TOXICOLOGY AND CARCINOGENESIS

STUDIES OF

SODIUM FLUORIDE

(CAS NO. 7681-49-4)

IN F344/N RATS AND B6C3F₁ MICE

(DRINKING WATER STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health
FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP chemical health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection per se is not an indicator of a chemical's carcinogenic potential.

These NTP Technical Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Technical Report are available without charge while supplies last from the NTP Public Information Office, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709 (919-541-3991).
NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF SODIUM FLUORIDE

(CAS NO. 7681-49-4)

IN F344/N RATS AND B6C3F₁ MICE

(Drinking Water Studies)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC  27709

December 1990

NTP TR 393

NIH Publication No. 91-2848

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health
CONTRIBUTORS

National Toxicology Program

J.R. Bucher, Ph.D., Study Scientist
C.J. Alden, Ph.D.
G.A. Boorman, D.V.M., Ph.D.
D.W. Bristol, Ph.D.
S.L. Eustis, D.V.M., Ph.D.
T.J. Goehl, Ph.D.
R.A. Griesemer, D.V.M., Ph.D.
J.K. Haseman, Ph.D.
J.K. Witt, M.S., Oak Ridge Associated Universities
G.N. Rao, D.V.M., Ph.D.
T.J. Goehl, Ph.D.
S.L. Eustis, D.V.M., Ph.D.
D.W. Bristol, Ph.D.
R.A. Griesemer, D.V.M., Ph.D.
J.K. Haseman, Ph.D.
G.N. Rao, D.V.M., Ph.D.
J.K. Witt, M.S., Oak Ridge Associated Universities

NTP Pathology Working Group

Evaluated slides, prepared pathology reports on 12-Jan-90

J.R. Leininger, D.V.M., Ph.D., Cochairperson
Pathology Associates, Inc.
S. Grumbein, D.V.M., Ph.D., Cochairperson
Pathology Associates, Inc.
G.A. Boorman, D.V.M., Ph.D.
National Toxicology Program
S.L. Eustis, D.V.M., Ph.D.
National Toxicology Program
M.P. Jokinen, D.V.M.
National Toxicology Program
J.A. Popp, D.V.M., Ph.D.
Chemical Industry Institute of Toxicology
K. Yoshitomi, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
B. Hamilton, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
J. Maurer, D.V.M., Ph.D., Observer
Procter and Gamble

Biotechnical Services, Inc.

Prepared technical reports

L.G. Cockerham, Ph.D., Principal Investigator
G.F. Corley, D.V.M.
J.L. Elledge, B.A.
J.A. Gregan, M.A.
J.L. Hoskyn, B.S.
K.D. Mencer, B.A.
P.E. Parmley, M.A.
B.B. Randolph, M.B.A.
P.R. Dennis, M.C.M.

Battelle Columbus Laboratories

Conducted studies, evaluated pathology findings

A.C. Peters, D.V.M., Principal Investigator

14-Day and 6-Month Studies

C.E. Chrisp, D.V.M.
G.S. Dill, Jr., D.V.M.
D.K. Gerken, Ph.D.
E.G. Leighty, Ph.D.

2-Year Studies

M.R. Hejtmancik, Ph.D., Study Director
B.D. Carlton, Ph.D.
M.J.W. Chang, Ph.D.
W.T. Ferner, D.V.M.
D.S. Herring, D.V.M., Ohio State University
P.L. Jepsen, D.V.M.
L.R. Marsh, M.S.
R.L. Persing, D.V.M.
J.D. Toft II, D.V.M., M.S.
S.E. Weisbrode, V.M.D., Ph.D., Ohio State University

Experimental Pathology Laboratories, Inc.

Provided pathology quality assessment

K. Yoshitomi, D.V.M., Ph.D.
H.R. Brown, D.V.M.

Integrated Laboratory Systems

Prepared quality assurance audits

J.C. Bhandari, D.V.M., Ph.D., Principal Investigator

Analytical Sciences, Inc.

Provided statistical analyses

S. Seilkop, M.S.
R. Fleenor, B.S.
CONTENTS

ABSTRACT ................................................................. 5
EXPLANATION OF LEVELS OF EVIDENCE .............................. 8
PEER REVIEW PANEL .................................................... 9
SUMMARY OF PEER REVIEW COMMENTS ............................... 10
INTRODUCTION .......................................................... 15
MATERIALS AND METHODS ............................................ 23
RESULTS ............................................................................. 33
DISCUSSION ............................................................... 67
REFERENCES ..................................................................... 77

APPENDIX A Summary of Lesions in Male Rats
in the 2-Year Drinking Water Study of Sodium Fluoride .......... 87

APPENDIX B Summary of Lesions in Female Rats
in the 2-Year Drinking Water Study of Sodium Fluoride .......... 157

APPENDIX C Summary of Lesions in Male Mice
in the 2-Year Drinking Water Study of Sodium Fluoride .......... 215

APPENDIX D Summary of Lesions in Female Mice
in the 2-Year Drinking Water Study of Sodium Fluoride .......... 281

APPENDIX E Summary of Lesions in Male and Female Rats
and Mice at the First Interim Evaluation
in the 2-Year Drinking Water Studies of Sodium Fluoride .......... 355

APPENDIX F Summary of Lesions in Male and Female Rats
and Mice at the Second Interim Evaluation
in the 2-Year Drinking Water Studies of Sodium Fluoride .......... 361

APPENDIX G Organ Weights and Organ-Weight-to-Body-Weight Ratios .......... 377

APPENDIX H Genetic Toxicology ........................................ 387
### APPENDIX I  
**Supplemental Studies** ............................................. 399  
6-Month Studies: Fluoride Concentrations in Bone, Plasma, and Urine . 400  
2-Year Studies: Fluoride Concentrations in Bone, Serum, and Urine .... 403  
2-Year Studies: Hematology and Clinical Chemistry .................... 408  
2-Year Studies: Urinalysis and Urine Concentration .................... 413  
2-Year Studies: Bioavailability ....................................... 416

### APPENDIX J  
**Chemical Characterization and Dose Formulation** .................... 417

### APPENDIX K  
**Ingredients, Nutrient Composition, and Contaminant Levels**  
in the Low Fluoride Rat and Mouse Ration ............................. 427

### APPENDIX L  
**Water and Compound Consumption**  
in the 2-Year Drinking Water Studies of Sodium Fluoride ............ 435

### APPENDIX M  
**2-Year Sodium Fluoride Studies Using a Low Fluoride,**  
Semisynthetic Diet .................................................................. 441

### APPENDIX N  
**Sentinel Animal Program** ............................................. 445
ABSTRACT

NaF

SODIUM FLUORIDE
CAS No. 7681-49-4
NaF
Molecular Weight: 41.99

Sodium fluoride is a white, crystalline, water-soluble powder used in municipal water fluoridation systems, in various dental products, and in a variety of industrial applications. Toxicology and carcinogenesis studies were conducted with F344/N rats and B6C3F1 mice of each sex by incorporating sodium fluoride into the drinking water in studies lasting 14 days, 6 months, and 2 years. In addition, genetic toxicology studies were performed with Salmonella typhimurium, with mouse L5178Y cells, and with Chinese hamster ovary cells.

14-Day Studies: Rats and mice received sodium fluoride in drinking water at concentrations as high as 800 ppm. (Concentrations are expressed as sodium fluoride; fluoride ion is 45% of the sodium salt by weight.) In the high-dose groups, 5/5 male and 5/5 female rats and 2/5 male mice died; one female rat given 400 ppm in the drinking water also died before the end of the studies. No gross lesions were attributed to sodium fluoride administration.

6-Month Studies: Rats received concentrations of sodium fluoride in drinking water as high as 300 ppm, and mice as high as 600 ppm. No rats died during the studies; however, among the mice, 4/9 high-dose males, 9/11 high-dose females, and 1/8 males in the 300 ppm group died before the end of the studies. Weight gains were less than those of controls for rats receiving 300 ppm and mice receiving 200 to 600 ppm.

The teeth of rats and mice receiving the higher doses of sodium fluoride were chalky white and chipped or showed unusual wear patterns. Mice and male rats given the higher concentrations had microscopic focal degeneration of the enamel organ. Rats receiving 100 or 300 ppm sodium fluoride had minimal hyperplasia of the gastric mucosa of the stomach, and one high-dose rat of each sex had an ulcer. Acute nephrosis and/or lesions in the liver and myocardium were observed in mice that died early, and minimal alterations in bone growth/remodeling were observed in the long bones of mice receiving sodium fluoride at concentrations of 50 to 600 ppm.

The sodium fluoride concentrations selected for the 2-year studies in both rats and mice were 0, 25, 100, and 175 ppm in the drinking water. These concentrations were selected based on the decreased weight gain of rats at 300 ppm and of mice at 200 ppm and above, on the incidence of gastric lesions in rats at 300 ppm in the 6-month studies, and on the absence of significant toxic effects at sodium fluoride concentrations as high as 100 ppm in an earlier 2-year study.

Body Weights and Survival in the 2-Year Studies: Mean body weights of dosed and control groups of rats and mice were similar throughout the 2-year studies. Survival of rats and mice was not affected by sodium fluoride administration. Survival rates after 2 years were: male rats—control, 42/80; 25 ppm, 25/51; 100 ppm, 23/50; 175 ppm, 42/80; female rats—59/80; 31/50; 34/50; 54/81; male mice—58/79; 39/50; 37/51; 65/80; female mice—53/80; 38/52; 34/50; 52/80.

Neoplastic and Nonneoplastic Effects in the 2-Year Studies: The teeth of rats and mice had a dose-
dependent whitish discoloration, and male rats had an increased incidence of tooth deformities and attrition leading on occasion to malocclusion. The teeth of male and, to a lesser degree, female rats had areas of microscopic dentine dysplasia and degeneration of ameloblasts. Dentine dysplasia occurred in both dosed and control groups of male and female mice; the incidence of this lesion was significantly greater in high-dose than in control male mice. Osteosclerosis of long bones was increased in female rats given drinking water containing 175 ppm sodium fluoride. No other significant nonneoplastic lesions in rats or mice appeared related to sodium fluoride administration.

Osteosarcomas of bone were observed in 1/50 male rats in the 100 ppm group and in 3/80 male rats in the 175 ppm group. None were seen in the control or 25 ppm dose groups. One other 175 ppm male rat had an extraskeletal osteosarcoma arising in the subcutaneous tissue. Osteosarcomas occur in historical control male rats at an incidence of 0.5% (range 0-6%). The historical incidence is not directly comparable with the incidences observed in this study because examination of bone was more comprehensive in the sodium fluoride studies than in previous NTP studies of other chemicals, and the diet used in previous studies was not controlled for fluoride content. In the current study, although the pairwise comparison of the incidence in the 175 ppm group versus that in the controls was not statistically significant, osteosarcomas occurred with a statistically significant dose-response trend, leading to the conclusion that a weak association may exist between the occurrence of these neoplasms and the administration of sodium fluoride. No other neoplastic lesions in rats or mice were considered possibly related to chemical administration.

**Genetic Toxicology:** Sodium fluoride was negative for gene mutation induction in *Salmonella typhimurium* strains TA100, TA1535, TA1537, and TA98 with and without S9. In two laboratories, sodium fluoride was tested for induction of trifluorothymidine resistance in mouse L5178Y lymphoma cells; results were positive both with and without S9. Sodium fluoride was tested for cytogenetic effects in Chinese hamster ovary (CHO) cells in two laboratories. In the first laboratory, the sister chromatid exchange (SCE) test was negative with and without S9, and the chromosomal aberration (Abs) test was positive in the absence of S9; in the second laboratory, the SCE test was positive with and without S9, but no induction of Abs was observed. The laboratory that reported a negative result for Abs tested at doses below those shown to be positive in the other laboratory. Similarly, the positive SCE result was obtained at a higher dose and longer harvest time than was used by the laboratory reporting the negative SCE response.

**Conclusions:** 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. "Equivocal evidence" is a category for uncertain findings defined as studies that are interpreted as showing a marginal increase of neoplasms that may be related to chemical administration. There was no evidence of carcinogenic activity in female F344/N rats receiving sodium fluoride at concentrations of 25, 100, or 175 ppm (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 25, 100, or 175 ppm in drinking water for 2 years.

Dosed rats had lesions typical of fluorosis of the teeth and female rats receiving drinking water containing 175 ppm sodium fluoride had increased osteosclerosis of long bones.

---

*Explanation of Levels of Carcinogenic Activity is on page 8. A summary of peer review comments and the public discussion on this technical report appear on page 10.
### Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Sodium Fluoride

<table>
<thead>
<tr>
<th>Variable</th>
<th>Male F344/N Rats</th>
<th>Female F344/N Rats</th>
<th>Male B6C3F1 Mice</th>
<th>Female B6C3F1 Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Doses</strong></td>
<td>Control, 25, 100, or 175 ppm in drinking water for 2 years</td>
<td>Control, 25, 100, or 175 ppm in drinking water for 2 years</td>
<td>Control, 25, 100, or 175 ppm in drinking water for 2 years</td>
<td>Control, 25, 100, or 175 ppm in drinking water for 2 years</td>
</tr>
<tr>
<td><strong>Body weights</strong></td>
<td>Exposed similar to controls</td>
<td>Exposed similar to controls</td>
<td>Exposed similar to controls</td>
<td>Exposed similar to controls</td>
</tr>
<tr>
<td><strong>Survival rates</strong></td>
<td>42/80; 25/51; 23/50; 42/80</td>
<td>59/80; 31/50; 54/50</td>
<td>58/79; 39/50; 65/80</td>
<td>53/80; 38/52; 34/50; 52/80</td>
</tr>
<tr>
<td><strong>Neoplasms</strong></td>
<td>Osteosarcoma of bone (0/80; 0/51; 1/50; 3/80)</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td><strong>Nonneoplastic lesions</strong></td>
<td>Dentine dysplasia, degeneration of ameloblasts, attrition, deformity, and discoloration of teeth</td>
<td>Osteosclerosis, dentine dysplasia, degeneration of ameloblasts, attrition, deformity, and discoloration of teeth</td>
<td>Tooth discoloration</td>
<td>Tooth discoloration</td>
</tr>
<tr>
<td><strong>Level of evidence</strong></td>
<td>Equivocal evidence</td>
<td>No evidence</td>
<td>No evidence</td>
<td>No evidence</td>
</tr>
<tr>
<td><strong>Genetic toxicology</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. typhimurium</em> (gene mutation):</td>
<td>Negative with and without S9</td>
<td>Positive with and without S9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LS178Y mouse lymphoma:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCE (CHO cells <em>in vitro</em>):</td>
<td>Positive with and without S9*</td>
<td>Positive without S9*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abs (CHO cells <em>in vitro</em>):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Positive results in this assay were obtained at higher doses than those that produced negative results in another laboratory.
EXPLANATION OF LEVELS OF EVIDENCE

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the 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. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

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 cannot be evaluated because of major flaws (inadequate study). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the technical report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **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.

- **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.

- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemically related.

- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemically related increases in malignant or benign neoplasms.

- **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. Such consideration 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:

- 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 incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.
Sodium Fluoride, NTP TR 393

PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the NTP draft Technical Report on sodium fluoride on April 26, 1990 are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

National Toxicology Program Board of Scientific Counselors
Technical Reports Review Subcommittee

Michael A. Gallo, Ph.D., Chair
Department of Environmental and Community Medicine, UMDNJ - Rutgers Medical School
Piscataway, NJ

Daniel S. Longnecker, M.D.
Department of Pathology
Dartmouth Medical School, Hanover, NH

Ellen K. Silbergeld, Ph.D.
University of Maryland Medical School, Baltimore, MD
Environmental Defense Fund
Washington, D.C.

Jay I. Goodman, Ph.D.
Department of Pharmacology and Toxicology
Michigan State University
East Lansing, MI

Ad Hoc Subcommittee Panel of Experts

John Ashby, Ph.D.
Central Toxicology Laboratory
Imperial Chemical Industries, PLC
Alderley Park, England

Gary P. Carlson, Ph.D.
Department of Pharmacology and Toxicology
Purdue University
West Lafayette, IN

Harold Davis, D.V.M., Ph.D.
School of Aerospace Medicine
Brooks Air Force Base, TX

Robert H. Garman, D.V.M.
Consultants in Veterinary Pathology
Murrysville, PA

Lois Swirsky Gold, Ph.D.
Lawrence Berkeley Laboratory
University of California
Berkeley, CA

David W. Hayden, D.V.M., Ph.D.
Department of Veterinary Pathobiology
College of Veterinary Medicine
University of Minnesota
St. Paul, MN

Curtis D. Klaassen, Ph.D.*
Department of Pharmacology and Toxicology
University of Kansas Medical Center
Kansas City, KS

Barbara McKnight, Ph.D.
Department of Biostatistics
University of Washington
Seattle, WA

Lauren Zeise, Ph.D.
California Department of Health Services/RCHAS
Berkeley, CA

*unable to attend
SUMMARY OF PEER REVIEW COMMENTS

On April 26, 1990, the draft Technical Report on the toxicology and carcinogenesis studies of sodium fluoride received public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

Dr. John Bucher, NIEHS, began the discussion by reviewing the experimental design, results, and proposed conclusions (equivocal evidence of carcinogenic activity in male rats, no evidence of carcinogenic activity in female rats or in male or female mice).

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.

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 from the discussion section 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." He noted that the propensity for fluoride to accumulate in bone made this tissue the most likely one for occurrence of a carcinogenic effect, yet the fact that fluoride accumulates in the bone of female rats and male and female mice to a similar extent as in male rats suggested caution in drawing general 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 that 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 remodeling. Dr. Garman suggested that any future studies include measurements of bone tensile strength.

Dr. Silberfeld noted the role of the NTP data in subsequent 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. Dr. Bucher agreed and said "Control" rather than "zero" would be used in table headings and better defined as to the level of fluoride in the diet of control animals. There was discussion by Dr. McKnight with Dr. J. Haseman, 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 suggested these 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 inquired 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.

Dr. John A. Yiamouyiannis, Director, Safe Water Foundation, Delaware, Ohio, stated that occurrence of a rare form of liver cancer, hepatocellular carcinoma, 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.

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, (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.

Dr. Robert d'Amato, Procter & Gamble Company, described their 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 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 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.

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 Technical 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."

Dr. John R. Lee, Marin County, 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.

Dr. Melvin Reuber, representing the Safe Water Foundation, Delaware, Ohio, commented on some of the tumors observed in the NTP studies as follows: (a) in commenting on the hepatocellular-giomas and hepatoblastomas 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 Zymbal'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.

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 note that the concentrations are much higher than the likely levels of fluoride in the human body. 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. He 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 sixth and twelfth months.

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) 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.

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." 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.

Dr. Gold noted 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 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. 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 difference 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 cited by Dr. Ashby be added to the conclusions. 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 conclusions. 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 to add this sentence was accepted by nine yes votes to two no votes (Drs. Silbergeld and Zeise).

Dr. Ashby moved that the proposed conclusions be accepted. Dr. Gold seconded the motion, which was accepted unanimously with 11 votes.