**NATIONAL TOXICOLOGY PROGRAM**
**BOARD OF SCIENTIFIC COUNSELORS**
**Committee Meeting**
**September 17-18, 2002**
***Summary Minutes***

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The National Toxicology Program (NTP) Board of Scientific Counselors (the Board) met on September 17 and 18, 2002, at the Radisson Governors Inn, Research Triangle Park, North Carolina. (*Attachment 1*: *Federal Register* meeting announcement; *Attachments 2* and *3*: Agenda and Roster of Members). Members of the Board are Drs. George Bailey (Chairperson), Kim Boekelheide, George Bonney, Hillary Carpenter, Harvey Checkoway, Samuel Cohen, Norman Drinkwater, Michael Elwell, Thomas Gasiewicz, Lynn Goldman, Donald Mattison, Rafael Moure-Eraso, Cheryl Walker, Stephen Roberts, Richard Storer, and Bruce Weir. All were present except Drs. Bonney, Mattison and Walker. Dr. Bailey asked members around the table and attendees within the room to introduce themselves.

## I. Welcome

Dr. Kenneth Olden, Director of the National Institute of Environmental Health Sciences (NIEHS) and NTP welcomed members and thanked them for their advice over the years. He noted that because of the human genome project, the fundamental tools are now available or can be developed to help answer many questions about gene-environment interactions. Further, the National Institutes of Health (NIH) has indicated support for toxicology studies related to gene-environment interactions, so it is up to the NIEHS to make a compelling case for such studies. Dr. Olden reported that the NIH assembled about 120 scientists from government, academia and industry, including two Nobelists and several members of the National Academy of Sciences, to discuss priorities for research in this area. The consensus from several breakout groups is that research on gene-environment interactions is important and should be a priority. The NIEHS is holding its own brain storming session in Dallas in October. Dr. Olden acknowledged the support of the Administration, the Congress and the NIH for good, timely, and cutting edge research.

Dr. Olden presented certificates and acknowledged the contributions of retiring members of the Board: Dr. Bailey, Dr. Drinkwater, Dr. Goldman, and Dr. Moure-Eraso.

## II. NTP Update

Dr. Christopher Portier, Director, Environmental Toxicology Program (ETP), NIEHS, also thanked the Board members for their efforts and advice as this input helps the NTP set priorities and directions for research that will impact public health. He briefly reported on the recent activity of the Board's Technical Reports Review Subcommittee which met September 5-6 to review NTP studies on seven chemicals, five done in standard two-year rodent bioassays and two evaluated in transgenic mice; details of the findings would be presented later in this meeting. Dr. Portier said that the *Tenth Edition of the Report on Carcinogens* (RoC) is nearing completion and would soon be sent to the Secretary's office soon for approval. Preparation of the 11$^{th}$ RoC has begun and the first group of 10 nominations would be reviewed at the next meeting of the

Board's Report on Carcinogens Subcommittee on November 19-20; information on the nominations would be presented later in this meeting. Dr. Portier reported that the NIEHS convened a panel of experts on July 24 to aid in reviewing the design of proposed carcinogenicity studies on hexavalent chromium in drinking water that address public concern regarding the levels of hexavalent chromium found in the water supplies of several western states. Two-year chronic studies in rodents will begin in the near future.

He noted the presentation next by Dr. Shelby, Director of the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR), and asked the Board to consider making nominations of agents that might have reproductive or developmental toxicological effects. Dr. Portier commented on the NTP Interagency Center for Evaluation of Alternative Toxicological Methods (NICEATM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), noting that the charter for a new NIEHS scientific advisory committee was approved in January 2002 and this committee would be meeting for the first time on December 5 in Washington, D.C.

Dr. Portier discussed NTP collaborative studies with its traditional partners at the Food and Drug Administration (FDA), where a number of studies are ongoing at the National Center for Toxicological Research (NCTR), and with the National Institute for Occupational Safety and Health (NIOSH), including a cosponsored meeting last year on new biotechnology and its role in occupational safety and health. The NTP has an ongoing collaboration with the Collegium Ramazzini in Italy, one of the largest toxicology testing programs in the world, to work toward developing common protocols for rodent studies. Dr. Portier reported that Dr. Robert Maronpot, Chief of the Laboratory of Experimental Pathology, NIEHS, and his staff had produced two CDs on the pathology of the mouse and genetically altered mice, and would be glad to provide copies. He noted that the NTP cosponsored a workshop last year with the Environmental Protection Agency (EPA) and the FDA on the allergenicity of modified foods, specifically looking at the science behind the evaluation of genetically modified foods. From that workshop, the NTP is looking for better screening methods for allergenicity. The NTP has collaborative efforts with the World Health Organization (WHO) and the National Institute of Standards and Technology (NIST) on the issue of possible toxicological effects of radio-frequency and electric and magnetic radiation from cellular phones. Finally, Dr. Portier reported that staff from the Korean National Toxicology Program attended the Technical Reports Review Subcommittee Meeting as part of an effort by the Korean program to avoid duplication with our long-term rodent studies.

## III. Draft Format for NTP-CERHR Monograph

Dr. Michael Shelby, Director of the Center for the Evaluation of Risks to Human Reproduction (CERHR), said the monograph is made up of three components: an NTP brief (the one for di-*n*-butyl phthalate is being discussed as an example), an expert panel report, and all public comments received on the panel report. He said the purpose of the brief is to present the NTP's interpretation and conclusions in a clear, concise and scientifically sound manner with the intended audience being the public and health and regulatory agencies. Although there are monographs on seven phthalates, he chose di-*n*-butyl phthalate (DBP) for presentation, because

there is evidence of reproductive and developmental toxicities and new human exposure data have become available since the expert panel completed its report.

The brief defines the chemical nature of DBP; discusses some sources of exposure and how much people are exposed to, and whether human development and/or reproduction might be affected at those exposure levels; summarizes supporting scientific evidence; and judges the level of concern raised by current exposures to DBP. Levels of concern include *serious concern, concern, some concern, minimal concern, and negligible concern.* Based on the available data, Dr. Shelby said the expert panel concluded that there is *negligible concern for reproductive effects* in adult humans and *minimal concern for adverse developmental effects*.

Since completion of the expert panel report, the Centers for Disease Control and Prevention (CDC) released new data showing higher exposures for some women than those used by the expert panel. The NTP concludes that these new data raise the level of concern in some exposure circumstances. The narrative conclusions in the NTP brief are: (1) based upon recent estimated DBP exposures among women of reproductive age, the NTP has *some concern* for DBP causing adverse effects to human development if pregnant women are exposed to DBP levels at the high end of the range reported by the CDC, particularly development of the reproductive system, and (2) based on current estimated exposure of the general population to DBP, the NTP concurs with the CERHR Phthalates Expert Panel that there is *negligible concern* for reproductive toxicity in exposed adults.

### A. Public Comments

#### 1. Dr. Raymond David representing the Phthalate Esters Panel of the American Chemistry Council

Dr. David said he and his organization are supportive of the CERHR and its open process for deliberations of the expert panel. However, he noted that there has been no public input into development of the NTP brief. Dr David stated that the conclusion is not supported by the data, is based on a single outlier data point from one of three studies, and contradicts the expert panel's conclusion.

## Board Discussion

Dr. Goldman and Dr. Boekelheide were lead discussants. Dr. Goldman said she attended the expert panel review and noted how this review contrasted that of NTP carcinogenesis studies where the findings are considered only for assessment of potential hazard. She noted that from the viewpoint of physicians and other knowledgeable persons, the monographs are quite readable, but wondered whether they might be too technical for the general public. Dr. Boekelheide said he was on the panel, and commented that the issue of significant new data becoming available after a panel reached its conclusions could be a continuing problem. Dr. Bailey observed that a reader might have difficulty differentiating between *weight-of-evidence* and the magnitude of potential human risk.

Several Board members asked about the maximum exposure levels. Dr. Shelby said the exposures levels reviewed by the expert panel were 2-10 ug/kg bw/day, but the subsequent Centers for Disease Control and Prevention (CDC) data in women of childbearing age were higher with the 95$^{th}$ percentile being 32 ug/kg bw/day. Dr. Portier acknowledged the long time between the expert panel's report and publication of the DBP monograph, and said the NTP hopes to shorten this interval to about two months with subsequent NTP-CERHR monographs. Dr. Carpenter suggested that it might be desirable to have the brief reviewed by experts in risk communication. Dr. Moure-Eraso said more attention needs to be given to monitoring exposures of women working with DBP and Dr. Goldman agreed. Dr. Boekelheide suggested that CERHR have its own external advisory panel. Dr. Shelby responded that the NTP relies on this Board to advise them on CERHR activities. Dr. Portier praised Dr. Shelby for his efforts in making the monographs concise and readable.

## IV. The Role of Transgenic Models in the NTP Testing Program

Dr. Portier said the International Life Sciences Institute (ILSI) panel, on which NIEHS participated, met about eight months ago to discuss the usefulness of transgenic animal models in carcinogenicity testing. Subsequently this group published a series of reports that the NTP has found quite useful in its own discussions about how it might use these models in its toxicological testing program. He said the NTP has considered including studies using transgenic models in its technical report series and brought the first two reports before the Board's Technical Reports Review Subcommittee for peer review on September 6. Dr. Portier said the NTP Executive Committee discussed use of transgenic models by the NTP at its meeting in August and recommended more open discussion about the utility of these models before moving forward with a specific strategy. He noted that the American Chemistry Council has raised questions about their use in the long-term rodent bioassay process and comments have also been received from animal protection organizations. Dr. Portier noted that the NTP had carefully considered all input on this issue and said that instead of asking the Board to review a specific strategy, they would ask for more general input about how the NTP might use these models in its testing program. He concluded by noting that the NTP plans to hold a multi-day, multi-disciplinary workshop to discuss these issues.

### A. Review of Available Transgenic Models

Dr. John French, NIEHS, said the NIEHS effort began several years ago as a collaborative effort by Dr. Raymond Tennant's laboratory with Dr. Philip Leder, Harvard Medical School. The initial two mouse strains used were not very effective for carcinogen identification so others were developed. He said the three current models are the Tg.AC (hemizygous), the RasH2 (hemizygous), and the p53 (heterozygous), although the NIEHS also has evaluated the usefulness of other models. A key component of a model should be the presence of an inducible proto-oncogene or inactivated tumor suppressor gene that alone does not cause cancer, but upon exposure to a carcinogen would induce cancer with reduced latency due to other induced genetic alterations. Dr. French said the models should have a set of criteria for judging their utility that would include (1) a broad range of susceptible tissues, (2) zero to low incidence of sporadic tumors, (3) zero to low frequency of false negatives and false positives, and (4) a mode or mechanism consistent with the development of human cancer.

He described characteristics of the v-Ha-*ras* transgene in the Tg.AC mouse and its expression in response to tumor promoters/enhancers. General conclusions for the Tg.AC include: (1) it is an effective reporter phenotype when chemicals are applied topically, (2) carcinogens induce and/or clonally expand cells expressing the transgene, and (3) carcinogens induce both skin papillomas and carcinomas. The second *ras* model, the RasH2 mouse was developed in Japan. Dr. French presented information about studies of point mutations induced in tumors in rasH2 mice by ethylnitrosourea and methylnitrosourea, (forestomach carcinogens) and urethane (a lung carcinogen). This model is susceptible to both genotoxic and nongenotoxic carcinogens, and induction of tumors is associated with overexpression of human *ras* genes. Dr. French also discussed a third model, heterozygous p53 deficient mice, which shows a low incidence of spontaneous tumors up to nine months of age and appears to be useful as an *in vivo* test for mutagenic carcinogens. He noted that p53 haploinsufficient mice are susceptible to mutagenic carcinogens and the loss of heterozygosity includes both p53 locus specific and chromosome 11 (genomic) losses. Dr. French concluded by briefly mentioning other promising models involving dysregulation of the cell cycle, cell proliferation, immunosuppression, and oxidative stress.

### B. Transgenic Models: Can They Contribute to NTP Carcinogen Identification?

Dr. John Pritchard, NIEHS, reported that the focus of this analysis would be to (1) review existing data on transgenic mouse models, (2) assess how well they do as tools for predicting human cancer, (3) compare possible strategies for their use in carcinogen identification, and (4) identify research needs. He said a total of 99 chemicals were studied in the three transgenic models described by Dr. French. The transgenic models were assessed for their ability to correctly identify the human carcinogenicity of the chemicals as currently classified by the International Agency for Research on Cancer (IARC) and/or the Department of Health and Human Services' Report on Carcinogens (RoC). The chemicals evaluated included *known human carcinogens, suspected human carcinogens, and uncertain/unlikely human carcinogens.* Dr. Pritchard noted that a variety of potential testing strategies (12 in all) were evaluated, ranging from individual transgenic models to combinations of these models with each other and with traditional rodent bioassays. He reported that the individual transgenic models made the *correct* calls (positive for known or suspected human carcinogens and negative for probable human noncarcinogens) for 74-81% of the chemicals and this increased up to 86% using combined strategies (e.g., Trp53 +/- for genotoxic chemicals and Tg.AC for nongenotoxic chemicals). For comparison, identical analysis of chemicals in this data set, which were tested in the NTP 2-species rodent bioassay, yielded *correct* calls for 69% of the chemicals.

Dr. Pritchard showed data evaluating performance of the models using IARC classifications. For Group 1 (known human carcinogens), the models overall showed a concordance of 79%, for Group 2A (probable human carcinogens) there was 76% concordance, and for Group 2B (possible human carcinogens) there was 63% concordance. He then reviewed performance among the various testing strategies. The first eight strategies used individual transgenic models or combinations of two models. All strategies, except the Tg.AC or RasH2 alone, gave 80% or greater concordance (positive findings) with IARC/RoC known/suspected carcinogens plus negative findings for IARC/RoC noncarcinogens. Likewise, the rodent bioassay by itself only

gave about 70% concordance, while in combination with any one of the transgenic models resulted in concordance well in excess of 80%. Dr. Pritchard commented on whether these results showed a pattern. First, transgenic models tended to miss some known/suspected human carcinogens (i.e., *false negatives*). Second, rodent bioassays tended to be positive for some chemicals not identified as known or suspected human carcinogens by IARC/ RoC (i.e., *false positives*). The best strategies seemed to be those combining the transgenic models with the rat bioassay; these gave no false negatives and reduced the number of false positives.

Dr. Pritchard identified some research needs, including (1) improving study designs for existing transgenic models (e.g., to reduce *false negatives*) by increasing study duration and/or sample size and developing a historical control database, (2) validating new models as they become available, and (3) developing models for specific uses (e.g., toxicity or carcinogenicity at specific sites via specific mechanisms).

## C. Transgenic Animals in Environmental Health Risk Assessment

Dr. Portier, NIEHS, began his presentation by asking - What is the question that we want to answer with regard to the use of transgenic and other alternative models? How do we validate against this question? He said the NTP hopes to use these models to evaluate toxicity endpoints besides cancer, predict human risk from exposure to chemical and physical agents, conduct dose-response assessment to set standards, and make species extrapolations. He asked the Board to help the NTP identify other studies, such as pharmacological, toxicokinetic, and toxicogenomic, where these models might be appropriate and provide data useful to environmental health risk assessment. Finally, he asked the Board to comment on how the NTP should contribute to and/or lead efforts to develop and assess models that might provide more rapid and more accurate predictions of the carcinogenic potential of environmental agents.

Dr. Portier next asked for the Board's input about whether or not transgenic studies are tests of carcinogenicity. He noted that the Board's Technical Reports Review Subcommittee spent time addressing their utility as well as whether the categories for evidence of carcinogenicity are applicable for evaluation of findings from transgenic studies. The Subcommittee was also asked to comment on whether these studies should be reported in a separate technical report series. He noted that members of the Subcommittee were present and hoped they would comment during discussion. Next, Dr. Portier identified potential benefits related to the use of transgenic models for hazard identification, such as a low background of tumor response in controls, an earlier tumor response (i.e., months versus two years), and the ability to evaluate more compounds in a shorter timeframe at possibly less cost. He asked the Board for its input about what would be needed to validate these models and whether studies negative for carcinogenicity in transgenic models need to be followed up with traditional two-year bioassays.

Dr. Portier then discussed the use of transgenics in dose-response assessments. He noted that the potential benefits of these assays being cheaper and faster that would permit the inclusion of more dose levels for obtaining more information about dose-response. However, he also noted there are still other issues, such as the number of animals needed per group and the length and the timing of exposure. Dr. Portier said more effort is needed toward the validation of these

models for dose-response assessment to answer questions about the comparability of the maximally tolerated doses (MTD) between transgenic and nontransgenic studies and how the shape of the dose-response curve in these models would correspond to the human response.

Finally, he asked whether it would be worth conducting a transgenic study if a rat chronic bioassay were still needed. Dr. Portier showed data on the carcinogenicity of 2,4-diaminotoluene (2,4-D) in a rat bioassay and transgenic mice; the most positive responses observed were 84% mammary tumors in female rats and 20% skin tumors in male Tg.AC mice. This 4-fold difference in response, under a linear model, would result in a 4-fold change in potency.

Dr. Portier said that in the area of transgenics and species extrapolation, issues needing evaluation include toxicokinetics, genomics, and age equivalency across species (i.e., how to compare the *sped-up assay* versus the *lifetime* rodent bioassay). Dr. Portier concluded by listing ways that the NTP might take a lead in this field, including (1) standardization of study protocols, (2) model validation especially for hazard evaluation and dose-response assessment, (3) use of models in toxicogenomics and structure-activity relationship assessments, and finally, (4) leading efforts to broaden the overall evaluation of models.

### D. Role of Transgenic Models in the NTP Testing Program – Charge to the Board

Dr. John Bucher, NIEHS, said he would address issues pertaining to the design and interpretation of studies conducted in transgenic models. These include: (1) selection of the model, (2) length of study - six to nine months seems best in terms of sensitivity and low background tumor incidence, (3) number of animals - usually 10/group, (4) route of exposure, (5) dose selection and spacing – in some early studies doses higher than the MTD were used, (6) the relationship between neoplastic and nonneoplastic lesions, (7) whether a historical control database is needed, (8) what is an *adequate* negative study, (9) what does a tumorgenic outcome mean, and (10) are these cancer studies.

Dr. Bucher presented the charge to the Board in the following questions:

1. Does the Board have recommendations regarding the issues to consider in choosing a transgenic animal for mechanistic research and in validating its use for screening?
2. Under what conditions would the Board feel a positive result in a single or in multiple transgenic model(s) sufficiently reflects a reasonable concern for carcinogenicity in humans? What additional research would be needed to "validate" those conditions?
3. Under what conditions would the Board feel a negative result in a single or in multiple transgenic model(s) sufficiently reflects little or no concern for carcinogenicity in humans? What additional research would be needed to "validate" those conditions?
4. Does the Board have suggestions concerning research the NTP could support to determine if positive findings in transgenic models can be used to predict risk (level of exposure vs. carcinogenic response) in human populations?
5. To what degree would the Board suggest that the NTP balance further research on development of transgenic animals for understanding mechanisms with validation of these animals for a carcinogenicity-screening program?

### Board Discussion

Dr. Goldman asked whether there had been attempts to examine the false negative compounds (e.g., estrogenic hormones) and understand the biologic reason(s) for the response. Dr. Bucher responded that the Tg.AC model correctly identifies estrogenic hormones, but acknowledged that it's puzzling why other compounds shown to give positive response in the traditional mouse bioassay do not respond similarly in transgenics. Dr. Pritchard noted that because most transgenic rodent models are mice, one proposed strategy would be to use one or two of these models along with the 2-year rat bioassay in an attempt to reduce the number of false negative outcomes. Dr. Portier said the NTP Executive Committee suggested a sequential analysis, instead of running the bioassay and a transgenic study in parallel. There ensued discussion as to whether having the rat bioassay only would reduce the number of false negatives. Dr. Storer asked how transgenics might fill a gap not addressed by conventional bioassays, such as uncovering a carcinogenic mechanism or biological response. Dr. Pritchard replied that there were certain tumor sites, e.g., brain, not identified very well by the bioassays.

## E. Agency Comments

### 1. Food and Drug Administration (FDA)

Dr. William Allaben, National Center for Toxicological Research (NCTR)/FDA, gave an overview of the FDA's policy on the use of transgenic animal data by its five product centers. He stated that the FDA should have been involved early in the NTP's discussion and decision on a strategy for using genetically altered animals in carcinogen identification because of its position as a core NTP agency. He requested that the NTP Steering Committee be reestablished to enable early input on programmatic initiatives.

Dr. Allaben reviewed the missions of the 5 FDA centers - Center for Biologics Evaluation and Research ("Biologics"), Center for Food Safety and Applied Nutrition ("Foods"), Center for Drug Evaluation and Research ("Drugs"), Center for Veterinary Medicine ("VetMed"), and Center for Devices and Radiological Health ("Devices"). He said that the rodent bioassay has become the standard animal model for supporting sponsor product submissions to the FDA and for assessing other product risk, and generally has served the public well in promoting and protecting public health. He asked that the Board in evaluating the use of transgenic rodent models in NTP testing paradigms consider whether they are appropriate tests for cancer or if the results are predictive of human cancer risk.

Dr. Allaben reported that Drugs would consider the submission of data from alternative tests as a possible replacement for one rodent bioassay or as an additional toxicology data set. He noted that alternative study protocols reviewed by Drugs have included the newborn mouse, ras2, p53, and Tg.AC. He commented that Foods must adhere to the Delaney Clause in the Food Drug and Cosmetic Act and therefore would continue to require 2-year rodent bioassays be conducted; transgenic animal data would be used as supplement for providing mechanistic information. VetMed must also consider the Delaney Clause and therefore generally requires a 2-year rat bioassay and an 18-month mouse bioassay. Dr. Allaben said VetMed would consider alternative tests as additional data sets and never as stand-alone assays. Biologics and Devices generally do

not require 2-year bioassays and are supportive of alternative toxicology tests as adjuncts for assessing safety and risk. Dr. Allaben said the FDA is concerned that incorporating transgenic models into the NTP testing paradigm would lengthen the process for obtaining toxicological data useful for the assessment of carcinogenic risk in humans. In summary, the FDA's position is that the transgenic assays have potential value, could provide adjunct toxicological data, and are still being evaluated. The acceptability of data from such assays would be on a case-by case basis.

### 2. National Institute for Occupational Safety and Health/Centers for Disease Control and Prevention (NIOSH/CDC)

Dr. Mark Toraason, NIOSH/CDC, said his agency has not conducted an independent evaluation of the use of transgenic models for testing chemicals for cancer hazard identification, has not established a policy for using data from transgenic animals, nor has it used data from transgenic animal studies in assessing potential hazards associated with occupational exposures. NIOSH's comments would address some of the questions submitted to the Board and earlier to the Technical Reports Review Subcommittee.

1. "Is the current language regarding levels of evidence for carcinogenicity adequate and appropriate to describe results?" – NIOSH believes levels of evidence used for evaluating the bioassay results are adequate, but may not be appropriate for studies involving only transgenics and suggests terms such as 'tumor response' or 'expression of neoplasia' might be used.
2. "Should the NTP establish a second report series?" – NIOSH believes results from transgenics should be reported in a second report series unless studies were done simultaneously with the traditional bioassay.

Next, Dr. Toraason addressed questions submitted to the Board.

1. "Does the Board have recommendations regarding the issues to consider 1) in choosing a transgenic model for mechanistic research and 2) in validating its use for screening?" – NIOSH believes that using transgenics for mechanistic research is reasonable, but if they are to be used as a screening tool, then a formal review process is needed to define the parameters of the screening process, including the criteria for selection of the animal model and for interpretation and validation of results.
2. "Under what conditions would the Board feel a positive result in single or multiple transgenics sufficiently reflect reasonable concern for carcinogenicity in humans?" – NIOSH believes a positive result would raise concern regarding carcinogenic potential. A confirmation of the results would be required before findings could be used in quantifying health risk to humans.
3. "Under what conditions would the Board feel a negative result in a single or in multiple transgenics sufficiently reflect little or no concern for carcinogenicity in humans?" – NIOSH believes a negative result would probably not affect concern, as additional evidence would be needed, and this would be especially true where human data suggest that a chemical may be a carcinogen.
4. "Does the Board have suggestions concerning research that the NTP should support to determine if positive findings in transgenics can be used to predict human risk?" – NIOSH

believes transgenic animal models should be validated using chemicals shown to be human carcinogens.

5. "To what degree would the Board suggest that the NTP balance further research on development of trangenics for understanding mechanisms with validation of the animals as part of a carcinogenicity screening program?" – NIOSH supports a balanced approach including both research and validation. Dr. Toraason noted that NIOSH typically uses a *weight of evidence* approach for evaluating issues in public health. If the data from studies conducted in transgenic models are sound and the methods used are accepted, then NIOSH would use the data in its evaluations. However, given the current state of knowledge in interpretation of transgenic animal results, Dr. Toraason said it is unlikely that NIOSH would use the results alone in deriving an occupational exposure limit until additional experimental testing and model validation are performed.

Dr. Toraason commented that NTP considers that the results from bioassays provide data for carcinogen hazard identification, but in practice, the results are used for quantifying human risk as well. He hoped that future study designs using transgenics would include doses relevant to human exposures in order to provide data useful for quantifying human risk. Dr. Toraason closed by saying that the evaluation process for transgenics seems to have been somewhat 'ad hoc' and that a formal evaluation needs to be undertaken to determine the acceptance of each model and to define the conditions for their use, the questions to be asked and how to interpret the results.

### F. Public Comments

#### 1. Dr. Richard Becker, representing the American Chemistry Council (ACC)

Dr. Becker stated that the ACC thinks it critical that the NTP continue to conduct research on transgenic models and is pleased that the NTP 'tabled' the draft strategy. He said the NTP should determine what role the ICCVAM would play in validating these methods. Dr. Becker said broader scientific discussion is needed, such as was demonstrated at the ILSI workshops, to aid in the development, application, and interpretation of these models.

#### 2. Ms. Mary Beth Sweetland, People for the Ethical Treatment of Animals (PETA)

Ms. Sweetland said she has no scientific comments on the use of transgenic animals except to say that it is quite unscientific, considering the problem of false negatives and the question raised about whether these are tests of carcinogenicity. She said PETA would request that the NTP use halothane or halothane followed by carbon dioxide as the most humane method of euthanasia for the animals. Ms. Sweetland urged more attention to development of human cellular assays.

### Board Discussion

Dr. Bailey identified the lead discussants from the Board: Drs. Cohen, Drinkwater, Elwell, Roberts, and Storer. Dr. Storer commented on the Technical Reports Review Subcommittee's

review of the studies on the two triacrylates in the Tg.AC model. He said one concern is whether the reporter phenotype is indicative of a carcinogenic effect of the test chemicals or whether it is a subset of the responses of some agents that are tumor promoters. He thought that there was not enough mechanistic evidence about how the model responds to use the label of 'clear evidence of carcinogenic activity' for the findings. Dr. Storer said he would be more confident of a carcinogenic effect in the p53 model than in the Tg.AC. In response to the NTP's question about when the Board would feel a positive result in single or multiple transgenic models reflects a reasonable concern for carcinogenicity in humans, he commented that although you have look at each model individually, he felt that a clearly positive result in two models especially in concert with other mechanistic information would raise serious concern about human risk. With regard to being able to replace 2-year bioassays with these models, Dr. Storer added that false negatives in these models are still a problem. Dr. Bailey suggested that using higher doses and longer study duration might reduce the number of false negatives. Dr. Roberts said confidence in the models would depend in part on how they are being used, i.e., as a screening tool to determine whether or not to perform a bioassay, as a model for studying mechanism of action, or as a replacement for the bioassay.

Dr. Drinkwater endorsed using a transgenic mouse model in concert with the rat 2-year bioassay, and noted that the difficult question would be deciding what model to use. He said the Technical Reports Review Subcommittee felt that the p53 heterozygous mouse is the best model for carcinogen identification. The Subcommittee pointed out that one problem with the p53 and other models is that there may be tissue-specific responses to tumor induction that would only show up in the 2-year bioassay and would be missed in the transgenic model. Dr. Bailey added that target organ specificity is also an issue for consideration. Dr. Cohen said transgenic models are virtually useless for predicting or understanding target organ response in humans and added that they would not be especially useful for determining dose-response unless the number of animals per study were greatly increased. He felt that the data presented by Dr. Pritchard demonstrated the usefulness of transgenic mouse models for hazard identification and if used in conjunction with gene toxicity assays and the 2-year rat bioassay could replace the 2-year mouse bioassay. He commented that a major advantage of replacing the mouse bioassay with a transgenic model would be to reduce false positives. Dr. Goldman cautioned against doing away with the mouse bioassay and added that the data in Dr. Portier's presentation suggest that there might be less power to detect small effects in the transgenic versus traditional mouse assay.

Dr. Bailey suggested that the NTP evaluate extending study duration to nine months to determine if the false negative rate decreases and to assess the impact of study duration on background tumor rate. Dr. Bucher said several studies conducted in the p53 model had been negative for tumors after both six and nine months, while background tumor rates in the Tg.AC tended to increase from six to nine months. Dr. French said strains differ in their susceptibility to tumor induction. He added that the dose administered, i.e., saturating versus nonsaturating, could also affect the tumor rate.

Dr. Portier said the NTP seeks the Board's advice about whether transgenic models should be used in its testing program and if so how. Dr. Cohen replied that it would depend in part on whether the chemical being tested is likely to be a human carcinogen; if so, a positive in the p53

model after six months might be enough to trigger beginning a risk evaluation; however, if dose-response or organ specificity information were needed, then additional studies would be necessary. Dr. Goldman expressed reservations about the value of transgenics for assessing carcinogenic risk to humans by agencies such as FDA and NIOSH. Further, she questioned the proposed savings in time and/or money if a 2-year rat bioassay were still needed and wondered whether the predictability of data from transgenics would be greater than structure-activity correlations. Dr. Olden remarked that it is premature to speculate whether studies in transgenic models would replace 2-year bioassays, but rather he envisioned development of a battery of assays to aid in detecting toxicity or carcinogenicity. He noted that the sequencing of the human genome, the availability of emerging technologies in genomics, and the identification of susceptibility genes would all aid in this effort. Dr. Boekelheide said an advantage of introducing transgenic models into the NTP testing program would be to increase its testing flexibility. For example, he commented that if the only information needed were whether a chemical is positive or negative for carcinogenicity, then a transgenic study might be sufficient, but if dose-response information were needed, that would necessitate using other tools. Dr. Drinkwater concurred, and added that if a chemical were evaluated in the p53 and was positive then that might be sufficient, but if the results were negative, then a 2-year rat bioassay would probably need to be run.

Dr. Carpenter said he believes transgenic models could be useful for hazard identification, but he sought input about how to apply the information from such studies to making public health decisions. He added that what is needed for hazard identification is information based on data using doses lower than those typically used in animal studies. Dr. Olden commented that at human exposure levels, hyper-susceptibility or genetic predisposition might likely be the important determinant for determining who would get cancer. He added that this is an area of current research at the NIEHS. In addition, the NIEHS has commissioned the Institute of Medicine to help advise the institute on how to appropriately use toxicogenomic data.

Dr. Portier said the NTP is looking broadly at available genetically altered mouse models. Dr. Bailey commented that other vertebrate models are also available and said the NIEHS Marine and Freshwater Biomedical Centers Program is conducting studies in aquatic species. Dr. Olden added that the NIEHS has established five university-based mouse genome centers for creating genetic models.

Dr. Drinkwater said if the NTP is serious about using transgenics in its toxicological testing program, then it must set high standards for the conduct of those studies. Both Dr. Boekelheide and he commented that a carefully designed process for validation of the transgenic models is needed. Dr. Gasiewicz inquired whether the NTP might follow the ICCVAM validation process. Dr. Portier said the requirements of the ICCVAM process might preclude its use for validating transgenic models. Dr. William Stokes, NIEHS and Director of the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods, commented that ILSI has done some validation studies. Dr. Goldman commented that there must be a standardized validation process for transgenic models in order for them to acceptable to regulatory agencies.

## V. Toxicogenomics
### A. National Center for Toxicogenomics

Dr. Raymond Tennant, Director of the National Center for Toxicogenomics (NCT) at the NIEHS, said the NCT was established in September 2000. The NCT is a concept for applying genomic technology to studying the adverse effects of environmental agents. It is a novel strategy for combining the scientific resources of the NIEHS, academia and the private sector for development of the knowledge database, Chemical Effects in Biological Systems (CEBS). Dr. Tennant said the NCT is needed because humans are exposed to numerous chemicals occupationally and through the environment that manifest their effects over dose and time, and any potential impact of those exposures is affected by an individual's genetic makeup. The short-term goals for toxicogenomics are to develop predictive assays, such as *signature* patterns of altered gene expression resulting from chemical exposure, to combine that information with proteomic analysis and to identify potential biomarkers of exposure. Long-term goals focus more broadly on development of a gene expression database, analytic and query tools, and relational interfaces and annotation.

Dr. Tennant said the NCT will function through: (1) the development and harmonization of gene expression and proteomic technology; (2) the development of academic and private sector consortia; (3) the conduct of *proof of principle* experiments that define altered patterns of gene expression associated with adverse effects of drugs, chemicals, and environmental agents; (4) the acquisition of data sets, storage, query, and analysis capabilities through resource contracts; and (5) the creation of the publicly accessible CEBS. Currently NCT has activities within the NIEHS intramural component complementary to the toxicogenomics research consortium at five universities, external partnerships with the private sector (e.g., ILSI), and resource contracts for development of CEBS.

Dr. Tennant said part of the process for acquiring data involves RNA extraction and hybridization to cDNA or oligonucleotide chips possessing 10 to 20,000 specific genes from rodents, humans or other species. Following hybridization, the chips are scanned to identify those genes whose expression is changed upward or downward. The identification of specific expression patterns or *signatures* is a potential predictive tool of carcinogenic effect. Initially NCT has focused on characterization of the acute toxicity of chemicals from various structural or functional classes by looking at patterns of gene expression and at the linking of these patterns to specific adverse effects of those toxicants or classes of toxicants. Dr. Tennant said the criteria for designing such experiments involves using well studied compounds for which there is significant human exposure and similar toxicity in rodents and humans, and for which clinical biosamples would be potentially available. Comparing the toxic endpoints and gene expression signature of unrelated compounds may lead to identification of biomarkers of toxicity. It is anticipated that both organ specificity and chemical specific deferential gene expression responses will be identified.

Dr. Tennant briefly described NCT studies with methapyrilene, a species-specific carcinogen, and pyrilamine, its non-carcinogenic analog. Methapyrilene has specific effects on the liver (e.g., bile duct hyperplasia), and by profiling target vs. non-target tissues, specific genes may be

identified that are related to those adverse effects. Dr. Tennant said they hope to identify proteins/peptides collected from tissues in parallel for microarray analysis and proteomic analysis that might be candidates for biomarkers of a specific toxicity. He concluded by detailing the goals of CEBS: 1) creation of a reference toxicogenomic information system of studies on environmental chemicals/stressors and their effects; 2) development of relational and descriptive compendia on toxicologically important genes, groups of genes, SNPs, mutants, and their functional phenotypes relevant to human health and environmental disease; and 3) creation of a toxicogenomics knowledge base to support hypothesis-driven research. Dr. Tennant acknowledged collaboration with Gene Bank associated with the Human Genome Project and with other components of the NIEHS and NTP.

## B. Toxicogenomics for the NTP – Links Between the NCT and NTP

Dr. Gary Boorman, NIEHS, said he would address toxicogenomics from toxicological and pathological viewpoints. He identified several NIEHS researchers involved in in-house studies, NTP circadian studies, and NTP chemical studies. The focus of current work is on acute effects of chemicals that affect the liver and especially those that cause hepatocellular necrosis. The first chemical selected for study was acetaminophen, chosen because liver toxicity is one of the most common responses in rodents and humans, liver metabolism is similar in both species and well studied, there is an opportunity for concurrent clinical studies, and the drug has both therapeutic and toxic effects. Dr. Boorman discussed the absorption, distribution, metabolism and excretion of acetaminophen in rats, pointed out the shift in metabolism to more toxic metabolites at higher doses, and described the pathologic, clinical chemical, and genetic profiles in low- versus high-dosed animals. He stated that the goals of the in-house studies were to (1) define critical time points for analysis, (2) define the genetic profile for toxic and therapeutic doses, (3) establish mechanisms of toxicity, and (4) evaluate procedures for NTP/NCT studies. Dr. Boorman said another variable needing evaluation is time of exposure, noting that some NTP studies are daytime exposures where technicians administer the chemical while others include nighttime exposure where the chemical is planed in the drinking water or feed. The acetaminophen study compares night versus daytime exposures. He briefly discussed other studies of hepatotoxicants, including the family of microcystins produced by algae found in surface water. Microcystins cause acute hepatocellular necrosis by protein phosphatase 1 and 2A inhibition (PP1 and 2A). The hypothesis is that different microcystins varying by one or two peptides, but with different levels of toxicity because they act through the same PP1 and 2A mechanism will give similar gene expression patterns when given at similar PP1 and 2A inhibition levels. This provides rationale for NTP evaluating only one of the nearly 60 microcystins and extrapolating the data to the others. Dr. Boorman concluded by noting the importance of genomic technology and of the NTP being poised to take advantage of it, and how NTP collaboration with NCT offers the best chance for effective use of this evolving technology.

## C. Practical Aspects of Integrating Toxicogenomics into the NTP

Dr. Bucher, NIEHS, stated that the goal is to integrate toxicogenomic information seamlessly with other data collected on a chemical and to enhance our understanding of toxic and carcinogenic mechanisms. The NTP offers some advantages in collection and use of this

information, including standardization of all aspects of studies, the capacity to handle large amounts of data, the capability to integrate diverse findings in studies' interpretations, adequate reporting capabilities through the technical report series, and the potential for linkage with physiologically based pharmacokinetic (PBPK) models. Temporary disadvantages are that the analyses are labor intensive and experience is not up to speed in our contract laboratories. Dr. Bucher listed a number of design and performance issues including: (1) selection of gene arrays, (2) selection of times and doses for analysis, (3) program should be hypothesis driven, (4) issue of how to handle and coordinate mRNA isolations, hybridizations, bioinformatics, and data analysis and interpretations, (5) integration of NCT and NTP databases, and (6) the coordination of the NCT Toxicology Pathology Group with NTP design groups. He said the current approach is to start with in-house studies and incrementally add training and evaluation of contractors and the integration of contracted studies with in-house components moving toward increasing contractor-dependent activities.

Dr. Bucher stated the questions for the Board:

1. Should the NTP place a higher priority on using these new tools to examine specific chemically induced mechanisms of response/toxicity, or focus on projects that would lead to a better understanding of the biology and human relevance of current models?
2. Are there unique opportunities for the NTP to contribute to development of biomarkers through use of toxicogenomic data?
3. When chemical specific studies are performed, would toxicogenomics be most useful as a screening tool for prioritization or as a tool for investigation of mechanisms of action/toxicity?
4. Should the NTP routinely incorporate the new "omic" technologies in our standard testing programs?
5. What level of "validation" of these technologies would need to be demonstrated before routine incorporation of these technologies into the testing program should be attempted? (6) How can the NTP best develop collaborative efforts with the extramural community? Dr. Portier clarified the "either/or" questions by saying that NTP would plan to do both but would appreciate guidance on priority setting.

### D. Public Comments

#### 1. Ms. Mary Beth Sweetland, People for the Ethical Treatment of Animals (PETA)

Ms. Sweetland stated that PETA believes that the assays needed to be validated via the ICCVAM process, and thought it slightly disingenuous of the NTP to rule out the process because of the purported number of animals that would be required.

## Board Discussion

Dr. Roberts started the discussion and said he thought the NTP should focus on projects leading to a better understanding of the biological and human relevance of current models. Dr. Drinkwater supported incorporating these new assays prospectively into bioassay protocols even though how to interpret the results is not currently understood and noted that having 90-day gene

expression data would likely be useful in the future. Dr. Goldman supported compiling the genomic/proteomic data in a database as a resource to the extramural community and noted the example of EPA/industry cooperation in sharing data from high volume chemicals. Dr. Cohen thought that design issues need to be carefully worked out and comparable so the "best" and meaningful data can be obtained. Dr. Storer raised the question of the stability of samples in long-term tissue storage. Dr. Bucher said the current limit is about six months because of issues with RNA quality in stored samples, but this area needs more study. Dr. Drinkwater stated that there should be emphasis on development of reliable methods for timely analysis of data from 2-year studies. Dr. Goldman said that the NTP should involve the extramural community in helping it work out issues with collection, analysis and storage.

Dr. Bailey asked Dr. Tennant to talk more about biomarkers and their potential utility. Dr. Tennant responded that there are clearly patterns of serum gene expression that have predictive value, so the question is whether we can refine more specific markers of adverse effects. Dr. Portier asked the Board how NTP studies might be used to interpret future use of biomarkers. Dr. Drinkwater replied that the NTP should consider tissue banking. Dr. Portier asked for advice on whether NTP should be taking biological samples during the course of its current chronic studies to look for changes in biomarkers. He asked if there would be value in linking early biomarkers within animals with cancer findings or if these should be evaluated in a separate group. Dr. Drinkwater observed that understanding the state of gene expression would be relevant and useful, but this would be a large undertaking. Dr. Gasiewicz added that the fingerprinting patterns should be evaluated at multiple doses and multiple time points. Dr. Goldman commented that identifying such biomarkers for use in cancer epidemiology studies would be invaluable.

Dr. Portier reiterated his earlier request that the Board advise the NTP on how it should move forward. The Board agreed that the extramural scientific community should have early access to the databases being developed.

## VI. NTP Testing Program
### A. Overview of Current Initiatives

Dr. Bucher, NIEHS, discussed some of the focused areas of toxicology research that the NTP is working on, including

1. DNA-based therapies –nominated by FDA for study because of concerns over the potential for some of these agents to insert into the genome and cause heritable effects.
2. Endocrine disrupting agents – two of the largest studies ever performed by the NTP are nearing completion. The first is the dioxin toxic equivalency factor cancer potency study with combinations of Ah receptor agonists and antagonists. This study is testing the theory that toxic equivalency factors can be used to predict cancer potency. The second is a multigenerational study of various estrogenic and antiandrogenic compounds being conducted at the NCTR through an NIEHS interagency agreement.
3. Herbal medicines and supplements – These studies include evaluations of the toxicity of substances such as aloe vera, kava, and comfrey.

4. Occupational exposures – The NIEHS has an interagency agreement with NIOSH to characterize worker exposure, identify health research gaps, and develop protocols for laboratory toxicology studies. Studies completed or planned include cellulose insulation, asphalt fumes, metal working fluids, and 1-bomopropane.
5. Water contaminants – The NTP is collaborating with EPA to assess potential risks from exposure to a wide range of mostly halogenated water disinfection by-products, and also agents occurring naturally or as contaminants in drinking water such as aluminum complexes, blue-green algae, microcystins, and organotins.
6. Phototoxicology – The NCTR has a state-of-the-art phototoxicology laboratory and is conducting studies on the potential chronic toxicity on mouse skin of α- and β-hydroxy acids combined with UV-containing light. Additional studies are planned to assess the phototoxic effects of products containing aloe vera and retinyl palmitate.
7. Polybrominated diphenyl ethers – These compounds are components of flame-retardants. This is a new initiative and studies under design are primarily with tetrabromodiphenyl and pentabromodiphenyl ethers.

### Board Discussion

Dr. Boekelheide asked whether any long-term studies with retroviral vectors are planned. Dr. Bucher replied that some were done, but were unable to show incorporation into DNA. The Board asked some general questions about specific chemicals or mixtures being studied in the various initiatives.

## B. Cellular Telephone Emissions

Dr. Ronald Melnick, NIEHS, said the FDA's Center for Devices and Radiological Health nominated cell phone radio-frequency radiation (RFR) for study of its potential health effects because of the large and increasing exposure of Americans, now in excess of 115 million Americans using wireless communication devices, and because there are no regulatory standards *per se* although the FCC has exposure guidelines (1.6 mWatts/gram) for protection from thermal effects. He said little is known about potential health effects of long-term exposure to non-thermal levels of RFR. Animal studies have been recommended for evaluating the carcinogenic potential of RFR since meaningful epidemiological data may not be available for several years. He noted that there are studies underway through the European Union at frequencies of 900 and 1747 megahertz (MHz), frequencies for analog and digital phones, respectively. There studies use B6C3F1 mice and Wistar rats in a Ferris wheel-type exposure system where the animals are restrained in tubes. The Ramazzini Cancer Research Institute in Italy is conducting a lifetime study in Sprague Dawley rats at 1800 MHz, with low-frequency modulation and exposure for 20 hours/day beginning at weaning. Dr. Melnick reported that because of the complexities of RFR, the NTP is working with the National Institute of Standards and Technology (NIST) to develop a reverberation chamber exposure system. This system will have a mechanical stirring device to create a homogeneous electromagnetic environment and animals will be individually caged and free roaming. Currently NIST is conducting feasibility studies to determine whether this is an appropriate approach.

## Board Discussion

The Board asked several questions about experimental design features and Dr. Melnick provided the details. Dr. Checkoway commented that human studies report brain tumors and maybe breast cancer, and wondered whether NTP studies would address cancers other than brain. Dr. Melnick responded that the NTP study would evaluate effects of whole body exposure and there would be complete histopathological evaluations. In response to a question from Dr. Toraason, Dr. Melnick said body temperature would be monitored during the prechronic studies.

## C. New Substances Nominated to the NTP for Toxicological Studies

Dr. Scott Masten, NIEHS, briefly outlined the nomination, review and selection process and noted that this process includes multiple opportunities for pubic comment. Once agents are selected, studies are initiated as time and resources permit. Dr. Masten highlighted the questions for the Board's consideration.

1. Is the background material provided in advance adequate for the Board to provide input and perspective?
2. In the context of overall importance to public health, what priority should the NTP place on the studies recommended?
3. Are there other issues the NTP should consider in addressing the recommended studies?
4. Does the Board have additional guidance for engaging the academic community and other public segments concerning study nominations?

Dr. Masten said that the Interagency Committee for Chemical Evaluation and Coordination (ICCEC) reviewed 19 new nominations (27 total agents) and recommended 14 for study. He identified the nominations:

1. Dietary supplements/consumer products – 5-amino-ortho-cresol, ephedrine alkaloids, Iso-E-Superâ
2. industrial chemicals – abrasive blasting agents, *tert*-butyl hydroperoxide, cobalt metal dust, magnesium oxide, methylolurea, nitrogen trifluoride, sodium metasilicate, turpentine, welding fumes
3. therapeutics and other agents – chloramine-T and *p*-toluenesulfonamide, ketamine hydrochloride, hexafluorosilicic acid and sodium hexafluorosilicate, thimerosal
4. environmental contaminants – hexachloro-1,3-butadiene, infrasound, 4-methylquinoline.

The ICCEC did not recommend the study of magnesium oxide, methyolurea, or the environmental contaminants.

Public comments were received for eight of the nominations and raised the following issues: (1) opposition to government-sponsored animal studies of agents with available health effects data and where *in vitro* methods are available, (2) opposition to NTP study of industrial chemicals included in the EPA testing program or voluntary testing programs, (3) citizen and academic support for the study of ephedrine alkaloids, fragrance chemicals, and fluorosilicates, and (4) submission by industry of unpublished toxicity studies.

## D. Public Comments

**1.** Dr. Edwin Bisinger, Akso Nobel Chemicals Inc., U.S. representative of Axcentive BV, current submitter of an FDA Investigational New Animal Drug Application for chloramines-T

Dr. Bisinger stated that there is no appreciable human exposure to chloramine-T or its primary degradation product, *p*-toluenesulfonamide (*p-TSA)* in aquaculture in the United States. Also there is no human exposure in the United States through its use as disinfectant because its registration was cancelled more than 12 years ago. Second, he said that FDA has concluded that all required genotoxicity data on chloramine-T or *p*-TSA have been submitted and the data have shown no genotoxic activity. These chemicals appear to pose a low risk to public health in the United States.

### Board Discussion

Dr. Goldman expressed concern about human exposure due to accidental release during transport of the chemical and thought modest testing is warranted.

**2.** Ms. Janet Reed Pettit, President of Concerned Citizens for Pure Water

Ms. Pettit said that hexafluorosilicic acid and sodium hexafluorosilicate were used for fluoridating the water supplies of her town and of about half the population of America. Although these chemicals have permeated our nation's foodstuffs and drinks, no one knows the average level of ingestion and no studies have proven silicofluorides safe. She said there is good evidence that silicofluorides facilitate uptake of lead and other heavy metals, such as aluminum, into the brain. Studies of small towns where water supplies are fluoridated suggest a higher level of learning disabilities in children and a higher incidence of violent crime than in towns where the water supplies are not fluoridated. Ms. Petit stated that testing of the silicofluorides is long overdue.

### Board Discussion

The lead discussants were Drs. Carpenter, Checkoway, and Moure-Eraso. Dr. Carpenter commented that the background documents on the NTP study nominations were informative, but he would like to have them sooner. Dr. Moure-Eraso said it would be helpful if the documents contained more specific information on occupational exposures and occupational health issues. Dr. Checkoway thought the complex mixtures reflected the reality of human exposures, but presented a daunting task for study. Dr. Portier agreed and noted that the NTP's collaboration with NIOSH is very helpful in determining what components of mixtures to study. Dr. Goldman questioned the rationale of not testing hexachloro-1,3-butadiene and 4-methylquinoline just because of low production volumes and exposure. She said the former chemical is found in adipose tissue of fish while the latter is found in tobacco smoke. Dr. Goldman endorsed the public comments on the silicofluorides calling for better characterization of the fluorine dissociation, interaction with metals and presence of contaminants. Dr. Bailey thought there was sufficient information about toxicological testing on hexachloro-1,3-butadiene in the background

document. Dr. Masten responded that the main reason for recommending *no testing* was the sufficient data on carcinogenicity. Dr. Bailey commented that with herbal supplements, not only are they complex mixtures, (e.g., ephedra alkaloids), but also have different compositions from different producers. Dr. Allaben reported that the FDA has a memorandum of understanding with the University of Mississippi to characterize a number of the dietary supplements, particularly ephedra, and this has been completed. Dr. Bailey said with regard to priority that should be given to the recommended studies, the Board has identified the issues of human exposure data and/or lack of background toxicology data as most important.

He then asked the Board whether there are other issues the NTP should consider when developing a research program. Dr. Carpenter responded that any studies to obtain mechanistic type information would be useful for future risk assessments. In response to the question asking for guidance regarding NTP's approach to engaging the academic community and other public sectors regarding study nominations, Dr. Bailey asked the NTP to explain its current process. Dr. Portier replied that the NTP has historically engaged the academic community through collaborations on study designs, evaluations of completed studies, and the RO3 grant mechanism. Dr. Moure-Eraso commented that contacts needed to be maintained with labor. Dr. Carpenter observed that many professionals in the public health field may be aware of the NTP, but are not aware that they can play a role in the NTP's research and testing activities. Dr. Roberts suggested that the NTP consider society newsletters and professional journals are sources to solicit broader input.

## VII. Concept Reviews

### A. MRI and Multimodality Imaging Support for NIEHS and NTP Research

Dr. Robert Maronpot, NIEHS, presented the concept (*Attachment*). The purpose of this proposed contract would be to provide more complete support for the morphological phenotyping of genetically altered mice designed to address basic biological questions relevant to the mission of the NIEHS and the NTP. He spoke of the Visible Mouse, high resolution imaging in the perfused fixed whole mice, and showed slides demonstrating this technology. The advantages of magnetic resonance microscopy (MRI), include (1) it does not destroy the sample, (2) it is inherently digital and three dimensional, (3) specimens can be examined in different planes, (4) it permits volumetric measurements of tissue and organ structure, and (5) it allows the acquisition of imaging data in live animals at multiple intervals over time. Dr. Maronpot said the brain is an ideal organ for applying this technology. He concluded by identifying three tasks and one option in the proposed work: (1) MRI imaging of whole animals or tissues, (2) multimodality imaging of whole live animals and/or *in vivo* imaging of specified organ systems, (3) some methods development, and (4) an option for allowing investigators to completely phenotype genetically engineered mice.

### Board Discussion

Dr Gasiewicz commented how marvelous this technology is and asked whether it would be used for only studying genetically altered animals. Dr. Maronpot said it would also be used to study normal mice and rats. Dr. Drinkwater asked whether other techniques such as PET would be

part of the project. Dr. Maronpot replied that one project a year under the second task would include PET. Dr. Drinkwater moved that the concept proposal be approved. Dr. Cohen seconded the motion, which the Board approved unanimously (11 yes/0 no).

### B. Genetic Toxicity in Animals

Dr. William Caspary, NIEHS, presented the concept (*Attachment*). He said the objective of this continuing contract effort is to characterize the *in vivo* genetic toxicity of environmental agents in rodents. Specifically, this would be accomplished by examining chromosomal mutations and DNA damage in these animals. He reported that two assays are used - the micronucleus (MN) assay and the comet assay. Slides with blood samples from 13-week old mice are scored for MN in chronic bioassays, and in acute studies, erythrocytes are harvested and scored. A positive MN test has high predictability for rodent carcinogenesis. Dr. Caspary said the comet assay measures single- and double-strand breaks, oxidative-induced base damage, -DNADNA and DNA-protein crosslinking, and DNA repair in single cells. The NTP is asking the Board for approval to continue this effort.

#### Board Discussion

Dr. Gasiewicz asked how quantitative and how sensitive is the micronucleus assay. Dr. Caspary said that 25-30% of chemicals examined are positive in the MN assay. Dr. Storer suggested evaluating the assay's specificity by testing well-characterized non-carcinogens. Dr. Caspary agreed that would be a good idea. Dr. Goldman moved that the concept proposal be approved. Dr. Gasiewicz seconded the motion, which the Board approved unanimously (11 yes/0 no).

### C. Mechanisms of Chemical Toxicity

Dr. Caspary presented the concept (*Attachment*). He said the objectives of this new contract effort are (1) to identify chemicals that induce specific endpoints or to modulate various pathways by which chemicals induce cancer, (2) to characterize possible genetic and biological lesions that may be responsible for various toxicological endpoints of interest to the NTP, and (3) to stimulate further research on some of these endpoints of toxicological importance. The concept has two parts, one being metabolism and the other being to examine effects on mammalian cells in culture.

With regard to metabolism, the mutagenic activity of chemicals will be evaluated in a *Salmonella* system using liver S9 as well as other mammalian activating systems. Using mammalian cells, the ability to detect a wider spectrum of mutations will be possible, including point mutations, chromosomal mutations, and premutagenic lesions, than by using mouse lyphoma or TK6 cells. The micronucleus and COMET assays will be used, and the effort will identify chemicals that modulate the cell cycle and affect apoptosis. Dr. Caspary said the purpose is to get a snap shot of possible mechanisms that would useful in interpreting bioassay results.

## Board Discussion

Dr. Drinkwater asked whether this effort would be a routine part of NTP genetic toxicity testing. Dr. Caspary responded that he anticipated all new NTP chemicals would go through the *Salmonella* and probably other activating systems and a subset – perhaps 75% – would be evaluated in the mammalian cell systems. Dr. Boekelheide asked how apoptosis would be evaluated and Dr. Caspary replied initially through flow cytometry and staining. In response to a question by Dr. Gasiewicz, Dr. Caspary noted that cell cycle and apoptosis studies might detect damage not due to a mutagenic event. Dr. Boekelheide moved that the concept proposal be approved. Dr. Carpenter seconded the motion, which the Board approved unanimously (11 yes/0 no).

## VIII. Update on the Technical Reports Review Subcommittee Meeting

Dr. James R. Hailey, NIEHS, summarized the uses and levels of evidence of carcinogenicity for toxicology and carcinogenesis studies reviewed and approved by the Board's Technical Reports Review Subcommittee on September 5-6, 2002.

- Dipropylene glycol – used in antifreeze, air fresheners, cosmetics, and found in groundwater – exposure in drinking water – *no evidence* in male and female rats or mice.
- Elmiron® – drug used for treatment of interstitial cystitis and thrombotic disorders – exposure by gavage – *no evidence* in male and female rats and *some evidence* in male and female mice (hemangiosarcomas and hepatocellular tumors).
- Decalin – used as a solvent, in fuel and stain removal– exposure by inhalation – *clear evidence* in male rats (kidney and adrenal medulla neoplasms), *equivocal evidence* in female mice (liver neoplasms and stromal polyps of the uterus), and *no evidence* in female rats and male mice.
- *trans*-Cinnamaldehyde – used in flavors and fragrances – exposure by microencapsulation in feed – *no evidence i*n male and female rats or mice.

Dr. Hailey discussed studies of urethane and ethanol conducted by the National Center for Toxicological Research (NCTR) of the Food and Drug Administration. IARC classifies urethane as category 2B, a *possible human carcinogen*. Urethane is used in the preparation of amino acids, manufacture of pesticides, fumigants, and cosmetics, and is a byproduct of fermentation. This study was designed to evaluate the effects of ethanol on urethane carcinogenesis, as well as effects of urethane alone and ethanol alone. Urethane was studied only in mice, where there was *clear evidence* in males and females (robust tumor responses in liver, lung and harderian gland, along with sex-specific lesser tumor responses). The studies on ethanol alone were determined by the Subcommittee to be inadequate to determine carcinogenic activity, while there was weak evidence of an interaction of ethanol on the carcinogenicity of urethane. Dr. Hailey also reported on six-month studies of trimethylolpropane triacrylate and pentaerythritol triacrylate in the Tg.AC transgenic mouse. These chemicals are used in UV-curable inks and acrylic glues. The NTP withdrew considerations of the conclusions for carcinogenic activity by the Subcommittee.

Dr. Hailey identified studies to be presented for Subcommittee review in spring 2003 including stoddard solvent, 2-methylimidazole, propylene glycol mono-*t*-butyl ether, and triethanolamine

(mice only), as well as two artificial sweeteners, aspartame and acesulfame potassium, studied in transgenic mice. Studies for review in fall 2003 include malachite green, leucomalachite green, and sodium chlorate, as well as four dioxin-like compounds that are being studied as part of the Toxic Equivalency Factor (TEF) initiative - TCDD (dioxin), PCB 126, a dioxin mixture, and 2,3,4,7,8-pentachlorodibenzofuran. The TEF initiative objectives are to (1) determine potency factors for individual classes of dioxins, (2) test the validity of the TEF method for predicting carcinogenicity of a simple mixture of dioxins, and (3) determine if non-dioxin-like PCBs antagonize the carcinogenicity of a dioxin-like PCB.

### Board Discussion

Dr. Moure-Eraso asked why the conclusions for the Tg.AC studies were withdrawn. Dr. Portier responded that this decision was made prior to the Subcommittee meeting and that the results were more appropriate for reporting in the toxicology report series and not the cancer technical report series. Dr. Drinkwater, who participated in the Subcommittee review, agreed that this was the appropriate mode for reporting at this time.

## IX. Update on the Report on Carcinogens

Dr. C. W. Jameson, NIEHS, briefly went over the review process for the Report on Carcinogens (RoC) and pointed out that the NIEHS Review Group (RG1) no longer has any involvement with drafting the background document. Rather, under a contract, the documents are now prepared and/or reviewed by expert consultants with specific expertise for the nomination.

Dr. Jameson noted that the final draft of the 10$^{th}$ Edition of the RoC is complete and would be submitted to the Secretary's office for approval in the near future. One entry, talc, both containing and not containing asbestiform fibers, was deferred. He said this deferral is pending a careful review of the literature on these materials to determine if a clear definition of the agent or agents involved in human exposures could be developed. Following this review, the nomination will be considered, possibly, for the 12$^{th}$ RoC.

Dr. Jameson reported that review of nominations to 11$^{th}$ RoC is under way. The Board's RoC Subcommittee would meet on November 19-20 in Bethesda, Maryland to review the first 10 nominations including 1-amino-2,4-dibromoanthraquinone, cobalt sulfate, diethanoamine, naphthalene, nitrobenzene, nitromethane, 4,4'-thiodianiline, and three selected heterocyclic amines – MeIQ (2-amino-3,4-dimethylinidazo[4,5-*f*]quinoline), MeIQx (2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline), and PhIP (2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine). Dr. Jameson concluded by announcing the second set of nominations for the 11$^{th}$ RoC that the Subcommittee would review in summer 2003. The nominations include: diazoaminobenzene, hepatitis B virus, hepatitis C virus, high risk human papillomaviruses, ionizing radiation (including X-radiation, gamma radiation, and neutrons), and lead and lead compounds.

## Board Discussion

Dr. Goldman asked for clarification as to why the talc listing for the 10th RoC was deferred, wondering if the background document was inadequate. Dr. Portier said the document was not inaccurate; however, the Subcommittee gave a strong recommendation that the document did not clearly define what is asbestiform and non-asbestiform talc and whether asbestos fibers were present during certain exposures. This led to the decision to defer the review of this nomination until these issues could be addressed.

**ADDRESSES:** Submit written comments on the collection of information to the Office of Information and Regulatory Affairs, OMB, New Executive Office Bldg., 725 17th St. NW., rm 10235, Washington, DC 20503, Attn: Stuart Shapiro, Desk Officer for FDA.

**FOR FURTHER INFORMATION CONTACT:** Denver Presley, Office of Information Resources Management (HFA–250), Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, 301–827–1472.

**SUPPLEMENTARY INFORMATION:** In compliance with 44 U.S.C. 3507, FDA has submitted the following proposed collection of information to OMB for review and clearance.

### Veterinary Feed Directive—21 CFR Part 558 (OMB Control Number 0910–0363)—Extension

The veterinary feed directive (VFD) drugs section of the Animal Drug Availability Act of 1996 (ADAA) (Public Law 104–250) established a new class of restricted feed use drugs that may be distributed without invoking State pharmacy laws. In order to implement the VFD drugs section of the ADAA, FDA issued regulations (65 FR 76924, December 8, 2000) that impose reporting and recordkeeping requirements on veterinarians, distributors of animal feeds containing VFD drugs, and clients using medicated feeds containing VFD drugs. All distributors of animal feed containing VFD drugs must notify FDA of their intent to distribute animal feed containing a VFD drug, and must maintain records of the distribution of all animal feeds containing VFD drugs (21 CFR 558.6).

In the **Federal Register** of April 30, 2002 (67 FR 21252), the agency requested comments on the proposed collection of information. FDA received one comment.

The comment asked if the proposed collection of information was necessary for the proper performance of FDA functions and whether the information will have practical utility. The answer is yes. As detailed, the VFD regulation ensures protection of public health while enabling animal producers to obtain and use needed drugs as efficiently and cost-effectively as possible.

Respondents to this collection of information are veterinarians, distributors of animal feeds containing VFD drugs, and clients using medicated feeds containing VFD drugs.

FDA estimates the burden for this collection of information as follows:

TABLE 1.—ESTIMATED ANNUAL REPORTING BURDEN[1]

| 21 CFR Section | No. of Respondents | Annual Frequency per Response | Total Annual Responses | Hours per Response | Total Hours |
|---|---|---|---|---|---|
| 558.6(a)(3) through (a)(5) | 15,000 | 25 | 375,000 | 0.25 | 93,750 |
| 558.6(d)(1)(i) through (d)(1)(iii) | 1,500 | 1 | 500 | 0.25 | 125 |
| 558.6(d)(1)(iv) | 20 | 1 | 20 | 0.25 | 5 |
| 558.6(d)(2) | 1,000 | 5 | 5,000 | 0.25 | 1,250 |
| 514.1(b)(9) | 1 | 1 | 1 | 3.00 | 3 |
| Total | | | | | 95,133 |

[1] There are no capital costs or operating and maintenance costs associated with this collection of information.

TABLE 2.—ESTIMATED ANNUAL RECORDKEEPING BURDEN[1]

| 21 CFR Section | No. of Recordkeepers | Annual Frequency per Recordkeeper | Total Annual Records | Hours per Record | Total Hours |
|---|---|---|---|---|---|
| 558.6(c)(1) through (c)(4) | 112,500 | 10 | 1,125,000 | .0167 | 18,788 |
| 558.6(e)(1) through (e)(3) | 5,000 | 75 | 375,000 | .0167 | 6,263 |
| Total | | | | | 25,051 |

[1] There are no capital costs or operating and maintenance costs associated with this collection of information.

These estimates are based on agency communication with industry. Other information needed to calculate the total burden hours are derived from agency records and experience.

Dated: August 13, 2002.

**Margaret M. Dotzel,**

*Associate Commissioner for Policy.*

[FR Doc. 02–20917 Filed 8–16–02; 8:45 am]

BILLING CODE 4160–01–S

## DEPARTMENT OF HEALTH AND HUMAN SERVICES

### Public Health Service

### National Toxicology Program; Notice of a Meeting of the NTP Board of Scientific Counselors

Pursuant to Public Law 92–463, notice is hereby given of a meeting of the National Toxicology Program (NTP) Board of Scientific Counselors on September 17–18, 2002, at the Radisson Governors Inn, 1–40 at Davis Drive, Exit 280, Research Triangle Park, North Carolina.

The NTP Board of Scientific Counselors (the Board) is composed of scientists from the public and private sector and provides primary scientific oversight to the NTP.

#### Agenda

The meeting being held on September 17–18, 2002, is open to the public from 8:30 a.m. to adjournment each day with attendance limited only by the space available. Persons needing special assistance should contact the NTP Executive Secretary (contact information below). A draft agenda with tentative schedule is provided below. Primary agenda topics include: (1) A draft format for the NTP brief that will be part of the NTP–CERHR Monograph prepared on each chemical reviewed by the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR); (2) a discussion of the proposed NTP

strategy for using genetically altered animals in carcinogen identification, (3) an overview of the NIEHS National Center for Toxicogenomics and its links with the NTP, and (4) recommendations of the NTP Interagency Committee for Chemical Evaluation and Coordination (ICCEC) for substances nominated to the NTP for study. There will also be an update about current NTP testing initiatives, the NTP Board of Scientific Counselors Technical Reports Review Subcommittee meeting on the September 5–6, 2002, and the status of the 10th and 11th Editions of the Report on Carcinogens. The Board will review a concept proposal for the continued use of a contract mechanism to investigate potential genetic toxicity of substances under study by the NTP. Time is allotted during the meeting for the public to present comments to the Board and NTP staff on agenda topics.

*NTP–CERHR Monograph*

The NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) serves as an environmental health resource to the public and to regulatory and health agencies for scientifically based, uniform assessments of the potential for adverse effects on reproduction and development caused by agents to which humans are exposed. Additional information about the CERHR is available at *http://cerhr.niehs.nih.gov.*

As a final step in its evaluation of a chemical, the CERHR will prepare an NTP–CERHR Monograph. The monograph will include an NTP brief, the expert panel's report on the chemical, and all public comments received on the expert panel report. The NTP brief provides the NTP's interpretation of the potential for exposure to the chemical to adversely affect reproduction and/or development in humans. NTP–CERHR monographs will be made publicly available.

As a prototype for the NTP–CERHR Monograph, the CERHR has available the draft NTP–CERHR Monograph on Di-n-butyl Phthalate (DBP) and will request comment from the Board on the proposed format for the NTP brief. The CERHR is interested in obtaining input about the brief's layout and presentation of information, it clarity, and its utility for the public. The draft monograph is available on the NTP–CERHR Web site (*http://cerhr.niehs.nih.gov*) or by contacting the NTP Executive Secretary.

*NTP Draft Strategy for Using Genetically Altered Animals in Carcinogen Identification*

The NTP has developed a proposed draft strategy for the routine use of genetically altered animals in carcinogen identification. The NTP is seeking broad external input on this strategy and will present it to the Board for review and comment. This meeting also provides an opportunity for the public to offer comment to the Board and NTP staff on the proposed draft strategy. The draft strategy is available on the NTP Web site (*http://ntp-server.niehs.nih.gov,* see What's New, Meeting of the NTP Board of Scientific Counselors) or by contacting the NTP Executive Secretary (contact information below).

*Toxicogenomics*

With the advent of novel molecular technologies, the NTP is moving into the arena of toxicogenomics, a new scientific field that examines how the entire genome is involved in biological responses of organisms exposed to environmental toxicants. Toxicogenomics studies the effect of toxicants on gene activity and specific proteins produced by genes in response to those toxicants.

In an effort toward centralizing activities in toxicogenomics, the NIEHS/NIH established the National Center for Toxicogenomics (NCT) in September 2000. The NCT's mission is to coordinate a nationwide research effort for the development of a toxicogenomics knowledge base. Additional information about the NCT is available on the NIEHS Web site at *http://www.niehs.nih.gov/nct* or by contacting the NTP Executive Secretary (contact information below). The NTP will describe some of its current efforts to incorporate toxicogenomics into its testing strategies through interactions with the NCT.

*NTP Testing Program*

Overview of Current Initiatives

The NTP seeks to maintain a balanced research and testing program that provides data addressing a wide variety of issues of importance to public health. Currently the NTP is focusing on several areas that have received inadequate attention in the past: for example, photoactive chemicals, contaminants of finished drinking water, endocrine-disrupting agents, and certain occupational exposures. The NTP is addressing potential safety issues associated with herbal medicines, radiofrequency radiation emissions from cellular telephones, hexavalent chromium, and DNA-based therapies. In general, these initiatives are broad-based and include the investigation of various health-related endpoints. Additional information about some current initiatives is available in the NTP Booklet, Current Directions and Evolving Strategies, available on the NTP Web site (*http://ntp-server.niehs.nih.gov,* select Publications).

ICCEC Recommendations for Substances Nominated for Future NTP Studies

Information about substances nominated to the NTP for toxicology and carcinogenesis studies and the ICCEC's recommendations was published in the **Federal Register** on June 12, 2002 (Vol. 67, No. 113, p. 40329–33). This notice is available on the Web (*http://ntp-server.niehs.nih.gov/htdocs/Liason/ICCECFinal02JuneFR.html*) along with supporting documents for each nomination (*http://ntp-server.niehs.nih.gov/htdocs/liason/BkgrSum02June.html*) or by contacting the NTP Executive Secretary (contact information below). This meeting provides an additional opportunity for the public to provide comment on these nominations and study recommendations to the Board and NTP staff. Comments submitted to the NTP in response to the June 2002 **Federal Register** notice are under consideration and do not need to be resubmitted or readdressed.

Substances recommended for study:

- Abrasive blasting agents—5 different industrial materials used as alternatives to sand.
- 5-Amino-o-cresol—permanent hair dye ingredient.
- tert-Butyl hydroperoxide—high production volume industrial catalyst.
- Chloramine-T and p-Toluenesulfonamide—active ingredient and metabolite of therapeutant used in aquaculture to control bacterial infections.
- Cobalt metal dust—important industrial material linked to lung problems in workers.
- Ephedrine alkaloid dietary supplements—widely used in herbal dietary supplements with numerous reports of adverse effects.
- Ethanone, 1-(1,2,3,4,5,6,7,8-octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)-(Iso-E-Super)—high-production-volume fragrance material.
- Hexafluorosilicic acid and Sodium hexafluorosilicate—primary agents used to fluoridate public drinking water system.
- Ketamine hydrochloride—approved anesthetic drug that causes brain lesions in developing rats.
- Mercury, ((o-carboxyphenyl)thio)ethyl-, sodium salt (Thimerosal)—organomercuryl-based

preservative used in vaccines and other biological products.

- Nitrogen trifluoride—cleaning and etching agent in the semiconductor industry.
- Sodium metasilicate—industrial cleaning agent.
- Turpentine—high-production-volume industrial solvent and raw material.
- Welding fumes—variable composition mixture responsible for respiratory and other adverse effects in exposed workers.

Nominations for which no studies are recommended at this time:

- Hexachloro-1,3-butadiene—industrial by-product and persistent environmental contaminant.
- Infrasound—low frequency acoustic energy present at low levels in community and occupational settings.
- Magnesium oxide—high-production-volume chemical with numerous industrial uses.
- Methylolurea—starting material for and impurity in urea-formaldehyde resins.
- 4-Methylquinoline—environmental pollutant structurally related to the rodent carcinogen quinoline.

**Public Comment Encouraged**

Public input at this meeting is invited and time is set aside for the presentation of public comments on any agenda topic. At least 7 minutes will be allotted to each speaker, and if time permits, may be extended to 10 minutes. Persons registering to make oral comments are asked to provide their name, affiliation, mailing address, phone, fax, e-mail, and sponsoring organization (if any). Each organization is allowed one time slot per agenda topic. To facilitate planning for the meeting, persons interested in providing formal oral comments are asked to notify Dr. Mary Wolfe, NTP Board Executive Secretary, NIEHS, P.O. Box 12233, MD A3–07, Research Triangle Park, NC 27709; telephone: 919–541–0530; and e-mail: (*wolfe@niehs.nih.gov*) by September 10, 2002. Persons registering to make oral comments are asked, if possible, to provide a copy of their statement to the Executive Secretary by September 10th, to enable review by the Board and NTP staff prior to the meeting. Written statements can supplement and may expand the oral presentation. Individuals will also be able to register to give oral public comments on-site at the meeting. However, if registering on-site and reading from written text, please bring 25 copies of the statement for distribution to the Board and NTP staff and to supplement the record.

Persons may also submit written comments in lieu of making oral comments. Written comments should be sent to the Executive Secretary and must be received by September 10th to enable review by the Board and NTP staff prior to the meeting. Persons submitting written comments should include their name, affiliation, mailing address, phone, fax, e-mail, and sponsoring organization (if any) with the document.

**Registration**

The NTP Board of Scientific Counselors meeting is open to the public. Attendance at this meeting is limited only by the space available. Due to changes in security policies at the NIEHS, individuals who plan to attend are asked to register with the NTP Executive Secretary (*see* contact information above). The names of those registered will be given to the NIEHS Security Office in order to gain access to the campus. Persons attending who have not pre-registered may be asked to provide pertinent information about the meeting, *i.e.*, title or host of meeting before gaining access to the campus. All visitors (whether or not you are pre-registered) will need to be prepared to show 2 forms of identification (ID), *i.e.*, driver's license and one of the following: company ID, government ID, or university ID. Also, those planning to attend who need special assistance are asked to notify the NTP Executive Secretary in advance of the meeting (*see* contact information above).

**Additional Information About Meeting**

Prior to the meeting, a copy of the agenda and a roster of the Board's members will be available on the NTP Web site at *http://ntp-server.niehs.nih.gov* and upon request to the Executive Secretary (contact information provided above). Following the meeting, summary minutes will be prepared and available through the NTP Web site and upon request to Central Data Management, NIEHS, P.O. Box 12233, MD E1–02, Research Triangle Park, NC 27709; telephone 919–541–3419; fax 919–541–3687; and email *CDM@niehs.nih.gov*.

**NTP Board of Scientific Counselors**

The Board is a technical advisory body comprised of scientists from the public and private sectors who provide primary scientific oversight to the overall Program and to the NTP Center for the Evaluation of Risks to Human Reproduction. Specifically, the Board advises the NTP on matters of scientific program content, both present and future, and conducts periodic review of the Program for the purposes of determining and advising on the scientific merit of its activities and their overall scientific quality. Its members are selected from recognized authorities knowledgeable in fields such as toxicology, pharmacology, pathology, biochemistry, epidemiology, risk assessment, carcinogenesis, mutagenesis, molecular biology, behavioral and neurotoxicology, immunotoxicology, reproductive toxicology or teratology, and biostatistics. The NTP strives for equitable geographic distribution and minority and female representation on the Board. Its members are invited to serve overlapping terms of up to four years and meetings are held once or twice annually for the Board and its two subcommittees (the Report on Carcinogens Subcommittee and the Technical Reports Review Subcommittee).

Dated: August 12, 2002.

**Samuel Wilson,**

*Deputy Director, National Toxicology Program.*

**Preliminary Agenda—National Toxicology Program (NTP) Board of Scientific Counselors, September 17–18, 2002**

*Radisson Governors Inn, 1–40 at Davis Drive, Exit 280, Research Triangle Park, North Carolina*

*September 17, 2002*

8:30 a.m. Welcome and Opening Comments

NTP Update

NTP–CERHR Monograph: Format of Draft NTP Brief
- Public Comments

NTP Draft Strategy for Using Genetically Altered Animals in Carcinogen Identification
- Evaluation of Transgenic Models for Use in Carcinogen Identification
- NTP Draft Strategy
- Agency Comments
- Public Comments

11:30 a.m.—Lunch

1 p.m. —NTP Draft Strategy (continued)

Toxicogenomics
- Overview of the National Center for Toxicogenomics (NCT)
- Links between the NCT and NTP
- Public Comments

5 p.m.—Adjourn

*September 18, 2002*

8:30 a.m.—Welcome and Introductions

NTP Testing Program
- Overview of Current Initiatives
- Testing Recommendations from the Interagency Committee for Chemical Evaluation
- Public Comments

Concept Review

Update on the NTP Technical Reports Review Subcommittee Meeting

Update on the Report on Carcinogens

Public Comments

Noon—Adjourn

[FR Doc. 02–20921 Filed 8–16–02; 8:45 am]

BILLING CODE 4140–01–P

## DEPARTMENT OF HOUSING AND URBAN DEVELOPMENT

**[Docket No. FR–4456–N–21]**

### Privacy Act of 1974, Deletion of a Privacy Act System of Records

**AGENCY:** Office of the Chief Information Officer, HUD.

**ACTION:** Notification of the deletion of a Privacy Act system of records.

**SUMMARY:** The Department proposes to delete one system of records from its inventory of records system subject to the Privacy Act of 1974 (5 U.S.C. 552a), as amended.

**DATES:** *Effective Date:* This proposal shall become effective without further notice September 18, 2002, unless comments are received on or before that date which would result in a contrary determination.

*Comments Due Date:* September 18, 2002.

**ADDRESSES:** Interested persons are invited to submit comments regarding this notice to the Rules Docket Clerk, Office of General Counsel, Room 10276, Department of Housing and Urban Development, 451 7th Street, SW., Washington, DC 20410–0500. Communications should refer to the above docket number and title. Comments submitted by facsimile (FAX) will not be accepted. A copy of each communication submitted will be available for public inspection and copying between 7:30 and 5:30 p.m. weekdays at the above address.

**FOR FURTHER INFORMATION CONTACT:** Jeanette Smith, Departmental Privacy Act Officer, Telephone Number (202) 708–2374. (This is not a toll-free number). A telecommunications device for hearing and speech-impaired persons (TTY) is available at 1–800–877–8339 (Federal Information Relay Services). (This is a toll-free number).

**SUPPLEMENTARY INFORMATION:** Pursuant to the Privacy Act of 1974 (5 U.S.C. 552a) as amended, notice is given that HUD proposes to delete a system of records identified as Single Family Casualty Damage Files, HUD/DEPT–9. The Department has determined that this system is no longer necessary. Accordingly, HUD/DEPT–9 is deleted from HUD's inventory of records subject to the Privacy Act.

**Authority:** 5 U.S.C. 552a: 88 Stat. 1896; 342 U.S.C. 3535(d).

Dated: August 13, 2002.

**Gloria R. Parker,**

*Chief Technology Officer.*

[FR Doc. 02–20938 Filed 8–16–02; 8:45 am]

BILLING CODE 4210–72–M

## DEPARTMENT OF THE INTERIOR

### Bureau of Land Management

**[OR–030–1020–PG; G 02–0348]**

### Meeting Notice for the Southeast Oregon Resource Advisory Council

**AGENCY:** Bureau of Land Management (BLM), Vale District, Interior.

**SUMMARY:** The Southeast Oregon Resource Advisory Council (SEORAC) will meet in the conference room at the Comfort Inn, 504 N. Highway 20, Hines, OR 97641, 541–573–3370 from 8 a.m. to 5 p.m., Pacific Time (PT), on Friday, October 18, 2002.

The meeting topics that may be discussed by the Council may include a discussion of issues within southeast Oregon related to Steens Mountain Resource Advisory Council, North Lake Recreation Plan, Burns Steens/Andrews Resource Management Plan, Birch Creek Management Plan, Wildland Fire Board, OHV, Rangeland Assessment, Federal officials' updates, and other matters as may reasonably come before the Council. The entire meeting is open to the public. Information to be distributed to the Council members is requested in written format 10 days prior to the start of the Council meeting. Public comment is scheduled for 11:15 a.m. to 11:45 a.m., Pacific Time on Friday, October 18, 2002.

**FOR FURTHER INFORMATION CONTACT:** Additional information concerning the SEORAC may be obtained from Peggy Diegan, Management Assistant/Webmaster, Vale District Office, 100 Oregon Street, Vale, OR 97918 (541) 473–3144, or *Peggy_Diegan@or.blm.gov* and/or from the following web site: *<http://www.or.blm.gov/SEOR–RAC>*.

Dated: August 12, 2002.

**David R. Henderson,**

*District Manager.*

[FR Doc. 02–20935 Filed 8–16–02; 8:45 am]

BILLING CODE 4310–33–M

## DEPARTMENT OF THE INTERIOR

### Bureau of Land Management

**[OR–130–1020–PH; GP02–0339]**

### Notice of Public Meeting, Eastern Washington Resource Advisory Council Meeting

**AGENCY:** Bureau of Land Management, Interior.

**ACTION:** Notice of public meeting.

**SUMMARY:** In accordance with the Federal Land Policy and Management Act (FLPMA) and the Federal Advisory Committee Act of 1972 (FACA), the U.S. Department of the Interior, Bureau of Land Management (BLM) Eastern Washington Resource Advisory Council (RAC), will meet as indicated below.

**DATES:** The Eastern Washington Resource Advisory Council (EWRAC) will meet on September 17, 2002, at the Spokane District Office, Bureau of Land Management, 1103 North Fancher Road, Spokane, Washington, 99212–1275.

**SUPPLEMENTARY INFORMATION:** The meeting will start at 9 a.m. and adjourn about 4 p.m.. Topics on the meeting agenda include: Update on Columbia Basin Shrub-Steppe Land Exchange; Development of ground rules for Public Input Process; Review of Proposed Resolution on Energy & Minerals; Status of Incoming RAC members; Future RAC meeting dates.

The entire meeting is open to the public. Information to be distributed to Council members is requested in written format 10 days prior to the Council meeting date. Public comment is scheduled for 11 a.m. to 12 noon.

**FOR FURTHER INFORMATION CONTACT:** Sandra Gourdin or Kathy Helm, Bureau of Land Management, Spokane District Office, 1103 N. Fancher Road, Spokane, Washington, 99212, or call (509) 536–1200.

Dated: August 12, 2002.

**Gary J. Yeager,**

*Acting District Manager.*

[FR Doc. 02–20948 Filed 8–16–02; 8:45 am]

BILLING CODE 4310–33–P

## DEPARTMENT OF JUSTICE

### Drug Enforcement Administration

### Importation of Controlled Substances; Notice of Application

Pursuant to section 1008 of the Controlled Substances Import and Export Act (21 U.S.C. 958(I)), the Attorney General shall, prior to issuing a registration under this Section to a

**NTP BOARD OF SCIENTIFIC COUNSELORS**
**SEPTEMBER 17 – 18, 2002**
**Radisson Governors Inn, RTP, NC**

**SEPTEMBER 17, 2002**

| | | |
|---|---|---|
| 8:30 AM | **WELCOME AND INTRODUCTIONS** | Dr. George Bailey, Jr., Chairman, Oregon State University |
| | · Recognition of retiring members | Dr. Kenneth Olden, NIEHS |
| 8:45 AM | Update | Dr. Kenneth Olden |
| 9:00 AM | Update on the NTP | Dr. Christopher Portier |
| 9:30 AM | **DRAFT FORMAT FOR NTP-CERHR MONOGRAPH** | Dr. Michael Shelby |
| | · Public comments | |
| | · Board discussion | |
| 10:30 AM | ***BREAK*** | |
| 10:45 AM | **THE ROLE OF TRANSGENIC MODELS IN THE NTP TESTING PROGRAM** | |
| | · A Review of Available Transgenic Models | Dr. John French |
| | · Transgenic Mouse Models: Their Role in Carcinogen Hazard Identification and Dose-response Assessment | Dr. John Pritchard and Dr. Portier |
| Noon | ***LUNCH*** | |
| 1:00 PM | **THE ROLE OF TRANSGENIC MODELS IN THE NTP TESTING PROGRAM** *(Continued)* | |
| | · Charge to the Board | Dr. John Bucher |
| | · Agency Comments | |
| | o FDA | Dr. William Allaben, FDA/NCTR |
| | o NIOSH | Dr. Mark Toraason, CDC/NIOSH |
| | · Public comments | |
| | · Board discussion | |
| 3:00 PM | ***BREAK*** | |
| 3:15 PM | **TOXICOGENOMICS** | |
| | · National Center for Toxicogenomics (NCT) | Dr. Raymond Tennant |
| | · Links Between the NCT and NTP | Dr. Gary Boorman |
| | · Practical Aspects of Integrating Toxicogenomics into the Testing Program | Dr. Bucher |
| | · Public comments | |
| | · Board discussion | |

***5:00 PM ADJOURN***

**SEPTEMBER 18, 2002**

| | | |
|---|---|---|
| 8:30 AM | **INTRODUCTIONS** | |
| 8:45 AM | **NTP TESTING PROGRAM** | |
| | · Overview of Current Initiatives | Dr. Bucher |
| | · NTP Nominations to the Testing Program | Dr. Scott Masten |
| | · Public comments | |
| | · Board discussion | |
| 9:45 AM | **CONCEPT REVIEW FOR MRI AND MULTIMODALITY IMAGING** | Dr. Robert Maronpot |
| | · Board discussion and ACTION | |
| 10:00 AM | ***BREAK*** | |
| 10:30 AM | **CONCEPT REVIEW FOR GENETIC TOXICITY IN ANIMALS** | Dr. William Caspary |
| | · Board discussion and ACTION | |
| 10:45 AM | **CONCEPT REVIEW FOR MECHANISMS OF CHEMICAL TOXICITY** | Dr. William Caspary |
| | · Board discussion and ACTION | |
| 11:00 AM | **UPDATE ON THE TECHNICAL REPORTS REVIEW SUBCOMMITTEE MEETING** | Dr. James Hailey |
| | · Board discussion | |
| 11:15 AM | **UPDATE ON THE REPORT ON CARCINOGENS** | Dr. C. W. Jameson |
| | · Board discussion | |

***11:30 AM ADJOURN***

George S. Bailey, Jr., Ph.D.
Professor and Director
Marine/Freshwater Biomedical Sciences Center
& Distinguished Professor of Food Toxicology
Department of Food Sciences & Technology
232 Wiegand Hall
Oregon State University
Corvallis, Oregon 97331

Kim Boekelheide, M.D., Ph.D.
Professor
Division of Biology and Medicine
Department of Pathology and Laboratory Medicine
Brown University, Box G-B587
97 Waterman Street
Providence, RI 02912

George E. Bonney, Ph.D. ****
Professor, Microbiology Director
Statistical Genetics and Bioinformatics Unit
National Human Genome Center at Howard University
2216 Sixth Street, NW, Suite 205
Washington, DC 20059

Hillary M. Carpenter, III, Ph.D.
Toxicologist
Minnesota Department of Health
121 East Seventh Place, Suite 220
P.O. Box 64975
St. Paul, MN 55101

Harvey Checkoway, Ph.D., M.P.H.
Professor
Department of Environmental Health
and Epidemiology, Box 357345
School of Public Health and Community Medicine
University of Washington
F-561 Health Sciences Bldg, 1705 NE Pacific St.
Seattle, WA 98105

Samuel M. Cohen, M.D., Ph.D.
Professor and Chair
Department of Pathology and Microbiology
University of Nebraska Medical Center
983135 Nebraska Medical Center, UMA 3516
Omaha, NE 68198-3135

Norman R. Drinkwater, Ph.D.
Professor
McArdle Laboratory for Cancer Research
University of Wisconsin-Madison
1400 University Avenue
Madison, WI 53706-1599

Michael R. Elwell, D.V.M., Ph.D.
Research Advisor
Pathology, Drug Safety Evaluation
Pfizer Global Research and Development
Eastern Point Road
Groton, CT 06340

Thomas A. Gasiewicz, Ph.D.
Professor
Department of Environmental Medicine
Environmental Health Sciences Center
University of Rochester School of Medicine
601 Elmwood Avenue
Rochester, NY 14642

Lynn R. Goldman, M.D., M.P.H.
Professor
Department of Environmental Health Sciences
The Johns Hopkins University School of Public Health
615 N. Wolfe Street, Room W8511
Baltimore, MD 21205

Donald R. Mattison, M.D. ****
Senior Advisor
National Institute of Child Health and
Human Development
National Institutes of Health
6100 Executive Blvd.
Rm 4B05
Rockville, MD 20852

Rafael Moure-Eraso, Ph.D., C.I.H.
Professor
Department of Work Environment
College of Engineering
University of Massachusetts Lowell
One University Avenue
Lowell, MA 01854

Cheryl Lyn Walker, Ph.D. ****
Professor of Carcinogenesis
Department of Carcinogenesis
The University of Texas
M.D. Anderson Cancer Center
Park Road IC
P.O. Box 389
Smithville, TX 78957

Stephen M. Roberts, Ph.D.
Professor
Center for Environmental & Human Toxicology
University of Florida, Box 110885
Bldg 471, Mowry Rd. Rm. 14
Gainesville, FL 32611-0885

Richard D. Storer, Ph.D., M.P.H.
Senior Investigator
Department of Genetic and Cellular Toxicology
Merck Research Laboratories, WP45-311
770 Sumneytown Pike
West Point, PA 19486

Bruce S. Weir, Ph.D.
William Neal Reynolds Professor of
Statistics and Genetics
Department of Statistics
North Carolina State University
Raleigh, NC 27695-7566

****not in attendance

# CONCEPT REVIEW

National Toxicology Program
Board of Scientific Counselors

*September 18, 2002*

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Background on Concept Review.....1
Title: MRI and Multimodality Imaging Support for NTP & NIEHS.....2
Presenter: Robert R. Maronpot, Chief, Laboratory of Experimental Pathology

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## BACKGROUND ON CONCEPT REVIEWS

NTP contracts, interagency agreements, and grants support a variety of activities toxicologic characterization, testing, methods development, and program resources (i.e., chemistry, occupational health and safety, animal production, pathology, quality assurance, archives, etc.).

Prior to issuance of a Request for Proposal (RFP) or a Request for Application (RFA), a project concept review is required. These project concepts in many instances may consist of more than one contract, interagency agreement, or grant. Concept reviews are needed for new projects, recompetitions with changes in statements of work, and projects ongoing for five years or more since the last concept review.

The project concept reviews are conducted by the NTP Board of Scientific Counselors **and are** open to the public so long as discussions are limited to review of the general project purposes, scopes, goals, and various optional approaches to pursue the overall program objectives. The meeting will be closed to the public, however, if the concept discussions turn to the development or selection of details of the projects or RFPs/RFAs, such as specific technical approaches, protocols, statements of work, data formats, or product specifications. Closing the session is intended to protect the free exchange of the advisory group members' opinions and to avoid premature release of details of proposed contract projects or RFPs/RFAs.

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a. scientific, technical or program significance of the proposed activity;

b. availability of the technology and other resources necessary to achieve required goals;

c. extent to which there are identified, practical scientific or clinical uses for the anticipated results; and

d. where pertinent, adequacy of the methodology to be used in performing the activity.

**NATIONAL TOXICOLOGY PROGRAM CONCEPT REVIEW**

**CONCEPT TITLE:** MRI and Multimodality Imaging Support for NTP & NIEHS

**PRESENTER:** Dr. Robert R. Maronpot, Chief, Laboratory of Experimental Pathology

## OBJECTIVES

The purpose of this proposed contract is to provide more complete support for toxicity and carcinogenicity studies in animals by the National Toxicology Program (NTP) as well as support for NIEHS investigators in morphological phenotyping of genetically altered mice designed to address basic biological questions relevant to the mission of the NIEHS and the NTP. These services are needed to characterize the toxicological effects of chemical, biological and physical agents, and genetic manipulations through studies using animals and to more fully identify phenotypic alterations in mice. These studies provide a rational basis and data on which a broad array of public health decisions are based for the protection of people from exposure to potentially hazardous substances.

## BACKGROUND

Recent advances in image acquisition permit magnetic resonance imaging of experimental laboratory rodents at microscopic resolutions. These advances in conjunction with the ability to rapidly acquire high resolution image arrays in far shorter times than previously possible will now permit practical application of this technology to toxicological research. Utilization of this technology for magnetic resonance microscopy (MRM) will potentially facilitate toxicology studies as well as permit an alternative and comprehensive means for phenotyping genetically altered mouse models for toxicology and cancer research.

MRM is a high resolution application of magnetic resonance imaging (MRI) that permits acquisition of images of the whole mouse at 100-micron isotropic resolution at 2.0T with image arrays of 256 x 256 x 1024. Higher resolution (50 x 50 x 50 microns) of limited volumes has been acquired at 7.1T with image arrays of 512 x 512 x 512. Even higher resolution images (20 x 20 x 20 microns) of isolated organs have been acquired at 9.4T. Images are typically acquired from perfused fixed specimens, although imaging of live animals for functional phenotyping is possible, but more technically challenging at comparable resolutions. The volume resolution represents an increase of 625,000x over conventional clinical MRI. This technology will allow basic scientists to begin using MRM as a routine ancillary method for fully characterizing toxicological effects in experimental animals and for morphologic phenotyping of the mouse.

The advantages of MRM derive from the fact that it does not destroy the sample, it is inherently digital and three dimensional, it allow examination of specimens in different planes of orientation, it permits volumetric measurements of tissue and organ structures, and, in live animals, it permits acquisition of imaging data at multiple intervals over time. These features are difficult and impractical to achieve by existing conventional pathology means. In addition, the application of contrast agents permits identification of tissue structures and composition analogous to utilization of special staining procedures in conventional pathology. Investigators

can view the acquired images over the internet on their personal computers, literally electronically slice through an entire animal, and can make side-by-side comparisons between treated and control mice or between genetically altered and wild type mice.

## PROPOSED WORK

The proposed work consists of three tasks and one option. Task 1 involves MRI imaging of whole animals or tissues and is based upon completely imaging an entire perfused fixed animal or specific tissue. Task 2 is a multimodality imaging acquisition of whole animals and/or in vivo imaging of specific organ systems. In addition to MRI, PET and microCT scanning are currently possible. Task 3 allows for taking advantage of new imaging acquisition techniques such as use of novel contrast agents by supporting a limited amount of methods development. The optional task, to be used in support of NIEHS investigators and pending availability of sufficient funds, involves morphological phenotyping of genetically altered mice.

**CONCEPT REVIEW**

National Toxicology Program
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Background on Concept Review…….....1
Title: Genetic Toxicity in Animals…….2
Presenter: Dr. William J. Caspary. ETP/TOB

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**NATIONAL TOXICOLOGY PROGRAM CONCEPT REVIEW**
**CONCEPT TITLE:** Mechanisms of Chemical Toxicity
**PRESENTER**: Dr. William J. Caspary, ETP/TOB

## OBJECTIVES

The objective of this contract effort is to characterize the in vivo genetic toxicity of chemical, biological and physical agents in rodents. Specifically, this will be accomplished by examining chromosomal mutations and DNA damage in these animals.

## BACKGROUND

The NTP uses the micronucleus assay in femoral bone marrow cells to determine if chemicals can induce chromosome damage in animals either under the scope or by measurement of micronuclei. Treatment of micronuclei with antibodies specific for chromosomal centromeres (e.g., CREST antibody reaction, anti kinetochore antibody, specific chromosome probes) distinguishes between those micronuclei containing centromeres from those containing acentric chromosome fragments.

The NTP also uses the micronucleus assay to measure chromosomal damage in other animal tissues. Cancer bioassay contract laboratories routinely prepare slides of peripheral blood from rodents used for 13-week studies for micronucleus analysis. For chemicals that are positive in this assay, there is a greater than 90% certainty that the compound will be carcinogenic in rodents.

Single cell gel electrophoresis, which is sometimes called the Comet Assay, is a sensitive technique using small amounts of tissue that permits assessment of DNA damage in many tissues. The NTP uses this technique to detect single- and double-strand breaks, oxidative-induced base damage, and DNA-DNA/DNA-protein crosslinking and DNA repair in individual cells.

## CONCEPT PROPOSAL

The present proposal is to continue to conduct in vivo genetic toxicity tests that measure DNA lesions in rodents.

## SIGNIFICANCE AND PRACTICAL USES OF RESULTS

*Scientific Significance*:

Some chemicals cause DNA damage which can lead to mutations. Mutations at critical genes can ultimately contribute to tumor formation. These mutations amplify, modify or abrogate protein function. Measurement of DNA damage and/or mutation in animals treated with

chemicals identifies compounds that have the potential of causing genetic damage and provides information on potential mechanisms leading to cancer. Tissue specificity of toxic effects can also be examined. For example, when measured in peripheral lymphocytes or lung cells, results can be compared to similar measurements in humans who are clinically, occupationally or environmentally exposed to the same chemical.

*Program Significance:*

Exposure to chemicals encountered in the environment, work-place or food supply can have profoundly negative impacts on human health. The NTP seeks to assess risks associated with possible acute, repeated or chronic exposure to chemicals by investigating a variety of biological effects including carcinogenicity induced on chronic exposure. Studies of the mechanism(s) of toxicity and the fate of chemicals in intact animals are an integral part of the range of NTP studies designed to characterize the toxicity of chemicals. Metabolites and structural analogs of NTP chemicals can be examined. The information provided by rodent studies on mechanisms of toxicity also facilitates extrapolation of risks to groups and/or classes of chemicals and thus extends the knowledge gained from individual studies. It also provides quantitative insights that can help in risk assessment and risk management. Data from these rodent studies provide information on possible genetic mechanisms leading to neoplasia and thereby supports the NTP bioassay program. The results from these studies may mitigate the need to run bioassays on NTP compounds. Often, these assays can be performed on the same animals being used for measurement of other toxicological endpoints, thereby reducing the numbers of animals used and improving the ETP s ability to integrate the different responses obtained from a particular chemical or treatment condition. Studies can be conducted on the same species and strains used in the NTP cancer bioassay program. For example, the contract will allow the determination of increased levels of micronuclei in blood samples taken from male and female mice from the NTP 13 week toxicity studies.

**AVAILABILITY AND ADEQUACY OF METHODOLOGY AND TECHNOLOGY**

At present the NTP has two contracts performing this work.

**PRACTICAL USES**

See scientific and program significance.

**PROPOSED CHANGES TO THE CURRENT WORK STATEMENTS:**

The work to be performed is expected to closely resemble in scope and effort the activities carried out under these contracts during the preceding period.

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Presenter: Dr. William J. Caspary, ETP/TOB

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**NATIONAL TOXICOLOGY PROGRAM CONCEPT REVIEW**
**CONCEPT TITLE:** Mechanisms of Chemical Toxicity
**PRESENTER:** Dr. William J. Caspary, ETP/TOB

## OBJECTIVES

The objective of this contract effort is to identify chemicals that induce specific endpoints or modulate various pathways by which chemicals induce cancer. This effort will identify chemicals that induce toxicity under different metabolic activation systems, chemicals that induce DNA damage and mutations in mammalian cells, and chemicals that modulate the cell cycle and apoptosis. All chemicals will be tested first in a bacterial assay system (e.g., *Salmonella typhimurium*) using various metabolic systems. Most of these chemicals will also be tested for their ability to induce mutations and micronuclei in either L5178Y mouse lymphoma or TK6 mammalian cells and for their ability to modulate the cell cycle and apoptosis. A select number of chemicals will also be tested for their ability to induce DNA damage in cultured mammalian cells. For selected chemicals that induce mutations in mammalian cells, the types of DNA lesions will be identified.

## BACKGROUND

Historically, the ETP has administered an extensive program to test chemicals for mutagenic activity using bacterial and mammalian cell systems. One of these, the *Salmonella typhimurium* mutation assay identifies chemicals that induce point mutations. Since this assay is unable to detect chemicals that produce exclusively other types of genetic damage such as chromosomal aberrations and recombination, the ETP has also used heterozygous mammalian cell mutation assays such as the L5178Y mouse lymphoma and TK6 mutations assays to detect chemicals that induce a broad array of mutations including chromosomal aberrations and recombination.

Mutations leading to cancer can be either point mutations or recombination events. These mutations amplify, modify or abrogate protein function. Mutations, in themselves, do not cause cancer or other forms of toxicity. Mutations have to be expressed and the modified protein products must act (or the missing protein must fail to act) on some biochemical function to effect a toxic response. For neoplasia, two targeted pathways are the cell cycle and the apoptotic response to chemical-induced damage.

## CONCEPT PROPOSAL

### Metabolism

Many chemicals do not elicit a mutagenic effect in bacteria in the presence of liver homogenate. However, limiting testing to preincubation of a chemical with a liver homogenate, the S9 fraction containing cytochrome P450 metabolizing enzymes, does not elicit information

on the ability of other activating systems, such as intestinal activation or conjugation, to express a chemical's mutagenicity such as a glutathione-S-transferase activation system. By linking different metabolic activation systems to a bacterial mutation assay and/or by predicting metabolic pathways by structural analysis, the ETP will both identify the mutagenic activity of chemicals in these bacterial systems and the activation conditions responsible for a chemical's mutagenic activity. This contract effort will examine the mutagenic activity of chemicals in a bacterial system using several activation systems.

**Mammalian Cell Mutagenesis**

By using cultured mammalian cells, the ability of a chemical to induce a wider spectrum of mutations than can be measured in bacteria can be assessed. These include point mutations, chromosomal mutations and premutagenic lesions such as DNA damage. These are measured by resistance to a selective agent, by the formation of micronuclei, by visualization of DNA extruded from cells (comet assay) and by the use of molecular techniques to identify point mutations and recombinational events.

In this contract effort, chemicals will be tested in either the L5178Y mouse lymphoma or TK6 mammalian cell line to detect a wide spectrum of chemical-induced mutagenic damage, from point mutations to recombinations at a specific gene. For a few chemicals, molecular techniques will be used to assess the spectrum of the mutations in the mutant colonies. Sequencing the mutated gene can determine the intragenic mutations that occurred. LOH and FISH analysis will provide information on recombination.

The micronucleus assay will be routinely used to assess whether chemicals can induce chromosome abnormalities in mammalian cells in culture. Treatment of micronuclei with antibodies specific for chromosomal centromeres (e.g., CREST antibody reaction, anti kinetochore antibody, specific chromosome probes) will distinguish between those micronuclei containing centromeres from those containing acentric chromosome fragments.

This contract effort will use single cell gel electrophoresis to identify chemicals that induce DNA damage (single- and double-strand breaks, oxidative-induced base damage, and DNA-DNA/DNA-protein crosslinking).

**Cell Cycle and Apoptosis**

The cell cycle is the cellular program for growth and division. The two cell division events that the cell carefully controls are entry into S-Phase during which DNA replication occurs and entry into mitosis during which chromosomal condensation occurs. Apoptosis is a mechanism by which an organism destroys cells that represent a threat to its integrity. Apoptosis is genetically controlled.

This contract effort will identify chemicals that modulate the cell cycle and affect apoptosis.

## SIGNIFICANCE AND PRACTICAL USES OF RESULTS

*Scientific significance:*

Cancer is a genetic disease in that mutations at critical genes ultimately contribute to tumor formation. The genes identified to date that are involved in the etiology of cancer influence DNA damage and repair, regulate cell proliferation and death, guard genomic integrity or express enzymes that modulate exposure to or metabolism of chemicals. This work provides a snapshot of selected chemical-induced lesions that may be involved in the toxicity of a chemical. Measurement of these endpoints identifies compounds that have the potential of causing genetic damage and cell cycle disruptions and provides information on potential mechanisms leading to cancer.

*Program Significance:*

Exposure to chemicals encountered in the environment, work-place or food supply can have profoundly negative impacts on human health. The NTP seeks to assess risks associated with possible acute, repeated or chronic exposure to chemicals by investigating a variety of biological effects including carcinogenicity induced on chronic exposure. The information provided by these studies of mechanisms of toxicity also facilitates the extrapolation of potential hazard to groups and/or classes of chemicals and thus extends the knowledge gained from individual studies. It also provides some quantitative insights that can help in risk assessment and risk management. Data from these cellular studies provide information on possible genetic mechanisms leading to neoplasia of NTP chemicals and thereby supports the NTP bioassay program. Thus, chemicals that generate genetic damage and possible mechanisms can be identified. In addition, metabolites and structural analogs of NTP chemicals can be examined which may mitigate or stimulate a need to conduct cancer bioassays on these compounds.

## AVAILABILITY AND ADEQUACY OF METHODOLOGY AND TECHNOLOGY

In vitro assays for all the endpoints are available. Metabolism will be examined by linking various activation systems to a bacterial test system for mutagenesis (e.g., the Ames test). Mammalian cell mutation assays such as the mouse lymphoma L5178Y or human TK6 mutation assays are available and have been used by this program in the past. DNA sequencing protocols and assays for measuring recombinations in mammalian cells using LOH analysis and FISH have been published and are widely used. Protocols for both micronucleus assays and the comet assay are readily available, as are protocols for measuring the disruption of the cell cycle and apoptosis.

## PROPOSED CHANGES TO THE CURRENT WORK STATEMENTS:

Not applicable. This is a new effort.