivatives of nitrosomethylaniline. Dr. W. Jameson, NTP, said a more definitive characterization would be made of the impurities. Dr. Beliczky asked whether there were uses for HC Blue No. 1 other than as a hair dye and whether there was more than one manufacturer. [Dr. J. Mennear subsequently determined there were no other uses or other manufacturers in the U.S.]

In other discussion, Dr. Turnbull commented on how animal cage assignment randomly by rows rather than by individual cages might affect the power of statistical inference. Dr. Kociba asked that the dosages which were expressed as ppm also be given as mg/kg/day in the report, and that the sections on clinical observations and non-neoplastic toxicology be expanded. Both requests were agreed to. Dr. Swenbera stated that the designation of clear evidence based on liver tumors in mice was strengthened by the lack of hepatotoxicity even in females where the maximal tolerated dose likely was exceeded, and because the mouse liver is a target organ for aromatic amines. The interpretation of lung tumors in female rats as to association with chemical was more difficult. Dr. G. Boorman, NTP, said the NTP Pathology Working Group that examined these lung lesions was in unanimous agreement that the findings supported a designation of a carcinogenic response.

Dr. Harper moved that the technical report on the toxicology and carcinogenesis studies of HC Blue No. 1 be accepted with the conclusions as stated, and with the modifications as discussed. Dr. Davis seconded the motion, and the technical report was approved by ten affirmative votes with one abstention (Dr. Turnbull).
8-Hydroxyquinoline. Dr. Van Ryzin, a principal reviewer for the technical report on the carcinogenesis studies of 8-hydroxyquinoline, agreed with the conclusions that: "Under the conditions of these studies, there was no evidence of carcinogenicity in male or female F344/N rats or in male or female B6C3F1 mice." Citing the comments in the discussion on marginally significant increases over control in low dose female mice with hemangiomas or hemangiosarcomas, Dr. Van Ryzin proposed that, in general, only tumor incidences significant by a one tailed test at p<0.025 should be commented on in discussion, thus reducing discussion of marginal results. Further, he noted that all of the statistical values are available in the Appendixes. Dr. J. Huff, NTP, reminded the Panel that incidence rates for neoplasms having a trend or pairwise statistic of P<0.05 were placed routinely in the results sections. For comparative purposes, the incidence of the same lesion for the other sex of that species was also recorded. Ordinarily, marginal effects in a single group received little mention in the discussion unless considered compound-related.

As a second principal reviewer, Dr. Kociba said he also agreed with the conclusions. He urged inclusion in future design of subchronic and chronic studies additional measurements of hematology, urinalysis, serum chemistry, organ weight, and other parameters to allow for a more complete assessment of both chronic toxicity and carcinogenicity. Dr. E. McConnell, NTP, indicated that these indices are included in most current studies and in those designed during the past two years or so. Dr. Kociba asked that dietary exposure levels expressed as parts per million (ppm) also be expressed as mg/kg body weight/day to aid in extrapolation. Dr. J. French, NTP Chemical Manager, responded that this information is available in the food consumption appendix, but stressed that these values lack accuracy because of the group housing used, and the food scattering which occurred. He said the Program will include exposure levels, as mg/kg, in the text routinely. Dr. Kociba stressed the importance of including negative as well as positive data on chemicals because negative data is important in knowing what parameters to evaluate in safety assessment and health surveillance programs.

As a third principal reviewer, Dr. Kotelchuck agreed in principle with the conclusions but noted there appeared to be a marginal increase in the rate of alveolar/bronchiolar neoplasms with persistent trends among all exposed groups, although in no individual case was there statistical significance. However, he said that aggregation of the incidence data from both sexes of rats or mice using Chi square analysis suggested there was equivocal evidence for association of the lung tumors with exposure to 8-hydroxyquinoline. In discussion about the usefulness or appropriateness of grouping lesions across sexes and/or species for analysis, Dr. J. Haseman, NTP, said that while these comparisons are useful statistically to ascertain support across sexes and/or species, the NTP does not consider this biologically appropriate so does not do such analyses routinely. Further, a previous Peer Review Panel recommended this not be done. Dr. Kociba observed that cross species comparisons may also cancel out or diminish overall incidences as well as enhance them. Dr. Davis agreed and said that in view of endocrinological differences there was not a good biological justification for combining the sexes. Dr. French stated that more clarification of the lung tumor data including the potential positive trends would be added to the discussion section.
Dr. Davis asked that there be more prominence given, perhaps in the abstract, to non-tumor effects or lack of effects reported by others including hepatic and neurologic toxicity, and especially in view of the chemical's use in preparations which come into intimate contact with the body, e.g., vaginal suppositories. In other discussion, Dr. Swenberg reiterated a previous Panel recommendation that non-NTP data not be included in the abstract. Dr. French said recently completed genetic toxicity results for in vitro unscheduled DNA synthesis in rat hepatocytes and for the BALB/C-3T3 in vitro transformation assay would be included in the report. The chemical gave negative results in both assays.

Dr. Van Ryzin moved that the technical report on the toxicology and carcinogenesis studies of 8-hydroxyquinoline be accepted with the modifications discussed. Dr. Slaga seconded the motion and the technical report was approved unanimously by the Peer Review Panel.