National Toxicology Program
Board of Scientific Counselors' Meeting
March 10, 11 and 12, 1982

Summary Minutes

The National Toxicology Program (NTP) Board of Scientific Counselors' met on March 10, 11 and 12, 1982, in the Auditorium, Building 101, South Campus, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina (Attachment 1: Federal Register Meeting Announcement; Attachment 2: Agenda).

The minutes of the October 22 and 23, 1981, Board of Scientific Counselors' meeting were approved. Dr. D. P. Rall, NTP Director, and Dr. N. Nelson, Board Chairman, noted that Dr. M. Mendelsohn's term on the Board would end on March 31, and thanked him for his contributions to scientific oversight of the NTP.

Dr. Nelson introduced the program which was to center on a review of the current chemical carcinogenesis programs of the NIH/NTP, detailed presentations and discussions of proposed modifications of the experimental design and pathology requirements for the two-year carcinogenesis and toxicology bioassay, and proposed development and utilization of an in vivo rat liver tumor model. The Board was supplemented for this meeting by a panel of expert consultants. The roster of Board members, expert consultants and participating NTP staff are given in Attachment 3.

Overview of the Current Carcinogenesis Bioassay Program: (Attachment 4: Introduction and Overview of Current Program Activities Relevant to Carcinogenesis). Dr. J. A. Moore said he hoped this meeting would be a participatory discussion among the peer reviewers and NTP staff including an examination of alternatives and options to the proposals presented by staff. He then described the current program activities in carcinogenesis. He discussed modifications that NTP had made in the bioassay design and analyses as well as in the technical reports and said this was an ongoing process. Dr. Moore discussed the three major types of studies which form the basis for setting priorities and establishing the experimental design for the two-year bioassay. The studies include basic toxicological characterization, basic chemical disposition, and a genetic toxicology screen (Attachment 4).

Optimal Experimental Designs of the Long-Term Carcinogenesis Bioassay: (Attachment 5; Attachment 6: Optimal Design of the Chronic Animal Bioassay; Attachment 7: Low-Dose Rate Extrapolation Using the Multistage Model). Dr. D. Hoel said that the Biometry and Risk Assessment Program had been addressing the issue of how to improve the basic experimental design of the two-year bioassay for low-dose extrapolation

while retaining the power of the bioassay for detecting carcinogenic effects. An optimal experimental design would be a design which minimizes the mean-squared-error (MSE) of the estimate of the virtually safe dose (VSD) while maintaining a high power for the detection of increased carcinogenic response. Dr. Hoel said they had considered several models and had settled on two forms of the Armitage-Doll multi-stage model for estimating the VSD, the linear model and the linear-quadratic model.

Dr. C. Portier then described in more detail the studies carried out on optimal experimental design (Attachment 5). He described the four design parameters, defined the VSD and the objectives, detailed the historical approaches used, and described the computer simulation process using the linear model and the linear-quadratic model. He stated that the simulation approach will accurately predict changes in the risk assessment resulting from modifications of the bioassay design. Using contour plots which allow comparison of changes in MSE and power on the same chart, Dr. Portier described simulations using both three-dose designs (control, low, and high dose; currently used by the NTP) and four-dose designs (control, low, mid, and high dose). Conclusions drawn were that an optimal three-dose design (where the low dose was ½ MTD) and optimal four-dose design (where the low dose was ¼ MTD and the mid dose was ½ MTD) with both designs using the same total number of animals would yield virtually the same results. However, if there were sufficient overt toxicity at the high dose to markedly reduce survival, then a four-dose design would be superior. If the group given the MTD is effectively lost to analysis, the four-dose design will still have enough dose groups to indicate the shape of the dose-response curve. Thus, a reasonable design strategy would be as follows (with D_0 = control, D_1 = low dose, D_2 = mid dose, and D_3 = MTD; while N_0, N_1, N_2 and N_3 = numbers of animals at each dose level):

> D_0 = 0; D_1 between 10 and 30% of MTD; D_2 between 50 and 60% of MTD; D_3 = MTD; while N_0 = 50 to 60 animals; N_3 = 40 to 60 animals; N_1 = 1/3 of remaining animals; N_2 = 2/3 of remaining animals.

During discussion following Dr. Portier's presentation, there was concern expressed as to the adequacy of the numbers of animals proposed for the low dose. Dr. Portier replied that the small number (~ 20) did not compromise the ability of the model to estimate the linear component. Dr. Haseman noted the need to maintain a balance between power and extrapolation. He said that adding more than 20 animals to the low-dose group would reduce power while not producing a corresponding gain in usefulness for low-dose risk estimation. Drs. Tomatis and Gehring pointed out the need for more emphasis on basic biology and biological-mechanistic considerations in experimental design. Drs. Breslow and Huff opined that the small number of animals at the proposed low dose (~ ¼ MTD) would rarely if ever give responses different from controls. Thus, more animals should be located in the lower dose group.

Dr. Gehring said that biological responses, e.g., liver weight changes, needed to be used in dose-setting. Dr. Rall responded that the biological responses measured during the 90-day study are used in design of the bioassay. Dr. Portier agreed that biological data should be used in choosing the best model. For example, the 'biologically effective' dose(s) rather than the administered dose(s) where possible might be used. Dr. D. Gaylor, NCTR, commented that they had been working on the same problems and found the four-dose design to be best using equal numbers of animals per group and doses of 0, ¼ MTD, ½ MTD, and MTD. Their findings agreed with the results of Drs. Portier and Hoel. Dr. W. Marcus, EPA, asked that time-to-tumor be given more consideration. In regulation, a significant decrease in latent period following chemical exposure was considered most important. Dr. Moore came back to why these simulation studies were initiated in the first place. It had to do with the frustration resulting from the current bioassay design giving only a YES-NO answer at best with respect to carcinogenic potential. He said that the NIEHS/NTP wanted to enhance the design to give more information about dose-response, biological mechanisms, and risk estimation while not losing power to detect carcinogenic potential.

Dr. Breslow expressed concern that the proposed experimental design discussed by Drs. Portier and Hoel is so close to the controversial area of low-dose extrapolation. He said that one could propose alternative methods of low-dose extrapolation. Some investigators say the slope of the dose-response curve is always positive at the origin and there are good theoretical arguments to support that. If true, he said the way to proceed might be to go to the lowest dose where a positive effect over control was observed and draw a line from that point to zero. Dr. Rall disagreed, it would never go through zero; further, this was not low-dose extrapolation but rather interpolation. Dr. Breslow replied that we were going to zero effect. Continuing, he would like to see the design of the bioassay for carcinogenicity uncoupled from low-dose extrapolation. He said the present design and data are used to try and resolve the issue of whether the slope at zero dose is positive or zero. This can't be done. If one believed in perfect linearity one could use all of the experimental points to fit the line but he believed that within the usual experimental range of measurements there was upward curvature which would lead to overestimation of the slope. Dr. Hoel said it is important to know whether there is curvature in the dose-response line such as with the formaldehyde studies. Dr. Whittemore stated there were other biologic mechanisms consistent with a zero slope at the origin rather than a positive one. She also suggested doing 'colony' controls when there is more than one bioassay starting at about the same time in a laboratory. Further, she expressed concern about taking animals from the high-dose group for special experiments where dosing is stopped short of two years for study of lesion reversibility. She was afraid this would compromise the power of the bioassay while providing information that had questionable relevance to human risk.

Statement by Officers of the American Industrial Health Council (AIHC):
Dr. D. Hughes, Proctor and Gamble, said the AIHC appreciated this opportunity to present its views on proposed modifications by the NTP of the experimental

design in the two-year bioassay. Dr. C. Weil, Union Carbide, presented these views. He was pleased that the NTP was studying improvements in the bioassay, and suggested that the purpose of the bioassay needed to be restated. Points he wished to make included: (1) the MTD (Maximum Tolerated Dose) needs to be redefined; (2) pharmacokinetics and metabolism data should be used in dose selection; (3) we should give more thought to using animal species appropriate for man; (4) the route of administration should be the same as that by which humans are primarily exposed; and (5) there should be awareness of pitfalls in the use of historical controls, especially from different laboratories. In discussion, Dr. Hoel said that if one switches from the standard strains there will be lacking a good historical control data base. Dr. Weil agreed but said concurrent controls are more important.

Proposed Strategy to Reduce the Volume of Pathology Required on A Chronic Bioassay: (Attachment 8). Dr. McConnell summarized the current pathology procedures. He observed that 46% of the total contract cost in current NTP bioassays can be attributed to pathology as compared with an average 33% for previous bioassays. He said the average time required to complete the pathology segment of a chronic bioassay is 278 days under the best conditions. He reviewed the types of tumors and frequency of types for the 27 most recent bioassays judged to be positive. Dr. McConnell then outlined the proposed NTP pathology strategy (Attachment 8, pages 11-13). The major change from current practice would be to do histopathology on a baseline list of 12 organs or tissues (15-17 sections) and only in controls and high-dose groups as compared to 31 organs or tissues (42 sections) per animal in all the animals under current practice. Organs or tissues other than in the baseline list would be examined based on route of exposure, presence of gross lesions or identified or expected target organs. In addition, organs from all lower-dose groups will be examined where neoplasms were found to be significantly increased over control, where rare tumors were found regardless of incidence, and where toxic lesions were observed.

Dr. McConnell then discussed an interim kill proposal (Attachment 9). This would be done mainly to better detect and characterize toxic (nonneoplastic) lesions and not ordinarily as a means to observe "early" tumor development (although preneoplastic lesions would be diagnosed). The interim kill should be useful in detecting toxic lesions which might not have appeared by the end of the 13-week prechronic study. An interim kill is necessary if the proposed reduction in pathology is to be effective.

To illustrate whether the proposed pathology strategy has merit for future studies, Dr. McConnell presented an analysis of tumor sites in rats/or mice and lists of organs in F344 rats with a frequency of greater than 4% tumors from recent bioassays as well as NCI/NTP historical background tumor rates of greater than 1% in 3,000 rats and 3,000 mice. (Attachment 8, pages 14-16). In very few cases would tumors have been missed using the new strategy. He also reviewed the less extensive NCI/NTP data base for spontaneous nonneoplastic lesions, and stated that although such lesions in several organs would be missed with the proposed strategy, the ones most frequently observed would be encompassed by the baseline list of organs (pages 17-19). Further, use of an interim kill would reduce the number of

spontaneous lesions missed. He said that for chemically-induced lesions, we would be less likely to miss them because of the higher incidence. He described implementation of the strategy, including time savings from use of the new toxicology data base management system (TDMS) which would be in place prior to implementation (pages 20-23). For laboratory turn-around-time savings to occur, good management would be essential. Finally, Dr. McConnell illustrated projected savings in the volume of pathology using as examples recent bioassays with positive or negative outcomes. In conclusion, he said changes proposed by NTP should realistically reduce the volume of pathology by from 40 to 70 percent.

General Discussion

Dr. C. Morris, EPA, and Dr. V. Alexander, OSHA, wondered what the proposed decrease in number and types of tissues examined microscopically would do from the regulatory agencies' point of view since they depend on NTP bioassay data in decision-making processes. Dr. Hitchcock added that from her experience with the peer review process, the quality of pathology was rate-limiting and feared a reduction in the power of the bioassay if the amount of pathology was reduced. Dr. McConnell replied that the analysis presented indicated to NTP that little more would be missed in the way of tumors than currently, and, in fact, with the interim kill there would be better detection of toxic (nonneoplastic) lesions. Dr. Alexander proposed that NTP do complete histopathology on high-dose animals and use the findings to guide which tissues to examine in lower-dose groups. Dr. McConnell said this had been considered and was the procedure used with tissues from the subchronic studies. Drs. Swenberg and Tarone stated that the reductions proposed would lay a much heavier burden of management and decision making on the pathologist. Dr. Moore disagreed except for rare tumors where there might be more management involved. Dr. McConnell noted that the modifications could not be effected until TDMS was fully in place which would help to minimize the additional management requirements. Dr. Nelson raised the question as to whether the success of the proposed modifications was too dependent on the quality of the pathologist, and said there might be a negative effect on pathological evaluations in agencies that followed NTP's lead but had less skilled pathologists.

There was some discussion about an NTP suggestion to consider including a recovery group in a bioassay protocol for purposes of assessing reversibility of lesions. Typically, such a group would be dosed for 18 months and sacrificed at 24 months. Dr. Breslow wanted to know how this would affect the power of the bioassay, although he would support such a proposal. Dr. Horning wondered if a liver model might give more information on reversibility. Dr. Pitot agreed, and said two of the models discussed show reversibility of foci.

Specific Comments and Recommendations by Peer Reviewers

Dr. Nelson - he asked what the money and time savings would be if the proposed modifications were effected. Dr. McConnell replied there would be about a 50% reduction in pathologists' time spent , but little change in turn-around time. Average dollar savings he estimated would be about $100,000/

per study or about 25% of the total cost of an average bioassay.

Dr. Swenberg - he supported the overall objectives. His points were: (1) He said NTP should go with 20-22 tissues rather than 12 in the baseline; this would reduce management problems while not losing that much in savings. (2) He supported the interim kill proposal but wanted clinical pathology added. Learning as much as possible about the animal, including gross necropsy, clinical behavior, and clinical pathology, would aid in reducing tissues that needed histopathology and also reduce the likelihood of missing lesions. (3) He would like to see addition of a group of animals for evaluation of progression vs. regression of benign tumors. Finally, (4) the protocols decided on also should be acceptable for use by industry in their data submissions to regulatory agencies. With regard to (4), Dr. Rall said NTP would be presenting these proposals to regulatory agency representatives on the Executive Committee. Dr. Moore stressed that a "call for comment" would be emphasized in the April issue of the NTP Technical Bulletin.

Dr. Gehring - he emphasized that good gross necropsy was the key to effectiveness of the new protocols and more time should be devoted to gross necropsy. He commented on the 'boredom factor' for pathologists and the need to improve quality of use of pathologists' time. Dr. Horning reiterated this latter point.

Dr. Tomatis - he supported Dr. Swenberg's recommendation for including a group of animals to evaluate progression vs. regression of lesions. He supported adding a few more selected tissues to the baseline group. He emphasized the need to separate mice and rats for pathological consideration.

Dr. Albert - he stated that tumor pathology should be keyed to gross lesions with an interim sacrifice focused on toxic lesions. He proposed taking the savings realized and putting them into research and development of improved bioassay methodology and risk estimation techniques.

Dr. Breslow - he stressed there should be more emphasis on looking at the mid-dose (or low-dose in current design) and there should be more followup on quality control.

Dr. Whittemore - she proposed considering approval of the interim kill proposal separately from the pathology reduction proposal. Dr. McConnell said that for the pathology reduction to be optimally effective it needed to be coupled with interim sacrifice.

Dr. Tarone - he said that he had previously communicated his concerns and comments to NTP staff.

Dr. Harper - he expressed concern that since NTP was the standard setter other agencies might feel obligated to adopt the NTP procedures, although their staff level of expertise might be less than optimal to effectively utilize the procedures.

Dr. Hitchcock - she had a number of comments as follows: (1) the quality of pathological diagnoses is the rate limiting factor in the current interpretation of a bioassay. (2) Will the proposed modifications reduce the power of pathological diagnoses for detecting all neoplasms and for differentiating between hyperplasia and neoplasia? (3) Will the proposed changes reduce the likelihood of missing rare and unusual tumors and/or toxicity. (4) Any changes in current protocols should increase the power of pathological diagnosis. Interim kills may do this for non-neoplastic lesions if a sufficient number of animals are used. (5) Recommendations and practices adopted by NTP are likely to become the standard and adopted by others with lesser expertise to effect them as well. (6) What is the confidence of obtaining 100% correlation of the proposed protocol with current protocols? What success rate of detection is good enough? (7) The impact of the proposed protocols on detection of negative trends should be examined.

Dr. Horning - she was quite supportive, and said the use of an interim kill will contribute to our knowledge of general toxicology, and the proposed modifications should provide tighter protocols.

Dr. Mendelsohn - he said NTP may need to make some compromises to enable reduction of potential management problems.

Dr. Nelson - he also expressed concern about the limited number of tissues in the baseline group, particularly, in view of NTP being regarded as the standard setter.

An Analysis of Hepatocellular Tumors in NCI/NTP Carcinogenesis Bioassays Using F344 Rats and B6C3F$_1$ Mice: (Attachment 10). Dr. Moore said the NCI/NTP bioassay data base was chosen as it is the largest available, and there was some degree of uniformity in the protocols used. The data base includes bioassays approved by peer review through December 1981. Equivocal tumor responses were considered as negatives. He commented on the controversy over the significance of liver tumors in B6C3F$_1$ mice especially in males which had a relatively high background incidence.

Seventy-nine bioassays fulfilled selection criteria with 48 including hepatocellular tumor response. In 17 (22%), the only tumor response was in the liver. He noted that in some of the earlier bioassay reports there was no distinction made between adenomas (mice) or neoplastic nodules (rats) and hepatocellular carcinomas. Dr. Moore described the tumor allocation among either rats or mice and among rats and mice, and the chemicals involved. Of five chemicals that produced only hepatocellular tumors in F344 rats, three were benzidine-derived dyes and for these chemicals tumors were observed within 90 days of exposure; these should not be included in the tally of those chemicals causing only liver tumors because tumors of other sites would likely have occurred if the exposure period was extended to two years. With all other chemicals tumor induction was observed during the chronic study. He also described his analysis of 27 earlier NCI bioassays where the rat strain was Osborne-Mendel. Overall, three chemical classes predominated in producing a hepatocellular tumor response--short chain chlorinated aliphatics, chlorinated hydrocarbon pesticides, and phenylenediamines. There was little genetic toxicology data available

except for Salmonella where most of the findings for these chemical classes were negative. This was not surprising since Salmonella is notably insensitive to halogenated chemicals. Finally, only 6% of the 79 positive bioassays were based on hepatocellular adenomas (mice).

Discussion

Dr. Swenberg suggested it would be worthwhile to reread the earlier bioassays using Osborne-Mendel rats (and $B6C3F_1$ mice) where there were positive hepatocellular responses in mice (10 in all) which were undifferentiated as to adenomas or carcinomas. There was discussion as to whether chemicals which induced adenomas only were promoters. Dr. Albert asked whether there was any correlation between the type of hepatic tumor produced and the induction of tumors in other organs or sites by a chemical. Dr. Moore replied that he would like to analyze whether there were such correlations. In response to a query by Drs. Tomatis, Dr. Moore said the finding of liver tumors in rats was not necessarily predictive for induction of liver tumors in mice by a chemical. Dr. McConnell reported that in some of the earlier studies the pathologists only recorded the most severe lesion (carcinomas) thus leading to a probable underreporting of adenomas. Dr. Swenberg stated that liver tumor formation was often related to the hepatotoxicity of a chemical. Drs. Mendelsohn and Gehring said the issues of the mechanisms of carcinogenesis assume more importance when trying to relate animal tumor formation to likelihood in humans. Dr. Breslow said the presence of metastasis should strengthen the case for carcinogenicity of a chemical. Dr. Moore said evidence for metastasis often is incomplete and its absence doesn't mean metastases didn't occur, it just was not diagnosed. Dr. Pitot observed that the issue of neoplasia vs. malignant neoplasia was academic if what we're trying to assess is neoplastic potential. He said in humans malignant tumors may be diagnosed and cured with no evidence of metastasis. In the bioassay, metastasis may reflect some indication of potency. There was considerable discussion of whether or not neoplastic nodules or adenomas progress to carcinomas or regress, and no consensus was reached. Dr. Whittemore implored that from a public health standpoint we shouldn't discount chemicals that may be acting through a promotional mechanism. Dr. Swenberg returned to the need for understanding mechanisms since chemicals which act through genotoxic, and presumably irreversible, mechanisms may be regulated differently than chemicals which are not genotoxic. Others did not agree with this concept. There was general agreement among the reviewers that potential tumorigenicity for humans should not be ruled out just because the only tumor site in rodents was the liver, and especially the livers of $B6C3F_1$ mice.

Concept Proposal for Utilizing In Vivo Liver Tumor Models: (Attachments 11 and 12). Dr. Maronpot opened his discussion by noting that one of the major mandates of the NTP was to develop test methods that will identify toxic effects, including carcinogenic potential, of chemicals in an efficient and economical manner. Within this framework, selected short-term in vivo

animal models purported to predict carcinogenicity have been reviewed for their applicability in chemical testing. In addition, short-term in vivo tests have potential utility for examining mechanisms of carcinogenesis.

Dr. Maronpot said the two prime advantages of a short-term in vivo model would be to help interpret organ-specific tumor responses and help elucidate mechanisms of carcinogenesis which may be broadly applicable within classes of chemicals. Such a model in conjunction with other data, e.g., genotoxicity, could in carefully selected cases be used in lieu of a two-year bioassay. NTP does not recommend this as a general substitute. He briefly discussed six short-term in vivo carcinogenesis models (Attachments 11 and 12) which had been considered by NTP, and the rationale for why the rat liver model was judged to have highest priority relative to program needs.

Dr. Maronpot described four of the rat liver models considered including the advantages and disadvantages of each model: A. Sequential feeding of carcinogen and promoter; B. Single treatment with a necrogenic dose of carcinogen followed by proliferative stimulation in the presence of growth suppression (selection model); C. Single treatment with carcinogen during liver regeneration, followed by phenobarbital treatment ("Pitot liver model"); and D. Initiation at birth with subsequent natural proliferative stimulation, followed by promotion after weaning ("baby rat" model). He said that while the production of hepatocellular tumors is the definitive endpoint in these models, an early indicator of effect is the presence of phenotypically altered foci of hepatocytes which can be identified by various histochemical markers. He listed initiators and promoters that had been used in one or the other of these models.

The specific liver model recommended for support through the NTP contracting mechanism was the "baby rat" liver model. The rational for selection is based upon its relative advantages (page 9, Attachment 11). It is proposed to test chemicals both as initiators and promoters in the system. Chemicals which are negative as liver tumor initiators in the "baby rat" model will be retested using the "Pitot liver model". He said the major objectives in funding work will be (1) to permit refinement of the model, and (2) to use the model to test selected chemicals. These activities would go on in parallel. Available resources would probably limit chemicals tested to six per year. Dr. Maronpot discussed the suggested priority scheme for selection of the chemicals to test (page 13, Attachment 11).

General Discussion

Dr. Hitchcock commented on the severe changes and extreme variability of enzymes in the liver during the neonatal period, and asked whether there were sex or substrate differences in the "baby rat" model. Dr. Maronpot replied that the female was more sensitive and that both diethylnitrosamine and benzo(a)pyrene had been used and found effective as initiators.
Dr. Swenberg expressed strong support for development of a liver model but had some reservations about the "baby rat" model since it had not been validated yet and there were no controls for initiation and promotion. He said a dose-response study had been done with phenobarbital so this didn't

need to be repeated. Further, the design uses an intraperitoneal dose which might be a problem in testing chemicals that require gut metabolism. He also reported CIIT had been working on a series of liver models, and found the Pitot model, with design modifications, to be a good model. Dr. Gehring seconded Dr. Swenberg's suggestions including going with the Pitot model, although the regenerating liver is similar to the neonatal liver in its metabolic state. Drs. Whittemore and Mendelsohn asked whether unknowns would be tested both for initiation and promotion activity and the answer was yes. Dr. Pitot said the "baby rat" model was potentially useful especially for looking at tumor promotion. He suggested using two models, e.g., the "baby rat" for promotion, and one based on his model for looking for complete or incomplete carcinogens. He mentioned their work with proflavin which was shown to be an incomplete carcinogen producing foci. Dr. Swenberg thought NTP was selling short model B or the "Farber system" (selection model) which has the advantages of displaying a rapid initial response (foci), and having a good data base in that 53 chemicals have been looked at as initiators. Dr. Albert proposed that the priority list for testing should include chemicals which are genotoxic but not carcinogenic. Dr. Tennant said validation needed to include determination of intra- and inter-laboratory reproducibility and variability. Dr. Tomatis asked whether NTP would use a negative result in the model to decide not to go with a bioassay on a chemical. Dr. Moore replied that chemicals chosen would be those for which long-term bioassay data is already available. The model would not be used at present to select or reject candidates for the bioassay. Dr. Pitot said one needed to look at both foci and frank tumors to get at mechanisms. He added that foci could be quantitated. Dr. Albert pointed out that from the standpoint of extrapolation to humans it would be preferable to develop model systems for several organs including skin. Dr. Maronpot agreed but said limited resources precluded this, while Dr. Tennant suggested taking the results obtained from the liver system and comparing them with known effects for a chemical in other systems, e.g., there is a large data base for promoters in skin. Dr. Mendelsohn said he questioned the underlying mechanistic power of the test in that many chemicals would be toxic to a neonatal animal at doses below an effective dose for tumor initiation. Drs. Tomatis and Gehring questioned how the liver model would help in understanding organ-specific tumor responses in other organs. Dr. Gehring suggested raising priority 6, 'produces organ-specific tumors other than liver', for chemical selection to near the head of the list. Dr. Rall said that validation was perhaps not the word at this point in time but rather NTP wanted the Board's approval to pursue development and refining of this and other selected models. Dr. Horning inquired as to which histochemical markers would be used to identify foci, such as gamma glutamyl transferase (GGT), diaphorase or expoxide hydrolase. She proposed that promotion and enzyme induction may be one and the same phenomena. Dr. Pitot said that for promoted foci about 90% could be detected by GGT plus some other marker such as expoxide hydrolase. Dr. Horning said a plus for the system is its apparent utility for connecting morphology with biochemistry.

Dr. Tomatis stressed support of the liver model concept and urged that it be kept on a research basis. Dr. Pitot said he was impressed and pleased that the NTP was exploring the process of neoplasia in more depth and not just as an endpoint. Dr. Mendelsohn said that besides study of foci formation and tumor formation, and initiation and promotion, the next steps with

the tumor models should not be to just screen chemicals but rather to incorporate studies on chemical dosimetry of initiation, e.g., DNA adduct formation, which define quantitatively potential genetic lesions, also a measure of mutagenicity in liver cells, plus attempts to better quantitate early foci formation by measuring phenotypically changed cells. These kinds of studies may better define initiation and cope with problems of metabolic activation, repair, and literal DNA damage, rather than just looking at genotoxicity. Some of these changes would convert the project into a proper mechanistic study. Drs. Horning and Nelson agreed that the proposed study should be research oriented. Dr. Moore agreed and reiterated from Dr. Maronpot's discussion that NTP did not propose to use the liver model in a testing mode.

Dr. Moore asked that there be more discussion of the suggested priorities for types of chemicals to be looked at in the tumor models (page 13, Attachment 11). He said also NTP would like to compare results obtained from the tumor models with results gotten using the Mersalis-Butterworth technique. This technique is a combined in vivo - in vitro assessment of unscheduled DNA synthesis. Dr. Tennant added that the rapid-response genotoxicity screening system approved previously for concept by the Board included in vitro unscheduled DNA synthesis in rat hepatocytes. In discussion, Dr. Swenberg recommended that priority #3 with modification be given highest priority, i.e., chemicals that produced liver tumors only and were negative for genotoxicity. Drs. Gehring and Tomatis said that #6, chemicals that produce organ specific tumors other than liver, was most important. Dr. Tomatis also felt #5, known human carcinogens, should receive higher priority. Dr. Albert said the top two priorities should be as listed since these would include chemicals positive for liver tumor initiation and positive for liver tumor promotion. Also a variety of chemical classes should be considered. Further, he suggested adding a category of chemicals which were positive for genotoxicity but negative for carcinogenicity. Also, cocarcinogens should not be excluded. Dr. Alexander, OSHA, and Dr. Mendelsohn said chemicals chosen should be looked at separately as initiators and promoters and then for both effects concurrently, thus allowing evaluation of possible synergistic effects.

Concept Review

Dr. Nelson explained the ground rules used by the Board for concept review. Only board members vote on a motion to approve or disapprove. The concept proposal was entitled "Proposed Funding of Short Term In Vivo Rodent Liver Models".

Dr. Mendelsohn was the leadoff reviewer. He said the primary issue that the proponents of the model hoped to clarify were initiation vs. promotion, genotoxic vs. non-genotoxic mechanisms, and organ specificity of tumor response. He said it was very important that this type of work be done but as written the concept proposal needed modification. He recommended the following: (1) the proposal should not be limited to just the two models discussed; (2) the focus should be more on method development, and

not just to optimize sensitivity but also to give better specificity and stability; (3) broader endpoints, just how well can one test for both initiation and promotion, e.g., the "baby rat" model may not be sensitive enough for study of initiation because of chemical toxicity. He liked the dual endpoints of hyperplastic foci and tumors for giving greater sensitivity of detection as well as more information on mechanisms; (4) he questioned the cost as possibly too high ($500,000/year for three years).

In discussion, Dr. Tomatis asked whether tumor responses in a rat model would predict for responses in mice. Dr. Pitot thought it would whereas Dr. Swenberg said maybe it would. Dr. Tomatis hoped the research aspects of the proposal would be emphasized. Drs. Gehring and Swenberg said they saw the model giving insight into the carcinogenic process. They stressed broadening the concept to allow flexibility for looking at other models and other organ systems. Dr. Mendelsohn summarized by saying that (1) a major objective of the concept is to help clarify the nature of the carcinogenic (not just hepatocarcinogenic) process; (2) the models to be considered should not be restricted to just the two discussed; and (3) the research and developmental aspects of the proposal should be stressed.

Dr. Mendelsohn moved that the concept be approved with the modifications discussed. Dr. Whittemore seconded the motion and the Board approved it unanimously. The modified concept proposal is attached (Attachment 13).

Status Report on the Strain A Mouse Lung Adenoma Validation Activity: Dr. Maronpot described the scientific basis, experimental protocol, and historical background of the study. This particular study originated with the National Cancer Institute, and then was transferred into the NTP. The study was initiated under a contract using 60 chemicals for which there were two-year bioassay data. Thirty of these chemicals were put on test in the lung adenoma system in a second laboratory so that interlaboratory reproducibility could be assessed. He then discussed the results obtained from the first laboratory on the 54 chemicals for which there was good previous bioassay data. For 20 chemicals (37%), the response in the lung adenoma system was the same as in the bioassay. However, there was a 44% "false positive" rate, i.e., defined as chemicals positive in the system that were negative in the bioassay, while there was a 71% "false negative" rate, i.e., defined as chemicals negative in the system that were positive in the bioassay. Dr. Maronpot described partial results that had been received from the second laboratory. The preliminary conclusions that could be drawn were that there was a lack of congruity between lung adenoma results and bioassay results, and there was a lack of consistency in results between the two laboratories. Possible reasons for the former included (1) different species and strains (mice), (2) different routes of administration, and (3) total dose received and duration of treatment were much greater in the bioassay. Possible reasons for the latter included (1) differences in the amount of chemical, (2) substrain differences, and (3) sex differences (the first laboratory used both sexes, the second used only males).

In discussion, Dr. Nelson said that this study appears to be quite inadequate. Dr. Tomatis said that differential sensitivity of the substrains and chemical selection bias may enter into the disparity of the results. Dr. Breslow remarked that the substrain used in the second laboratory must have been much less sensitive in that there was only one positive among the 16 chemicals reported. This chemical was also positive in the first laboratory and in the bioassay. Dr. Nelson stated that routes other than intraperitoneal needed to be looked at in this system. There was some agreement that although this preliminary analysis was not very reassuring, there needed to be more indepth analysis including attempts to quantitate the degree of response in the Strain A mouse.

Selection of Chemicals for Development of A Reference Data Base: Dr. Moore said that the rationale for doing carcinogenesis studies, such as the bioassay, derives from the fact that almost all chemicals known to be human carcinogens have been shown to be carcinogenic in animals. One of the aims in developing shorter term tests is to enhance the predictiveness for humans. The question is how effective are animal bioassays in predicting whether or not a chemical is carcinogenic for humans. He said not too many of the chemicals on the IARC (international Agency for Research on Cancer) list of human carcinogens have been run in a NCI/NTP bioassay. So, he said, we need a good reference data base, as good as possible, so we will have a basis of comparison when developing and validating new tests. The most conservative approach is to start with IARC lists as the core, and then add data to fill out the information base on chemical classes. Dr. Swenberg asked with respect to the data base on animal carcinogens, for how many are there reasonable epidemiologic studies which show they aren't carcinogenic in humans. Dr. Tomatis said good epidemiologic studies of this type are rare. Dr. Nelson commented that OSHA has defined criteria for an adequate epidemiology study. Dr. Albert said comparisons of animal and human studies need to be done on a more sophisticated basis, perhaps with considerations of relative potency.

Concept Proposal to Determine Chemical Disposition Parameters of Compounds Selected for Toxicological Characterization: (Attachment 14). This proposal was presented to affirm the concept that basic knowledge of absorption, metabolism, distribution and excretion are important elements in determining the toxicological characteristics of a chemical, and that the approach used by NTP is appropriate (Attachment). Second, NTP proposed to expand the routes of exposure employed in chemical disposition studies to include the inhalation route. Dr. Horning, as principal reviewer said she was quite comfortable with the concept. The other Board members agreed. Dr. Moore said approval would allow NTP to recompete for replacement of two existing contracts which expire in the first and fourth quarters of FY 1983 and to add the capability for inhalation studies to complement chronic designs using that route of chemical administration. Dr. Horning moved that the concept be approved. Dr. Whittemore seconded the motion and it was approved unanimously.