NATIONAL TOXICOLOGY PROGRAM

BOARD OF SCIENTIFIC COUNSELORS

June 29, 1995

Summary Minutes

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SUMMARY MINUTES
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The National Toxicology Program (NTP) Board of Scientific Counselors (the Board) met on June 29, 1995, at the National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina. (Attachment 1: *Federal Register* meeting announcement; Attachment 2: Agenda and Roster of Members.) Members of the Board are Drs. Curtis Klaassen (Chairman), Arnold Brown, Elaine Faustman, David Hoel, Claude Hughes, Fumio Matsumura, Franklin Mirer, Kenneth Reuhl, John Stegeman, and Peter Working. Expert Consultant to the Board is Dr. Hiroshi Yamasaki. All were present except Dr. Faustman.

I. Report of the Director, NTP: Dr. Kenneth Olden, Director, NTP and NIEHS, discussed the status of the FY 1996 NIEHS and NIH budgets, noting that the House and Senate conferees had proposed a 1% decrease (from 1995) in the NIH budget for 1996 and an additional 3% decrease (from 1996) in FY 1997 with the budgets from 1998-2002 to remain at the 1997 level. Since this does not account for inflation (~ 4%/year), the decrease in 1996 would really be about 5%, a bleak outlook indeed. Dr. Olden said that a retreat of Institute Directors with Dr. Varmus and other senior NIH staff was scheduled for September at which in light of budgetary problems there may be discussions of different ways to fund research and training. As an example of the impact on the 1995 NIEHS budget, he said that of 201 new and competing grant applications recommended for NIEHS, only 18 could be funded.

Dr. Olden said he was pleased to announce that Dr. Varmus had approved the selection of Dr. Carl Barrett as Scientific Director on June 23. He stated that Dr. Barrett had already provided outstanding leadership as Acting Scientific Director. Dr. Olden reported that the search committee for the position of Director, Environmental Toxicology Program, had presented to him two finalists who were being interviewed by laboratory chiefs, NIEHS Assembly of Scientists, and the NIEHS Women Scientists group. He expected to make his choice in the near future and since this is a Senior Executive Service (SES) position final approval would be by Dr. Varmus.

Dr. Olden announced that a new advocacy group for environmental health research had been established. The Environmental Health Foundation is based in Phoenix, Arizona, and is intended to play a role analogous to the American Cancer Society with cancer research. Their primary objective would be lend support and obtain funding for environmental health sciences research. He said he and others had met recently with the Foundation to provide input in helping them establish a research agenda.

Dr. Olden commented on the recent report of the review of the National Cancer Institute (NCI) by a panel co-chaired by Drs. Michael Bishop and Paul Calabresi. He said the Director, NIH, may have other institutes undergo similar reviews. Dr. Olden said that in the near future we would form our own external review committee to help us ensure that we are addressing the right environmental health problems. The Chairs of the NTP and NIEHS Boards would be asked to serve on this committee. Dr. Olden reported that the annual NIEHS Leadership Retreat would be held at summer's end and like last year's, outside persons including members of our advisory boards would be invited to participate, as outside input was quite valuable. Dr. Olden thanked Dr. Arnold Brown for chairing the meeting of the Board's *ad hoc* working group in their April review of the criteria for inclusion of substances in the *Biennial Report on*

Carcinogens (BRC), noting the openness of the review process and the opportunity for a balance of views to be considered.

Dr. Olden announced that we may have approval of a new program for Senior Biomedical Research Scientists (SBRS) which will make NIH more competitive in attracting and retaining outstanding senior scientists. He said the NIEHS had received approval for seven of these positions and has selected three intramural scientists to go forward for NIH-wide approval as SBRS.

During the meeting, Dr. Olden presented certificates and acknowledged the contributions of retiring members of the Board: Dr. Hughes, Dr. Klaassen, Dr. Matsumura, and Dr. Working. In addition to serving as Chair, Dr. Klaassen had served concurrently on the Technical Reports Review Subcommittee where he also had served a term as Chair of that group.

II. Report of the Director, Environmental Toxicology Program (ETP), NIEHS: Dr. George Lucier, Director, ETP, provided the Board with an update on a number of completed, ongoing, and planned activities and initiatives of importance to the NTP and the NIEHS. These included:

- Workshops: A number had been held or would be held in 1995, including the "NTP Workshop on Mechanism-Based Toxicology in Cancer Risk Assessment" in January, the NTP-sponsored workshop on "Principles of Dose Selection for NTP Studies" at the Society of Toxicology meeting in March, the Board's *ad hoc* working group on criteria for the BRC in April, and a workshop on "Validation and Regulatory Acceptance of Toxicological Test Methods" proposed for the Fall.
- Continuing to attain proper balance in our programs, such as studies of cancer vs. noncancer endpoints, between chronic testing and mechanistic studies, and improving balance between rodent studies and obtaining information in humans.
- Research grants that would complement more applied intramural and contract studies. Most recently, the NIEHS Council had approved the use of R03 grants to engage the extramural community in performing more mechanistic studies with animals or materials from NTP bioassays or short-term studies on high-priority chemicals. Awards resulting from an RFA for research in Mechanistically-Based Alternative Methods in Toxicology were now being made .
- Chemical Nomination and Selection: The NTP continues actively to seek nominations of chemicals for study through large mailings and other methods. He noted that for 16 of the top 50 production chemicals in the U.S., there is virtually no toxicity data.
- Environmental Hormones: We are continuing our efforts to look at multigenerational effects of this large class of 'endocrine disrupters,' especially with regard to reproductive and developmental and carcinogenic effects (the Board had approved a concept at the last meeting). Dr. Lucier cited specific examples.
- Issue of Mixtures: We are trying to develop tractable approaches to studying mixtures and would welcome any input.
- Transgenic Initiative: The Board had been sent a copy of the proposal whereby transgenic animals will be evaluated as alternatives to the bioassay in studies with genotoxic and nongenotoxic carcinogens and noncarcinogens to determine sensitivity and specificity of transgenics. Comments are welcomed.
- Newsletter: He noted we had begun sending out a newsletter several times a year and asked for suggestions as to items that might be added.

III. Report of the NTP Workshop on "Mechanism-Based Toxicology in Cancer Risk Assessment: Implications for Research, Regulation, and Legislation": Dr. Lucier said the overall goals of the workshop held January 11-13 were: (1) to assess the scientific foundation for using mechanism-

based toxicology to address critical issues in risk assessment; (2) to identify and propose solutions to the regulatory problems which may emerge by the use of mechanistic toxicology in conducting risk assessments; and (3) to determine the applicability of mechanism-based toxicology in conducting risk assessment to current legislative issues. He said there had been concern that noncancer endpoints were not included but it was felt to do so would dilute the effort and preference would be given to look at other forms of toxicity in separate workshops. Dr. Lucier said the workshop began with a plenary session to define the issues, followed by breakout sessions with five workgroups reflecting the five uses of mechanistic toxicology: (1) for screening chemicals and setting priorities for carcinogen testing; (2) in hazard identification; (3) for determining dose-response relationships; (4) for species extrapolation; and (5) for determination of distributions of risk. The workgroups presented their findings and recommendations in another plenary session and the workshop closed after a public session. Dr. Lucier summarized the overall recommendations from the workshop and gave examples of how NTP is implementing them:

(1) Mechanistic and risk assessment considerations should be incorporated into all aspects of chemical selection, experimental design, and data analysis.

(2) Less expensive, faster, and more accurate methods for setting priorities and providing toxicological evaluations need to be developed and validated.

(3) The NTP should continue long-term bioassays which provide valuable information, and NTP and regulatory reliance on them should diminished only when alternative methods are appropriately validated.

(4) Acquisition of molecular and biochemical data from exposed populations is critical for risk assessment. The NTP should improve the linkage between experimental toxicology and molecular epidemiology.

(5) The NTP, working closely with stakeholders, should develop guidelines for achieving regulatory acceptance of mechanistic information in risk assessment. These guidelines should be flexible in order to accommodate the wide variety of mechanisms likely involved in chemical carcinogenesis, the recognition that our knowledge of mechanisms will never be complete, and the continued evolution of our scientific knowledge.

(6) The NTP should play a more active role in coordinating overall approaches to incorporate mechanistic information in risk assessment including sponsoring workshops on specific topics.

In conclusion, he said we need to develop chemical-specific strategies for providing toxicological evaluations, and then use the knowledge gained in development of biological and risk assessment models. Comments will be welcomed until mid-July and a final report will be prepared by the end of July and sent to all invited participants for any further comments. A meeting report will be published in *Environmental Health Perspectives.*

Discussion: Dr. Henry inquired about which of the many recommendations the NTP would implement. Dr. Lucier noted that we were strongly committed to mechanism-based toxicology which underlies many of the recommendations. He said we need to do a better job of improving the linkage between experimental toxicology and molecular epidemiology. Also, we need to do a better job of involving our diverse stakeholders. Dr. Hughes stressed the need for linking clinical molecular endocrinology and laboratory animal toxicology, observing that he has been involved in this and these linkages really can work well.

IV. Report on the Meeting of the *Ad Hoc* Working Group to Review Criteria for Listing of Substances in the *Biennial Report on Carcinogens* (BRC): Dr. Arnold Brown, Chair of the *ad hoc* Working Group, made note of the wide representation of participants from academe, industry, labor, public interest, environmental groups, State government, and representatives of the NTP

Executive Committee. He said that the level of discussion in the three breakout groups was vigorous yet the discourse was polite. He thanked all participants for a productive meeting, and especially Drs. Jameson and Lucier for their leadership.

Dr. C. W. Jameson, NIEHS, cited the 1978 legislative authority for publishing the *Annual Report on Carcinogens* (ARC), which was amended in 1993 to the *Biennial Report on Carcinogens* (BRC), and said there have been seven reports published to date. He read the current criteria (Attachment 3) used for selecting agents for inclusion in the seven volumes under the categories of "Known to Be Carcinogens" or "Reasonably Anticipated to Be Carcinogens." Dr. Jameson emphasized that the ARCs were geared to identify hazard, to alert the public to possible carcinogenic risk, and did not constitute a risk assessment. He said the Board had passed a resolution on April 6, 1994, supporting the formation of a new Board subcommittee which would be involved in the review process for agents nominated for inclusion in the BRC. Further, the new subcommittee would begin by convening a working group to review the criteria for listing in the BRC. Because of the time needed to establish the new Subcommittee and the urgency to review the criteria for listing substances in the BRC, an independent *ad hoc* Working Group of the NTP Board was established to review the criteria. The review of the criteria was the focus of the current Board discussion. Dr. Jameson then reported on the meeting of the *ad hoc* Working Group of the Board held in Washington, D.C. on April 24-25. The 45-member Working Group divided into three breakout groups in order to consider two issues: (1) the adequacy of existing criteria for listing substances in future Reports; and (2) the incorporation of mechanistic data as part of the criteria for listing substances in future Reports which may include the consideration of sensitive sub-populations as well as procedures to upgrade or downgrade the evaluation of the results of animal bioassay or epidemiology studies. As this was an open meeting, comments were received from the public ranging from "retention of current criteria with no change," to "minor revision of existing criteria to incorporate mechanistic information," or "major revision of existing criteria to incorporate all available mechanistic data." Dr. Jameson reported that the main discussion in the breakout groups was concerned with the degree of prescription. A majority of the members of the groups felt the criteria: (1) should be revised; (2) should include mechanistic information; (3) should not be overly prescriptive; (4) should not add additional categories; and (5) should not substitute for expert judgment.

Proposed Revised Criteria: Dr. Jameson said that based on the Working Group discussions and recommendations, the NTP staff composed proposed revised criteria, and he presented a comparison between the current criteria (Attachment 3) and proposed revised criteria (Attachment 4). Under Category 1 - Known to Be Carcinogens, the word "Human" was inserted before "Carcinogens," and in the criteria, "substance" replaced "agent." The Working Group stressed that listing under Category 1 should be based on human data only. Under Category 2 - Reasonably Anticipated to Be Carcinogens, the word "Human" was again inserted before "Carcinogens," while under 2.a., there were no changes. There were major changes proposed in the 2.b. criteria including: (1) inclusion in the first sentence between "malignant" and "tumors" of "and/or combined benign and malignant"; (2) changing "(a) in multiple species or strains" to "(a) in multiple species or at multiple tissue sites"; (3) changing "(b) in multiple experiments (preferably with different routes of administration or using different dose levels)" to "(b) by multiple routes of exposure"; and (4) the last sentence of 2.b. in the current criteria was removed and added to an explanatory paragraph to follow 2.a. and 2.b. (Attachment 4). Dr. Jameson presented two additional recommendations by the Working Group, one being that there should be a formal delisting procedure established, and secondly, that the NTP should foster discussion on use of mechanistic data in toxicology and risk assessment. He noted the latter was already being effected as reported by Dr. Lucier. In closing, Dr. Jameson said that following the Board

review, the revised criteria would be reviewed by NTP Executive Committee Working Group for the BRC, then by the PHS' Environmental Health Policy Committee, and finally by the NTP Executive Committee. The final recommendations will be presented to Dr. Olden for submission to the Secretary, DHHS.

Board Discussion: Dr. Klaassen posed the question that if the only animal tumor data was $\alpha_{2\mu}$-globulin-associated renal tumors in male rats would the chemical be listed. Dr. Jameson said it would not be listed as covered under the last sentence of the Explanatory Paragraph. Dr. Klaassen asked whether a chemical could be upgraded based on mechanistic data, and Dr. Jameson responded that the third sentence in the Explanatory Paragraph covered this, i.e., there may be substances for which there is less than sufficient evidence of carcinogenicity in humans or laboratory animals but for which there are compelling data indicating that the substance could reasonably be anticipated to cause cancer in humans. Dr. Klaassen asked about the use of data from transgenic animals in the listing process. Dr. Lucier said there were some who think transgenics may be too sensitive in detecting potential carcinogens. Their predictivity must be validated with chemicals for which there is good bioassay data for carcinogenicity and noncarcinogenicity. Dr. Goldsworthy said definition must be made of what constitutes "compelling data," while Dr. Hoel asked for examples of compounds where there was mechanistic data that would support their being listed. Dr. Lucier replied that this was an example of where the Working Group was trying not to be overly prescriptive, and added that there had to be scientific judgment entering in, such as would be provided by the new BRC Subcommittee of the Board. Dr. Lucier stated that a point of discussion might be whether there could be compelling mechanistic data that would allow a chemical to be classified a human carcinogen even though it may lack good epidemiologic data. Dr. Carl Barrett, NIEHS Scientific Director, contended that a chemical or agent could be placed in Category 1, lacking convincing epidemiologic evidence, if there was a consensus of experts that available mechanistic data strongly supported the chemical being a human carcinogen. Dr. Matsumura wondered why 'chemical metabolism' as a factor was not spelled out in the Explanatory Paragraph. Dr. Lucier said if there was a consensus for this it could be put in the criteria. Dr. Mirer thought that less weight was given to benign tumors in the revised 2.b. than in the levels of evidence used in assessing carcinogenicity in NTP Technical Reports, and asked for consistency. Dr. Stegeman asked what kind of mechanistic data would be used to downgrade (delist) a chemical. Dr. Lucier said a single tumor site in a single experimental group with a discrete chemical where the mechanism was not applicable to humans would qualify, e.g., α2u-globulin associated with renal tumors in male rats exposed to *d*-limonene. Dr. Henry said she was surprised that there was not more support for adding subcategories to Category 2. She was troubled by the "combined benign and malignant" addition and also by lack of discussion about tobacco, alcohol, and ionizing radiation. Dr. Barrett said the intent of the addition was to be more consistent with current NTP practices. Regarding replacement of "agent" with "substance," Dr. Hughes preferred "agent" as it was more inclusive. He thought the wording of the second sentence of the Explanatory Paragraph should be stronger in support of what goes into scientific judgment. Dr. Lucier responded that both points should be discussed by the Board. Dr. Working said the issue is not whether mechanistic data are used but rather how they are used. Dr. Mirer commented that we can't use mechanistic data under the current process. Dr. Lucier said that a purpose of adding another level of peer review of nominations, i.e., a BRC Subcommittee, would be to aid in evaluating mechanistic data. Dr. Barrett said the sentence pertaining to scientific judgment was carefully crafted as part of not making the criteria overly prescriptive and formulaic.

Public Comments: Dr. Klaassen announced that there had been requests by six individuals for time to make formal comments.

(1) Dr. Charles Axten, North American Insulation Manufacturers Association (NAIMA), stated that the current criteria do not permit listings to be based on a balanced review of all the data, and, therefore, NAIMA commends the NTP for undertaking this review. Although the proposed revisions are a step in the right direction, they recommend the following additional changes: a) In the second sentence of the Explanatory Paragraph, replace "may" with "should"; b) Make the standards equivalent for upgrading and downgrading classification of a substance; c) Change the requirement for "compelling data" to upgrade or downgrade to "weight of the evidence"; d) Create a new category in the BRC for "animal carcinogens not likely to be human carcinogens"; and e) The criteria should include a specific provision for delisting substances.

(2) Dr. George Cruzan, ToxWorks, representing the Styrene Information and Research Center (SIRC), said that SIRC supports the use of relevant mechanistic data when there are defined guidelines in the application of such data to evaluation of carcinogenic potential. Their concern has to do with what constitutes "compelling data," and he noted that individuals and groups differ on this, leading to inconsistent classifications. Therefore, SIRC urges the NTP to propose guidelines for determination of "compelling data," to solicit broad review and comment, and to accept a scientific consensus.

(3) Dr. Clay Frederick, Rohm & Haas, representing the American Industrial Health Council (AIHC), commented that AIHC supported clarification of the role of the BRC as a hazard identification document, noting that prior ARC/BRCs referred to listing as an initial step in hazard identification but were equivocal as to whether this was a big step or little step. He said the AIHC supports the use of mechanistic data in the listing criteria as integral to hazard identification. Dr. Frederick said that AIHC had concerns about 'Accuracy in Labeling' in use of mechanistic data for listing in the absence of a positive bioassay or human data, and proposed that such a listing be placed in a separate category with an appropriate description of the accuracy of the carcinogenicity prediction performance. In discussion, Dr. Mirer asked whether AIHC would support listing of a chemical based only on mechanistic information. Dr. Frederick answered affirmatively, citing benzidine dyes as a case in point, but only under a separate category. Dr. Barrett said a more likely situation might be where there is some animal data, but not as a standard bioassay, along with mechanistic data. This would be a situation where scientific judgment becomes important.

(4) Dr. John Keller, representing the International Society of Regulatory Toxicology and Pharmacology (ISRTP), stated that the BRC listings are blindly or intentionally misused by Federal and State regulatory agencies with no distinction made between known, probable, or possible carcinogens. He opined that the BRC and International Agency for Research on Cancer (IARC) connection needs to be decoupled. With regard to the Working Group meeting, Dr. Keller suggested that the five categories proposed by breakout group 2 and accompanying definitions be endorsed by the Board. Finally, he said the NTP should accept recommendations of the NAS/NRC committee on risk assessment that listing of substances in the BRC should be based on a weight-of-evidence approach to hazard identification.

(5) Dr. Loretta Schuman, Occupational Safety and Health Administration (OSHA), stated that OSHA does not support the revised criteria as proposed by the NIEHS/NTP and agrees with the majority of the Working Group members that only minor revisions to the current BRC criteria are needed and the criteria must support complete flexibility in use of <u>all</u> scientific evidence to arrive at the best scientific judgment. She said it was recommended that mechanistic data be included as only <u>one</u> type of additional information. Dr. Schuman said OSHA agreed with Categories 1 and 2.a., but not with 2.b. and the Explanatory Paragraph, which were overly prescriptive and did not allow the most flexibility to consider all available information. Dr. Stegeman asked whether a majority opinion was obtained by polling all the members of the NTP Board and *ad hoc* Working Group. Dr. Schuman replied that two of the three breakout groups recommended only minor revisions. Dr. Jameson reported that the summary meeting report

was sent to all 45 members and only six did not agree with it. In response to queries about the meaning of "complete flexibility," Dr. Schuman agreed that this was equivalent to scientific judgment.

(6) Dr. James Sherman, Monsanto, representing the Chlorobenzene Producers Association (CPA), said the CPA supports the direction of the Working Group review, especially as it gives more emphasis to inclusion of mechanisms of action and to the importance of scientific judgment. They recommended that the Board accept the proposed revised criteria after the following changes are made: a) In the second sentence of the Explanatory Paragraph where it is indicated that "Consideration may be given" to mechanistic data, this should be changed to "Consideration should be given"; b) The criteria should not create unreasonably high thresholds that mechanistic data must satisfy before being considered in the decision-making process as implied by "compelling data," since mechanism data can never show a substance is not a carcinogen; and c) The proposed criteria do not recognize the role of mechanism data in determining when animal data constitute "sufficient evidence" for predicting human risk, and conversely, when mechanism data demonstrate an animal study is not predictive of human hazard to permit downgrading from "sufficient" to "limited evidence."

Dr. Bryan Hardin, NIOSH, a member of the *ad hoc* Working Group and facilitator for breakout group 1, commented that the Meeting Summary leaves the impression that the current criteria were inadequate when in fact two of the three breakout groups recommended only minor changes. He thought there may be an overemphasis on mechanistic data possibly leading to a situation whereby if the mechanism isn't known, a substance would not be listed. He opined that the Explanatory Paragraph was too long and complex, and noted that his group suggested adding the following sentence at the end of 2.b.: "Additional evidence relevant to hazard identification may be used to influence the scientific judgment regarding sufficiency of evidence of human carcinogenicity."

V. Further Discussion and Development of Recommendations by the Board Concerning the BRC Selection Criteria: Dr. Klaassen opened the session by asking if a member would make a motion concerning inclusion of wording pertaining to mechanism studies in the criteria. **Dr. Brown moved that mechanistic information should be included in the selection process for agents to be listed in the BRC. Dr. Hughes seconded the motion.** In discussion, Dr. Yamasaki commented that mechanistic information has been used all along as part of an evolving process. Dr. Mirer said consistent decision rules cannot be followed concerning the use of mechanistic data in formation of the BRC. Dr. Matsumura said he supported inclusion of mechanistic data. Dr. Henry also was supportive but said this must be combined with a commitment by the agencies to provide additional resources. Dr. Klaassen agreed and asked if the NTP would allot additional resources. Dr. Lucier answered affirmatively, noting that a new Subcommittee of the Board was being formed to provide peer review, including evaluation of mechanistic data. As to resources, the recent reorganization of the NIEHS intramural programs was helping to maximize resources. Dr. Brown stressed that if mechanistic data are not included we do not have a contemporary document. **The Board voted unanimously to accept the motion.**

Dr. Hughes moved that the listing criteria be revised. Dr. Henry seconded the motion. The Board voted unanimously to accept the motion. Dr. Brown moved that the number of categories be left as in the proposed revised criteria. Dr. Hughes seconded the motion. In discussion, Dr. Matsumura said he favored adding another category to account for chemicals "reasonably anticipated" based on data from transgenic animals and other nontraditional assays. Dr. Barrett said it was not the intent of the BRC to classify every chemical. Rather, the review subcommittee might suggest more mechanistic or animal studies

be done. Dr. Stegeman thought it would be desirable to have a listing of candidates that are possible or probable carcinogens which might stimulate research. Dr. Lucier agreed such a list could help in setting priorities for study but if comprehensive would add significant resource cost. Dr. Barrett said it was our intent to open up the process for nominations of substances for listing. Dr. Henry said if our intent is to inform the public then this additional category might be useful. **The Board voted by seven yes to two no votes (Henry, Matsumura) to accept Dr. Brown's motion. Dr. Mirer moved that the Board recognizes that the process of changing the criteria including use of mechanistic information will require expanded review and resources and supports this. Dr. Henry seconded the motion, which was accepted unanimously. Dr. Brown moved that an explicit process be defined and described in the BRC for listing or delisting of an agent. Dr. Reuhl seconded the motion, which was accepted unanimously.**

There then ensued a discussion about the Explanatory Paragraph. Dr. Stegeman observed that this paragraph is really a description of the process. **Dr. Stegeman moved that the Explanatory Paragraph be modified in light of comments by the Board and public. Dr. Brown seconded the motion.** Dr. Lucier said since other governmental committees would be evaluating the revisions we needed guidance on specific parts of the paragraph that the Board would like to see modified. Dr. Mirer suggested that a revised paragraph be sent to the Board for their review. Dr. Henry said that since it was not part of the criteria and perhaps part of the process, the paragraph could be used as a preamble. Dr. Barrett said it could be added to or replace the current Note to the Reader. Dr. Brown stated that there seemed to be considerable support for changing the wording of the second sentence from "Consideration <u>may</u>..." to "Consideration <u>should</u>, or <u>must</u>..." Dr. Mirer said he could not support that change. Dr. Goldsworthy said that all of the factors in the second sentence entered into scientific judgment and suggested that the first sentence and beginning of the second sentence be merged as follows: "Conclusions regarding carcinogenicity in humans or experimental animals <u>will</u> be based on scientific judgment, with consideration given to all relevant information." There was discussion around leaving this sentence, along with perhaps some specific examples of "relevant information," with the criteria while placing the rest of the Explanatory Paragraph in a preamble. Dr. Barrett concluded that the Board had endorsed four fundamental principles including that scientific judgment is required, that mechanistic data is important, and that the data can be used to upgrade or downgrade a listing. The larger paragraph could be placed in a preamble which could be modified as needed in response to scientific knowledge. Dr. Barrett stated that the NTP would revise the criteria and paragraph to reflect the principles expressed. **The Board voted by eight yes votes with one abstention (Mirer) to accept Dr. Stegeman's motion.**

Dr. Mirer moved that the wording of Category 1 be modified as follows (additions underlined): "There is sufficient evidence of carcinogenicity from studies in humans that indicates a causal relationship between <u>exposure to</u> the agent, <u>substance or mixture</u> and human cancer." Dr. Brown seconded the motion, which was accepted by five yes votes (Brown, Hoel, Hughes, Mirer, Stegeman) to four no votes (Henry, Matsumura, Reuhl, Working). Dr. Goldsworthy reiterated that specific examples of "relevant information" should be retained along with the modified first sentence for the criteria to give the reader some guidance. Dr. Lucier said the criteria revised to reflect the Board's actions along with a proposed Preamble would be FAXed to members for comments and approval or disapproval. Dr. Klaassen announced that members of the public could also receive this material on request. **Dr. Hughes moved that the Board endorse inclusion of**

"Human" in the titles of categories 1 and 2. The motion was seconded and accepted unanimously.

VI. Report on Technical Reports Review Subcommittee Activities: Dr. Gary Boorman, NIEHS, reported briefly on the Subcommittee meetings of November 29, 1995, and June 20-21, 1995, in which the draft Technical Reports for 12 long-term toxicology and carcinogenesis studies were peer reviewed. He noted that four of these studies were by the inhalation route, four by dosed feed, two by dermal exposure, and two by gavage, contrasting with studies earlier in the Program where many more studies were by the gavage route. Of the 12 studies, five were overall negative and seven were overall positive. There were not many differences between the sexes and species in tumor responses. Dr. Boorman said that current study designs are incorporating more mechanistic considerations.

VII. Chemicals Nominated and Recommended for Study by the Interagency Committee for Chemical Evaluation and Coordination (ICCEC): Dr. Errol Zeiger, NIEHS, described the chemical nomination and selection process, noting that after the ICCEC makes testing recommendations with priority, the chemicals are brought to the Board for comments and recommendations prior to being presented to the NTP Executive Committee for action. Dr. Zeiger reported that the ICCEC recommended five chemicals for study at their meeting on December 14, 1994. The chemicals with ICCEC recommendations and supporting information are listed in Attachment 5. In discussion, Dr. Zeiger explained that in studies on arsenic trioxide, we would propose to take the available data and conduct mechanistic studies, including looking at low doses and developing human biomarkers. Dr. Klaassen stated that this research is important as many drinking water supplies, especially in the western U.S., have arsenic in them, and suggested NTP contact the American Water Works Association regarding their research plans. Dr. Mirer asked that a listing of nominations over the past 10 years or so and the status of these nominations be prepared for the Board. Dr. H. B. Matthews, NIEHS, gave an update on our efforts to determine the most important chemicals for study by the NTP resulting from our review of a large number of exposure-related databases.

VIII. Concept Review, ETP, DIR, NIEHS:

***In Vitro* and *In Vivo* Genetic Toxicity Testing** — (Attachment 6) Dr. Errol Zeiger, NIEHS, presented the concept, and Dr. Peter Working, Board member, served as principal reviewer. Dr. Zeiger said the extensive NTP genetic toxicology databases compiled over many years are considered benchmarks by scientists around the world. Currently, the two primary assays are the *Salmonella* test and the *in vivo* micronucleus test in rat and mouse bone marrow. He said individual assays had been brought for concept review in the past, which is not very efficient. Dr. Zeiger stated that the aim of this concept proposal was to provide better integration and expansion of the NTP genetic toxicology program and development of more sensitive measures of genetic toxicity. He proposed using the current assays while evaluating and adding new systems, e.g., tests for aneuploidy, the single cell gel assay, and use of transgenic rodents.

Dr. Working noted that the proposed activity is an extension and modification of an ongoing and productive program that has demonstrated its scientific and technical merit and significance, and as such is worthy of support by the NTP. He said the proposal to add flexibility with new assays could be both good and bad, and said it was important that sufficient validation of new endpoints be incorporated into the study design to ensure that the results are scientifically valid and relevant. For example, use of the single-cell assay might be an example of premature incorporation of a procedure. He advised that the Board or a panel of genetic toxicologists be asked to help evaluate usefulness of particular assays before incorporation. Dr. Working

recommended approval of the concept. Dr. Yamasaki emphasized the importance of incorporating studies of chromosome changes in human cells. Dr. Working moved that the concept be approved. Dr. Brown seconded the motion, which was approved unanimously by the Board.

IX. Alternative Methods - Status and Plans:

(1) RFA for Research in "Mechanistically-Based Alternative Methods in Toxicology" — Dr. William Stokes, NIEHS, said the RFA was issued in October 1994 and the purpose was to foster the development, validation, and use of mechanistically-based methods and models for toxicology research and testing that either do not require the use of animals, that reduce the use of animals, or that involve the use of alternatives such as non-mammalian species. He said there had been 94 applications received. There were three priority areas — carcinogenicity/mutagenicity, reproductive/developmental toxicity, and neurotoxicity. Eleven grants were awarded to be effective July 1, 1995, and these were distributed with five in the area of carcinogenicity/mutagenicity, three in reproductive and developmental toxicity, and one each in immunotoxicity, nephrotoxicity, and computer modeling.

(2) Small Grants Program for Research on National Toxicology Program Chemicals — Dr. Stokes said the purposes of this new program were to broaden the scope of NTP contract studies by including additional endpoints that will address risk assessment and mechanistic questions, and to utilize the scientific expertise of the extramural community in the NTP testing process. The program would work through the RFA mechanism and be targeted to specific chemicals with the award per investigator being $50,000 for one year. The process would involve the NTP developing the protocol for a specific contract study. During the request for contract and award period, an RFA would be developed to request projects (R03s) that can utilize animals, sera, or tissues from the NTP contract study to answer questions related to dose-response analysis, metabolism/disposition, biomarker development, sex and species differences, and mechanism of action. Dr. Stokes concluded by noting that the model for this program was the collaborative study between NTP and the Health Effects Institute on ozone. There were questions as to how much could be expected from the modest funding. Dr. Stokes said these grants would allow investigators to study a chemical using a particular technique or by incorporating it into a type of research that was already ongoing. Dr. Lucier saw these grants as especially valuable where the Program would want toxicokinetic information or biologically-based dose-response models. Dr. Klaassen said he thought these new NTP-related grant programs were examples of where suggestions from the Board had positively influenced the Program.

(3) Proposed Workshop on "Alternative Test Methods in Toxicology: Validation and Regulatory Acceptance" — Dr. Stokes reported that the NIEHS had received a mandate under the NIH Revitalization Act of 1993 which basically incorporated ongoing NTP activities in this general area. Among the activities NIEHS was directed to carry out was to establish criteria for the validation and regulatory acceptance of alternative testing and to recommend a process through which scientifically validated methods can be accepted for regulatory use. In response, an *ad hoc* Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) was established. Dr. Stokes reviewed the composition and goals of ICCVAM. The Committee has met almost monthly over the past year and has obtained relevant information from the public via a *Federal Register* notice. Existing agency validation and regulatory acceptance criteria and processes were obtained from a survey of the 14 participating Federal agencies and programs. A preliminary draft report is being prepared, and will form the nucleus of a workshop tentatively scheduled for October 30-31, 1995, at NIH. The aims of the workshop are to obtain comments and recommendations that will strengthen usefulness of the report for scientists in government agencies, industry, and academe, and that are relevant also to effective

implementation of the procedures and criteria described in the report. A final report will be developed after the workshop. Dr. Olden commented that because of possibly not having a budget in place at the beginning of the FY 1996, the workshop might have to be postponed from the proposed October 30-31 dates. (Ed. Note: The ICCVAM Workshop will be held on December 11-12, 1995, at the Crystal Gateway Marriott, Arlington, Virginia.)

issued to notify the applicant of the approved application.

Components of a Complete Application. A complete application consists of the following items in this order:

1. Application for Federal Assistance (Standard Form 424, Revised 4–88);
2. Budget Information—Non-construction Programs (Standard Form 424A, Revised 4–88);
3. Assurances—Non-construction Programs (Standard Form 424B, Revised 4–88);
4. Table of Contents;
5. Budget Justification for Section B—Budget Categories;
6. Proof of non-profit status, if appropriate;
7. Copy of the applicant's approved indirect cost rate agreement if necessary;
8. Project Narrative Statement, organized in five sections addressing the following topics:
   (a) Understanding of the Effort,
   (b) Project Approach,
   (c) Staffing Utilization, Staff Background, and Experience,
   (d) Organizational Experience, and
   (e) Budget Narrative;
9. Any appendices/attachments;
10. Certification Regarding Drug-Free Work place;
11. Certification Regarding Debarment, Suspension and Other Responsibility Matters; and
12. Certification and, if necessary, Disclosure Regarding Lobbying;
13. Supplement to Section II—Key Personnel; and
14. Application for Federal Assistance Checklist.

Dated: May 22, 1995.

David T. Ellwood,

Assistant Secretary for Planning and Evaluation

[FR Doc. 95–13220 Filed 5–30–95; 8:45 am]

BILLING CODE 4151–04–M

Public Health Service

National Toxicology Program; Board of Scientific Counselors' Meeting

Pursuant to Public Law 92–463, notice is hereby given of a meeting of the National Toxicology Program (NTP) Board of Scientific Counselors, U. S. Public Health Service, in the Conference Center, Building 101, South Campus, National Institute of Environmental Health Sciences (NIEHS), 111 Alexander Drive, Research Triangle Park, North Carolina, on June 29, 1995.

The primary agenda topic will be concerned with the report and recommendations of the *ad hoc* working group of the NTP Board from their review of the criteria for listing substances in the *Biennial Report on Carcinogens* (BRC) (formerly *Annual Report on Carcinogens*) on April 24 and 25, 1995. Specifically, the Board will:

(1) review the report and recommendations of the *ad hoc* working group;

(2) receive public comments on the report; and

(3) develop Board recommendations concerning the selection criteria.

The preliminary agenda topics with approximate times are as follows:

8:30 a.m.–8:45 a.m.—Report of the Director, NTP.

8:45 a.m.–9:15 a.m.—Report of the Director, Environmental Toxicology Program (ETP).

9:15 a.m.–9:45 a.m.—Report of the NTP Workshop on "Mechanism-Based Toxicology in Cancer Risk Assessment: Implications for Research, Regulation, and Legislation," held January 11–13, 1995.

10:00 a.m.–10:20 a.m.—Report on the Meeting of the *Ad Hoc* Working Group to Review Criteria for Listing of Substances in the BRC.

10:20 a.m.–11:00 a.m.—Board Discussion of the Working Group Report.

11:00 a.m.–12:00 p.m.—Public Comments on the Report.

1:15 p.m.–2:15 p.m.—Further Discussion and Development of Recommendations by the Board Concerning the BRC Selection Criteria.

2:15 p.m.–2:25 p.m.—Report on Technical Reports Review Subcommittee Activities.

2:25 p.m.–2:55 p.m.—Chemicals Nominated and Recommended for Study by the Interagency Committee for Chemical Evaluation and Coordination (ICCEC) on December 14, 1994, will be presented for discussion and time will be allowed for public comment. Chemicals evaluated by the ICCEC were (with CAS Nos. in parentheses): (1) Arsenic Trioxide (1327–53–3); (2) Ethidium Bromide (1239–45–8); (3) 5-(Hydroxymethyl)furfural (67–47–0); (4) Isoamyl Acetate (123–92–2); and (5) MX [3-chloro-4-(dichloromethyl) 5-hydroxy-2-(5H)-furanone] (77439–76–0). One chemical previously evaluated was re-reviewed: Hexamethyldisilazane (999–97–3).

3:15 p.m.–3:45 p.m.—Concept Review: *In Vitro* and *In Vivo* Genetic Toxicology Testing.

3:45 p.m.–4:30 p.m.—Alternative Methods—Status and Plans:

—RFA for Research in "Mechanistically Based Alternative Methods in Toxicology."

—Proposed Workshop on "Alternative Test Methods in Toxicology: Validation and Regulatory Acceptance."

Adjournment

Public Comments Encouraged

The meeting is open to the public, and public input concerning the criteria for listing a substance in the *Biennial Report on Carcinogens* is encouraged. A brief summary of the *ad hoc* working group meeting, including the current and proposed revised criteria, is available on request from the NTP Liaison Office, P.O. Box 12233, MD B3–01, Research Triangle Park, NC 27709, phone: (919) 541–0530, FAX: (919) 541–0295. This summary also will be published in the Federal Register in late May or early June. Written comments can be submitted to Dr. Larry G. Hart, Executive Secretary. Formal oral comments during the meeting will be limited to five minutes to permit maximum participation. Written comments accompanying oral statements are encouraged. To assure consideration by the Board at the meeting, written comments must be received by June 23, 1995. Registration to attend is not required; however, to ensure adequate seating, we ask that those planning to attend let us know. To register, submit written comments or announce intention to make oral comments, receive information on the agenda, or be put on the mailing list for summary minutes subsequent to the meeting, please contact: Dr. L. G. Hart, P.O. Box 12233, Research Triangle Park, NC 27709; telephone: (919) 541–3971; FAX: (919) 541–0719.

Dated: May 18, 1995.

Kenneth Olden,

Director, National Toxicology Program.

[FR Doc. 95–13284 Filed 5–30–95; 8:45 am]

BILLING CODE 4140–01–P

DEPARTMENT OF HOUSING AND URBAN DEVELOPMENT

Office of Administration

[Docket No. N–95–3922]

Notice of Submission of Proposed Information Collection to OMB

AGENCY: Office of Administration, HUD.

ACTION: Notice.

SUMMARY: The proposed information collection requirement described below has been submitted to the Office of

ATTACHMENT 2

AGENDA
BOARD OF SCIENTIFIC COUNSELORS
NATIONAL TOXICOLOGY PROGRAM

June 29, 1995

Conference Center, Building 101, South Campus
National Institute of Environmental Health Sciences (NIEHS)
Research Triangle Park, North Carolina

8:30-8:45 a.m.	Report of the Director, NTP	Dr. K. Olden, NIEHS
8:45-9:15 a.m.	Report of the Director, ETP	Dr. G. Lucier, NIEHS
9:15-9:45 a.m.	Report of the NTP Workshop on "Mechanism-Based Toxicology in Cancer Risk Assessment: Implications for Research, Regulation, and Legislation," held January 11-13, 1995	Dr. G. Lucier
9:45-10:00 a.m.	*Coffee Break*	
10:00-10:20 a.m.	Report on the Meeting of the *Ad Hoc* Working Group to Review Criteria for Listing of Substances in the *Biennial Report on Carcinogens* (BRC)	Dr. A. Brown, Board Chair/ Dr. C. Jameson, NIEHS/ Dr. G. Lucier
10:20-11:00 a.m.	Board Discussion of the Working Group Report	Board
11:00-12:00 p.m.	Public Comments on the Report	
12:00-1:15 p.m.	*Lunch*	
1:15-2:15 p.m.	Further Discussion and Development of Recommendations by the Board Concerning the BRC Selection Criteria	Board
2:15-2:25 p.m.	Report on Technical Reports Review Subcommittee Activities	Dr. G. Boorman, NIEHS
2:25-2:55 p.m.	Chemicals Nominated and Recommended for Study by the Interagency Committee for Chemical Evaluation and Coordination (ICCEC) on December 14, 1994 — Public Comment — Other Actions	Dr. E. Zeiger, NIEHS
2:55-3:15 p.m.	*Break*	
3:15-3:45 p.m.	Concept Review — *In Vitro* and *In Vivo* Genetic Toxicology Testing	Dr. E. Zeiger

3:45-4:30 p.m.	Alternative Methods - Status and Plans: — RFA for Research in "Mechanistically-Based Alternative Methods in Toxicology" — Proposed Workshop on "Alternative Test Methods in Toxicology: Validation and Regulatory Acceptance"	Dr. W. Stokes, NIEHS Dr. W. Stokes

Adjournment

National Toxicology Program
Board of Scientific Counselors

June 29, 1995

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Senior Scientist
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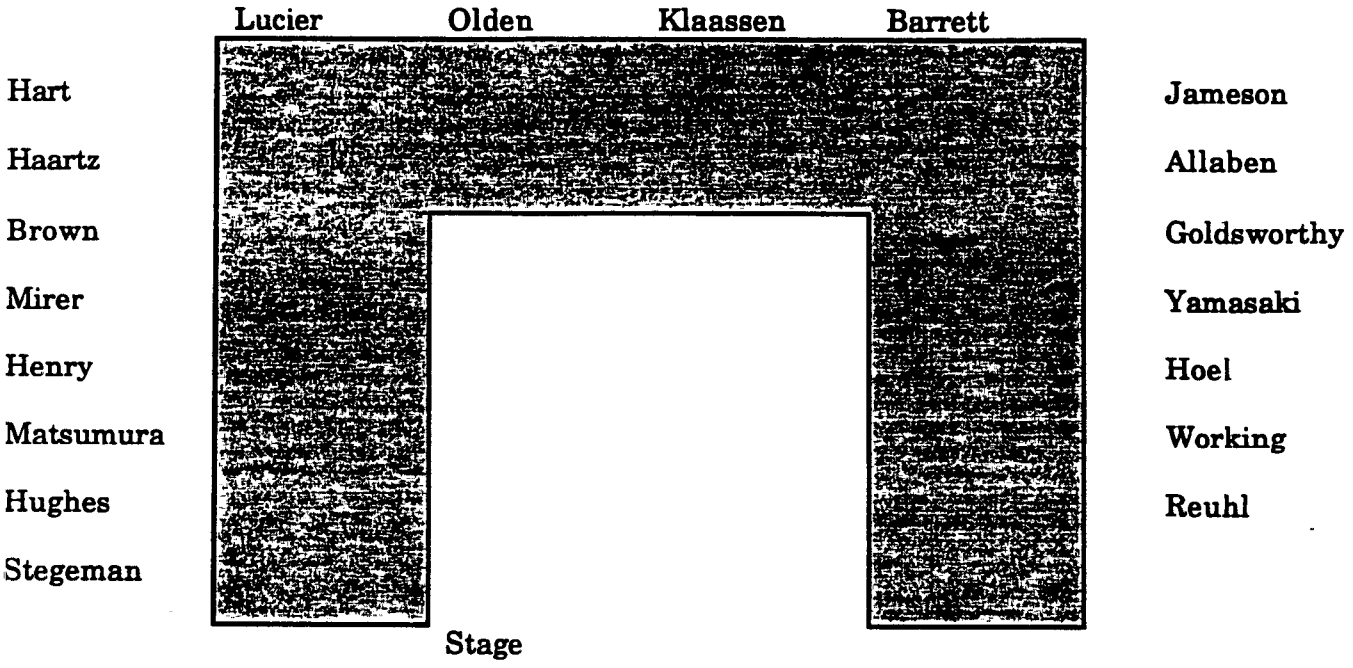
* Not present

National Toxicology Program
Board of Scientific Counselors' Meeting

National Institute of Environmental Health Sciences
South Campus
Conference Center, Building 101
Research Triangle Park, North Carolina

June 29, 1995



CURRENT CRITERIA FOR LISTING SUBSTANCES IN THE BIENNIAL REPORT ON CARCINOGENS

For the purpose of the BRC, the degrees of evidence are as follows:

1. **Known To Be Carcinogens:**
   There is sufficient evidence of carcinogenicity from studies in humans that indicates a causal relationship between the agent and human cancer.

2. **Reasonably Anticipated To Be Carcinogens:**
   a. There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias or confounding, could not adequately be excluded, or

   b. There is sufficient evidence of carcinogenicity from studies in experimental animals that indicates that there is an increased incidence of malignant tumors: (a) in multiple species or strains, or (b) in multiple experiments (preferably with different routes of administration or using different dose levels), or (c) to an unusual degree with regard to incidence, site or type of tumor, or age at onset. Additional evidence may be provided by data concerning dose-response effects, as well as information on mutagenicity or chemical structure.

PROPOSED REVISED CRITERIA FOR LISTING SUBSTANCES IN THE BIENNIAL REPORT ON CARCINOGENS

For the purpose of the BRC, the degrees of evidence are as follows:

1. **Known to be Human Carcinogens:**
   There is sufficient evidence of carcinogenicity from studies in humans that indicates a causal relationship between the substance and human cancer.

2. **Reasonably Anticipated to be Human Carcinogens:**
   a. There is limited evidence of carcinogenicity from studies in humans which indicate that causal interpretation is credible but that alternative explanations such as chance, bias or confounding could not adequately be excluded, or

   b. There is sufficient evidence of carcinogenicity from studies in experimental animals that indicates there is an increased incidence of malignant and/or combined benign and malignant tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor or age at onset.

Conclusions regarding carcinogenicity in humans or experimental animals should be based on scientific judgment. Consideration may be given to relevant information on dose response, route of exposure, chemical structure, sensitive sub populations, genetic effects or other data relating to mechanism of action, and/or factors that may be unique to a given substance. There may be substances for which there is less than sufficient evidence of carcinogenicity in humans or laboratory animals but for which there are compelling data indicating that the substance could reasonably be anticipated to cause cancer in humans. Conversely, there may be substances for which there is sufficient evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore reasonably be anticipated not to cause cancer in humans.

Chemicals Selected for Testing by the NTP

Chemical (CAS Number)	Nomination Source	Testing Recommendations (Priority)	NTP Chemical Selection Principles	Rationale/Remarks
1. Arsenic trioxide (1327-53-3)	NIEHS	Mechanistic toxicity studies (Moderate)	6	- Known human carcinogen - Rodent models inadequate for human risk assessment
2. Isoamyl acetate (123-92-2)	NIEHS	Carcinogenicity; Metabolism; SMVCE; Neurotoxicity (High)	3	- Human exposure - Limited toxicity data - High production volume
3. 5-(Hydroxymethyl)furfural (67-47-0)	NIEHS	Carcinogenicity (Moderate)	1, 2	- Consumer exposure - Natural product
4. MX [3-Chloro-4-(dichloromethyl) 5-hydroxy-2-(5H)-furanone] (77439-76-0)	American Water Works Association Research Foundation	Carcinogenicity (Moderate) *(see Remarks)*	1, 2	- Formed during chlorination of water - Mutagenic - NTP may collaborate with National Public Health Institute in Finland on MX rat bioassay in progress.
5. Ethidium bromide (1239-45-8)	Private individual	Prepare position paper Carcinogenicity (Moderate) *(see Remarks)*	2, 6	- Laboratory reagent - Human exposure - Mutagenic - Probable carcinogen - Recommend for inclusion in Biennial Report on Carcinogens - NTP to write position paper on carcinogenicity of EB - Carcinogenicity testing be performed if NTP position paper not written

ATTACHMENT 5

Nomination Principles for NTP Studies

The NTP will solicit nominations for NTP studies from the following categories:

1. Chemicals found in the environment that are not closely associated with a single commercial organization.

2. Biological or physical agents that may not be adequately evaluated without Federal involvement.

3. Commercial chemicals with significant exposure that were first marketed before current testing requirements or those that generate too little revenue to support further evaluations.

4. Potential substitutes or existing chemicals or drugs that might not be developed without Federal involvement.

5. Substances that occur as mixtures for which evaluations cannot be required of industry.

6. Chemicals or agents that will aid our understanding of chemical toxicities, or our understanding of the use of test systems to evaluate potential toxicities.

7. Chemicals that should be evaluated to improve the scientific understanding of structure-activity relationships and thereby help limit the number of chemicals requiring extensive evaluations.

8. Emergencies or other events that warrant immediate Government evaluation of a chemical or agent.

Environmental Toxicology Program

Division of Intramural Research

National Institute of Environmental Health Sciences

CONCEPT REVIEW

Prepared for:

National Toxicology Program
Board of Scientific Counselors

June 29, 1995

Title: In Vitro and In Vivo Genetic Toxicity Testing
Presenter: E. Zeiger
Primary Reviewer: P. Working

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.

The Board members are asked to review the project concepts for overall value and scientific relevance as well as for fulfilling the program goal of protecting public health. Specific areas should include:

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: In Vitro and In Vivo Genetic Toxicity Testing

PRESENTER: Errol Zeiger, Chemistry Branch, Environmental Toxicology Program

OBJECTIVES: To test chemicals for mutagenicity, DNA, and chromosome damaging effects in microbes and mammalian cells in vitro, and in rodents in vivo.

BACKGROUND: Genetic toxicity testing is an integral component of the NTP's program to evaluate the adverse biological activity of chemicals. The ETP is currently testing chemicals in contract laboratories for mutagenicity in bacteria, for chromosome aberrations in cultured Chinese hamster ovary cells, and for chromosome aberrations and micronuclei in bone marrow, and occasionally other cells of rats and mice treated in vivo. Other in vitro and in vivo mutagenicity and chromosome damage tests had been routinely used during the first 10 years of this testing program, but they were discontinued after studies showed that they had not lived up to their original promise to identify carcinogens and distinguish them from noncarcinogens, or because they were redundant with other, more facile, test systems.

Mutagenic events are implicated in the process of carcinogenesis, either as the initiating events or as mediators of subsequent cellular changes in the progression from normal to metastatic tumor cells. Studies conducted using the NTP genetic toxicity and carcinogenicity data bases have supported this relationship between genetic toxicity and rodent, as well as human, carcinogenicity.

Results from bacterial mutagenicity (Salmonella; Ames) tests have a high positive predictivity for carcinogenicity, and for rodent germ cell mutagenicity. Results from in vitro and in vivo chromosome damage tests also have a high positive predictivity for carcinogenicity. Although not all chemicals that produce chromosome damage in bone marrow produce detectable germ cell damage, all chemicals that have been shown to be mutagenic in mouse germ cells also produce damage in bone marrow cell chromosomes. However, chemicals negative for bone marrow damage are, as a rule, not tested for germ cell effects. The induction of chromosome aberrations and micronuclei correlate well with the induction of mutations in Salmonella, but represent different mechanisms of action.

In recent studies using the NTP database, the predictivity of a positive Salmonella test for rodent carcinogenicity was 89%. However, approximately 52% of the rodent carcinogens were not mutagenic in Salmonella. For this reason, a negative Salmonella test response, in the absence of other information, was not predictive for noncarcinogenicity. Among the chemicals that were carcinogenic in two species, 69% were mutagenic, whereas 60% of the nonmutagenic carcinogens were

carcinogenic only or at a single tissue site in a single sex of a single species, usually the mouse liver. The mutagenic carcinogens were also highly correlated with the ability of the chemicals to form electrophilic reactive species. With respect to the IARC human carcinogens, 88% of the Group 1, organic chemical carcinogens were Salmonella mutagens, as were 83% and 67% of the Group 2a and 2b carcinogens, respectively; and 90% of the Group 1 carcinogens were positive in the mouse bone marrow micronucleus test.

The genetic tests described here can all be used as rapid screening tests to characterize chemicals of interest and provide information for setting priorities for further testing. The in vivo test endpoints can also be incorporated into standard rodent toxicological testing procedures to minimize the use of animals and to allow better integration of the various test results. Additionally, the knowledge that a chemical is an in vitro and in vivo genetic toxin, coupled with knowledge of its structure and pharmacokinetics, can spare the use of long-term carcinogenicity tests on chemicals likely to be positive, or can lead to the selection of carcinogenicity test protocols that use reduced numbers of animals. This genetic toxicity information is also relevant to an understanding of the mechanism of action of the chemical.

PROPOSED CHANGES TO THE CURRENT STATEMENTS OF WORK: The Statements of Work in the Salmonella contracts will not change significantly. The protocol is sufficiently flexible to accommodate changes in the test procedures, or incorporate new tester strains or metabolic activation procedures.

The Statements of Work for the in vivo micronucleus and chromosome aberration procedures will be extended to allow the examination of tissues other than bone marrow cells for micronuclei and chromosome aberrations. For example, lung cells or peripheral lymphocytes can be examined to study effects at the site of administration (lung cells if by inhalation) or a cell type that can also be studied in exposed humans (peripheral lymphocytes). Micronuclei can be produced by chromosome breakage or by chromosome malsegregation during mitosis, both of which effects have are important for the initiation or progression of cancer and for the production of heritable damage. Chromosome malsegregation can be caused by chemical action on the chromosome itself, or on tubulin, which controls the chromosome segregation process. Procedures, such as kinetochore staining, will be incorporated to identify chemicals that produce micronuclei primarily via the induction of aneuploidy. This procedure will provide additional information on the mechanism of action of the genetically-active chemicals.

This program will also have the capability of incorporating other in vitro and in vivo test procedures as they become available, or if they are determined to be relevant and useful for the evaluation of specific chemicals, or to address specific toxicological questions. Similarly, the use of assay systems or endpoints that are no longer considered necessary or effective for their proposed purpose can be

discontinued. Among the new genetic toxicity test procedures and endpoints that can be used, two have been the subjects of much recent interest.

The single-cell gel assay (also known as the comet assay) measures DNA strand breaks, or alkali-labile sites, in single cells in vitro or from tissues in vivo, and appears to be highly sensitive to low levels of DNA damage. This procedure allows cells from various tissues to be examined, and can be performed using the same animals as are used in the other toxicity procedures. For example, cells for analysis can be obtained from peripheral blood, bone marrow, liver, lung, kidney, testes, etc.; many of these tissues are not readily used in standard cytogenetic procedures, and serial sampling of animals can be performed. The procedure is relatively new, and its use in combination with other tests will permit validation of its usefulness for prediction of genetic and carcinogenic events in target tissues.

Transgenic mice and rats in which gene mutations can be measured in cells from the various body tissues show promise for routine genetic toxicity testing. These animals will allow a complete evaluation of a substance's genetic toxicity because both gene mutations and chromosome damage can be measured in selected target tissues, and the results of these measurements can be correlated with the standard toxicological and histological measurements in the same tissues. Transgenic B6C3F1 mice and F344 rats are also available, so that the same animals can be used to measure mutations and other toxicological endpoints.

This program will allow the ETP to adopt new testing procedures and systems, as necessary, to take advantage of advances in the science of genetic toxicity testing, and current data needs of the program. Chemicals tested will include those nominated to the NTP for genetic toxicity or other toxicity testing, chemicals on test for short-term or chronic toxicity, and chemicals of interest to NIEHS personnel. All test results and data are entered into the NIEHS database and are accessible by all personnel. The summary results will also be available to the general community through the Gopher internet system, and distributed in response to requests from other government agencies, private organizations, and private individuals.