The review meeting began at 8:30 a.m. on April 26, 1990 in the Conference Center, Building 101, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. Members of the Subcommittee are: Drs. Robert Scala (Chairperson), Jay Goodman, Daniel Longnecker, and Ellen Silbergeld. Members of the Panel of Experts are: Drs. John Ashby, Gary Carlson, Harold Davis, Robert Garman, Lois Gold, David Hayden, Curtis Klaassen, Barbara McKnight and Lauren Zeise. Drs. Klaassen and Scala were unable to attend this meeting. Dr. Michael Gallo, former Board chair and member of the Subcommittee, served as Chairperson for this meeting. These minutes have been reviewed and approved by all members of the Subcommittee and Panel present. They were written by Dr. Larry G. Hart, Executive Secretary.

When available, a limited number of final NTP Technical Reports for the studies may be obtained free of charge from the NTP Public Information Office, MD B2-04, P. O. Box 12233, Research Triangle Park, NC 27709. Telephone: (919)541-3991; FTS: 629-3991. Subsequently, they may be purchased from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, Virginia 22161, (703)487-4650.

The next NTP technical reports peer review meeting will be held November 19-20, 1990, in Research Triangle Park, North Carolina. For information, contact Dr. Hart, (919) 541-3971; FTS 629-3971.
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Dr. J.R. Bucher, NIEHS, NTP Study Scientist, introduced the toxicology and carcinogenesis studies of sodium fluoride by reviewing the uses of fluorides and the experimental design and results for the 14-day and 6-month studies in male and female F344/N rats and B6C3F1 mice. Factors considered important in dose selection for the 2-year studies in rats were decreased weight gain and stomach lesions in a male and a female rat in the top dose groups from the 6-month studies (300 ppm). Factors considered important in dose selection for the 2-year studies in mice were decreased weight gain and deaths at 200 ppm and higher doses. Dr. Bucher put the doses used in the 2-year studies into perspective (0, 25, 100, 175 ppm sodium fluoride, equivalent to 0, 11, 45, 79 ppm fluoride) by noting that optimal levels of fluoride ion in public water supplies are considered to be about 1 ppm and that mice and rats drink more water proportionate to body weight than do humans. Also, rodent diets routinely contain higher amounts of fluoride than the human diet. For the 2-year studies, Dr. Bucher discussed primary nonneoplastic lesions of the teeth in rats and mice and bone lesions (osteosclerosis) in female rats. He commented on differences in the incidences of neoplasms between dosed and control animals at a number of tissue sites, in particular, tumors of the oral cavity, thyroid gland and skin of rats, and of the hematopoietic system of mice and concluded that there was insufficient evidence to consider the small increases related to sodium fluoride administration. The only other neoplasms believed to warrant consideration were osteosarcomas of the bone in mid and high dose male rats. There are a number of factors entering into determination of whether this is a chemically related effect, or whether this incidence occurred by chance. Dr. Bucher reviewed these, including several differences in the protocol used to sample and examine bone in this study compared to typical protocols, factors pertaining to the use of historical control data, and the expectation of a neoplastic response in an organ that accumulates fluoride, such as bone. However, fluoride levels in bone in high dose male rats did not differ from those in high dose female rats or male or female mice, and there were no osteosarcomas in these groups. Dr. Bucher concluded that the evidence was weakly supportive of an association between osteosarcomas and the administration of sodium fluoride to male rats. The evidence was thought to be inconclusive and best described by the equivocal evidence category in the NTP classification scheme (attached), which is defined as a marginal increase in neoplasms that may be chemically related. The conclusions as written on page 2 of the Technical Report are as follows:

Under the conditions of these 2-year dosed water studies, there was equivocal evidence of carcinogenic activity of sodium fluoride in male F344/N rats, based on the occurrence of a small number of osteosarcomas in dosed animals. There was no evidence of carcinogenic activity in female F344/N rats receiving sodium fluoride at concentrations of 0, 25, 100, or 175 ppm (0, 11, 45, or 79 ppm fluoride) in drinking water for 2 years. There was no evidence of carcinogenic activity of sodium fluoride in male or female mice receiving sodium fluoride at concentrations of 0, 25, 100, or 175 ppm in drinking water for 2 years.
Dosed rats had lesions typical of fluorosis of the teeth and high-dose female rats had increased osteosclerosis of long bones.

Dr. Longnecker, a principal reviewer, agreed with the conclusions. He thought this to be a well written report reflecting a carefully done study. He said the doses chosen were appropriate, yielding clear evidence of biologic effects without severe effects on animal growth. The photomicrographs in the report were of good quality and supported the diagnoses made.

Dr. Ashby, the second principal reviewer, agreed with the conclusions. However, he considered the definition for equivocal evidence of carcinogenic activity to be insufficiently precise for male rats and suggested that a statement on the top of page 93 in the Report be used instead, this being: "Taken together, the current findings are inconclusive, but are weakly supportive of an association between sodium fluoride administration and the occurrence of osteosarcomas in male rats." Noting the propensity for fluoride to accumulate in bone, this tissue was the most likely one for occurrence of a carcinogenic effect, yet the fact of fluoride accumulating in the bone of female rats and male and female mice to a similar extent as in male rats was indicative of caution in drawing simple conclusions. Dr. Ashby commented that sodium fluoride clearly has some genetic activity, but probably by an indirect or secondary effect on chromosome structure. He thought future acquisition of male rat bone marrow genotoxicity data was indicated.

Dr. Garman, the third principal reviewer, agreed with the conclusions. He stated that this was an outstanding report which covered the findings of a thorough, well-conducted study. He speculated that because of a possible link between fracture formation and subsequent development of osteogenic sarcomas in humans and animals, and because increased levels of dietary fluoride may result in increased fragility of certain bones, there might be a connection between osteogenic sarcoma formation and bone remodelling. Dr. Garman suggested that any future studies include measurements of bone tensile strength.

Comments by Other Members of the Panel: Dr. Silberfeld noted the role of the NTP data as being part of the first step in the complicated process of risk assessment, and pointed out that the doses used were not orders of magnitude above human exposure levels. She supported further research on genotoxicity and on mechanisms of sex differences seen. Dr. Davis underscored the observation of nonneoplastic lesions of the bone in female rats (osteosclerosis) in the absence of bone tumors. Dr. Goodman said the Abstract should point out the extra scrutiny given to the evaluation of bone tissue in this study. Dr. Hayden also commented on the thoroughness of the study and report. Dr. Gold noted that this was an unusual study in that there was not a zero control group, and related to this is the fact that fluoride is a naturally occurring chemical in the standard rodent laboratory diet. She emphasized that both control and dosed animals in all NTP studies received fluoride doses in the laboratory diet that were higher than the low dose tested. She said the use of '0 ppm' as the heading in tables for control values was misleading. Dr. Bucher agreed and said 'Control' would be used and better defined as to the level of fluoride in the diet of control animals. There was discussion by Dr. McKnight with Dr. J. Hasemen, NIEHS, as to why data from paired (age-matched) controls were not used in primary data tables. Dr. Zeise pointed out two rare tumors of the nasal mucosa found in high dose male rats and indicated these should be discussed in
the body of the report. She reiterated the need expressed by other Panel members for designing another study with higher top doses. Dr. Zeise noted that the fluoride concentrations in high dose rats were within the range observed in humans and the differences in pharmacokinetics and deposition of fluoride in bone between humans and animals should be studied. Dr. Carlson wondered about the possible mechanism for induction of the oral cavity tumors. Dr. Bucher responded that there was no overall statistical significance for the oral tumors even if the P values for female rats were combined statistically with the corresponding values for male rats. Additionally, there was a squamous cell carcinoma of the oral cavity in a female control as well as one in a paired male control. Thus, the level of confidence that the oral lesions might be associated with chemical exposure was less than that for the bone lesions. Dr. L. Hart, NIEHS, read into the record comments received from Dr. C. Klaassen, a Panel member who could not be present. Dr. Klaassen thought information in the Abstract about the historical control rate of osteosarcomas in male rats should include not only the mean (0.5%) but also the range (0-6%). Dr. Gallo concluded the initial discussion by emphasizing that there was a major need for looking at the mechanisms of toxic action of fluoride at the various sites in any future studies.

Public Comments: Advance notice had been received by nine persons that they wished to make statements at the meeting, and written material in support of their statements had been received prior to the meeting for distribution to Panel members, staff and interested attendees. Sufficient time was allowed for Panel members to question each of the speakers. Brief descriptions of the statements (in order of presentation) follows:

(1) Dr. John A. Yiamouyiannis, Director, Safe Water Foundation, Delaware, Ohio -- He stated that occurrence of a rare form of liver cancer, hepatocholangiocarcinomas, in fluoride-treated male and female mice in the NTP studies provided clear evidence of carcinogenic activity in mice. Further, he said a dose-dependent relationship between fluoride and the number of male rats with oral squamous cell tumors and a dose-dependent relationship between oral squamous cell metaplasia and tumors in female rats along with the increased incidence of osteosarcomas in male rats supported a finding of clear evidence of carcinogenic activity of fluoride in rats.

(2) Dr. James W. Bawden, University of North Carolina School of Dentistry, representing the American Association for Dental Research and the American Association of Dental Schools -- His presentation (a) contended that plasma fluoride levels reported for the six month studies in rats were in error due to the assay method used, (b) expressed concern about the terms "low", "mid" and "high" used to describe the doses used in the study, stating that a comparison of plasma levels of fluoride from animals in the study with those observed in humans would support terming the doses as "high", "very high", and "extremely high", (c) questioned the appropriateness and relevance of the rat model, noting that in humans osteosarcoma as a primary lesion is predominately associated with long bones and occurs almost exclusively in young people, and (d) agreed with the NTP conclusions. He opined that the results of the NTP study do not indicate that the fluoridation of municipal water supplies is ill advised.

(3) Dr. Robert d'Amato, the Procter & Gamble Company (P & G), described the P & G chronic carcinogenicity studies with sodium fluoride in Swiss CD mice
and Sprague-Dawley rats. The high dose for the rat study was 2 to 3 times greater than the NTP study high dose on a mg/kg body weight basis. The mouse studies, not yet reported, showed dose-related increases in the incidences of osteomas, but were flawed by a C-type retroviral infection in all groups. He speculated that increased incidences of osteomas (observed in the mouse study) might be due to a biological interaction between virus and fluoride ion. Their rat study indicated there was no evidence that sodium fluoride altered the incidences of preneoplastic or neoplastic lesions at any site in either sex. The results of the rat study have been accepted for publication in the Journal of the National Cancer Institute. Dr. d'Amato said the results of their studies supported the wide body of data which indicates that sodium fluoride does not cause cancer and that human lifetime exposure to fluoride via dentrifice usage, as well as from the environment, is safe.

(4) Ms. Susan Pare, Center for Health Action, questioned the objectivity of a study apparently designed to confirm a negative and stated that it had taken 13 years from the decision to do carcinogenesis studies on sodium fluoride to the present, leading her to wonder about the efficiency of the test system. Ms. Pare commented on the lack of a 'true' control diet, i.e., one free of fluoride, and the difficulties this could cause in comparisons with other studies. She contended that rare liver cancers originally diagnosed in exposed mice had been reclassified. Finally, she objected to 'sweeping' statements in NTP news releases and the Report about the effectiveness of water fluoridation against tooth decay.

A statement was read into the record from Dr. James A. Popp, Chemical Industry Institute of Toxicology, responding to comments attributed to him in written material provided to the NTP by Ms. Pare prior to the meeting which stated that Dr. Popp had expressed to a "reliable source" that the evidence in the NTP studies linking fluoride to osteosarcomas in rats was "clear". Dr. Popp had been a member of the Pathology Working Group evaluating the studies. In his statement, Dr. Popp said that he did not recall making such a comment, and added that "without complete information I believe it is impossible for me or any other member of the Pathology Working Group to make a determination of the appropriate level of evidence assignment for the sodium fluoride study."

(5) Dr. John R. Lee, Marin Country, California, representing the Center for Health Action spoke to the need for further studies which he thought should include (a) adequate controls, (b) better assessment of age-related nephropathy which can lead to decreased excretion of fluoride, (c) more balanced treatment in reporting of the deleterious effects of fluoride and considering the risks as well as the benefits of fluoride, (d) lumping subcutaneous and bone osteosarcomas together, and (e) a better evaluation of the genetic toxicology.

(6) Dr. Melvin Reuber, representing the Safewater Foundation, Delaware, Ohio, commented on some of the tumors observed in the NTP studies, as follows: (a) in commenting on the hepatoblastomas and hepatocholangiomas observed in mice, he said more sections of liver should have been cut; (b) the osteosclerosis reported should be considered a preneoplastic lesion; (c) squamous dysplasia should be considered a preneoplastic lesion; and (d) neoplasms of the Zymbalt's gland, skin, and kidney received insufficient pathologic evaluation. Dr. Reuber claimed there were discrepancies between the diagnoses made by the original laboratory pathologist for several lesions and the diagnoses made by the laboratory performing pathology Quality Assurance.
(7) Dr. Gary M. Whitford, Department of Oral Biology, Medical College of Georgia, suggested that statements in the Report about in vitro genotoxic effects of fluoride should be put into the perspective of likely levels of fluoride in the human body by inclusion of a qualifying phase such as: "...at concentrations much higher than those which occur in humans...". Dr. Whitford summarized the findings from a recently completed chronic toxicity study in which Sprague-Dawley rats were administered fluoride in the form of dentifrices. It was concluded that administration of 0.25 or 2.5 mg fluoride/kg for 18 months caused no consistent evidence of toxicity of any kind that distinguished these dosed groups from the control groups. All animals in the high dose groups, 12.5 mg/kg, died, usually in renal failure, between the 6th and 12th months.

(8) Dr. John W. Stamm, School of Dentistry, University of North Carolina, representing the American Dental Association (ADA) stated that the ADA disagreed with the NTP's conclusions for male rats based on four issues: (a) the criteria used by the NTP to assess strength of experimental evidence appeared to depart from the norms used by the NTP and NCI over many years; (b) the NTP interpretation appeared to have given insufficient attention to the relative contributions of increased and decreased incidences of tumors in the rat studies; (c) he said there was a recent suggestion that some NIEHS investigators themselves may regard compounds categorized as "equivocal" to be more properly seen as noncarcinogenic; and (d) extensive epidemiological studies in humans have consistently shown no link between water fluoridation and cancer.

(9) Dr. Edward Remmers, American Council on Science and Health (ACSH), noted that the ACSH had held a press conference on April 24, 1990, to present their pro-fluoridation position for drinking water. He asked the Panel to acknowledge that high-dose rodent studies are not infallible predictors of cancer risk in humans, and to reject the recommendation of those who allege that the EPA should classify fluoride as a "probable human carcinogen", and ban water fluoridation. Dr. Remmers concluded by reporting that the ACSH planned a press conference in the fall of 1990 on the limits of extrapolating cancer risk from animals to humans and on the possibility of considering seeking Congressional redress of the increasing misuse of animal studies to needlessly terrify the American consumer about safe technologies and products.

Further Panel Discussion

Dr. Gold asked that it be noted in the report that younger animals received a higher dose because they drink a larger amount of water in proportion to their body weight than older animals. Dr. Zeise questioned whether a high enough dose was used in mice. Dr. Bucher replied that the primary factor considered in selection of sodium fluoride concentrations for 2-year studies in mice was a reduction in body weight gain at higher doses in the 6-month studies. Dr. Zeise asked for a statement in the Abstract to the effect that mice could have tolerated higher doses. Dr. Bucher agreed saying that based on the 2-year results it appeared that mice might have been able to tolerate higher doses. Dr. Haseman agreed to Dr. McKnight's request that statistical analyses including the paired control data for the more important tumors be added to the tables. Dr. Silbergeld offered to provide references pertaining to sex-related differences in mineral metabolism which might aid in explaining sex differences in tumor response.
Motions

Dr. Goodman moved that the conclusion in male rats be changed to no evidence of carcinogenic activity based on the following points: (1) the number of osteosarcomas observed in male rats was within the historical control range; (2) scrutiny of bone and bone tissue was more rigorous than in previous studies; (3) fluoride accumulation was similar in all four experiments, and actually highest in female rats where there were no tumors; and (4) there was no statistical differences in pairwise comparisons between control and treated male rat groups. Dr. Davis seconded the motion. Discussion against the motion noted that the tumors at issue (osteosarcomas) were found in a target organ for fluoride (bone), they are uncommon tumors, and a further supporting factor was the observation of a subcutaneous osteosarcoma. The motion was defeated by 10 no votes to 1 yes vote (Dr. Goodman).

Dr. Longnecker moved that the draft Technical Report on sodium fluoride be accepted with the editorial changes and revisions as discussed by the Panel and with the conclusions as written for male rats, equivocal evidence of carcinogenic activity, and for female rats and male and female mice, no evidence of carcinogenic activity. He asked that the statement at the top of page 93 be added to the conclusions, i.e., "Taken together, the current findings are inconclusive but are weakly supportive of an association between sodium fluoride administration and the occurrence of osteosarcomas in male rats." Dr. Ashby seconded the motion. In discussion, there was concern that "weakly supportive" was too positive when viewed in the context of the NTP definition of equivocal evidence. Dr. Gold stated that the uncertain nature of the findings in male rats needed to be emphasized, and after further discussion, she proposed that the definition for equivocal evidence be included in the conclusion. This would replace the sentence from page 93. Dr. Longnecker and Dr. Ashby agreed. The statement which would be inserted after the first sentence of the conclusions read: "Equivocal evidence is a category for uncertain findings demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemically related." The motion was accepted by nine yes votes to two no votes (Silbergeld, Zeise). It was noted that the no votes were based on opposition to adding the sentence rather than on objections to the level of evidence.

To clarify this, Dr. Zeise asked for a motion on the conclusion in male rats alone. Dr. Ashby moved that the conclusion in male rats, equivocal evidence of carcinogenic activity, be voted on. Dr. Gold seconded the motion which was accepted unanimously with 11 votes.

The meeting was adjourned.
Explanation of Levels of Evidence of Carcinogenicity contained in this report:

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results ("Clear Evidence" and "Some Evidence"); one category for uncertain findings ("equivocal Evidence"); one category for no observable effects ("No Evidence"); and one category for experiments that because of major flaws cannot be evaluated ("Inadequate Study"). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Reports series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each experiment (male rats, female rats, male mice, female mice), one of the following quintet is selected to describe the findings. The categories refer to the strength of the experimental evidence and not to either potency or mechanism.

CE Clear Evidence of Carcinogenic Activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.

SE Some Evidence of Carcinogenic Activity is demonstrated by studies that are interpreted as showing a chemically-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.

EE Equivocal Evidence of Carcinogenic Activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemically related.

NE No Evidence of Carcinogenic Activity is demonstrated by studies that are interpreted as showing no chemically-related increases in malignant or benign neoplasms.

IS Inadequate Study of Carcinogenic Activity is demonstrated by studies that because of major qualitative or quantitative limitations cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. This should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- The adequacy of the experimental design and conduct;
- Occurrence of common versus uncommon neoplasia;
- Progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- Some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- Combining benign and malignant tumor incidences known or thought to represent stages of progression in the same organ or tissue;
- Latency in tumor induction;
- Multiplicity in site-specific neoplasia;
- Metastasis;
- Supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- The presence or absence of dose relationships;
- The statistical significance of the observed tumor increase;
- The concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasia;
- Survival-adjusted analyses and false positive or false negative concerns;
- Structure-activity correlations; and
- In some cases, genetic toxicology.