February 27, 2015

EPA-HSRB-15-01

Thomas A. Burke, Ph.D., MPH
EPA Science Advisor
Office of the Science Advisor
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Subject: January 14, 2015 EPA Human Studies Review Board Meeting Report

Dear Dr. Burke,

The United States Environmental Protection Agency (EPA or Agency) requested that the Human Studies Review Board (HSRB) provide scientific and ethics reviews of three items: a recent study of airway inflammation in asthmatics repeatedly exposed to nitrogen dioxide (Ezratty *et al.,* 2014), a pre-Rule publication by Spak *et al.* (1989) of gastric mucosal effects after ingestion of fluoride, and a pre-Rule publication concerning the effects of fluoride and calcium on spinal bone mineral content (Hansson and Roos, 1987). The Board's key responses to the charge questions are summarized in this letter and are detailed in the enclosed final meeting report.

A published report: Ezratty, Veronique *et al.* (2014) Repeated Nitrogen Dioxide Exposures and Eosinophilic Airway Inflammation in Asthmatics: A Randomized Crossover Study

Science

- If the statistical analysis cited was the methodology used, the Board finds that parts of the results may not be scientifically sound. If the authors, in fact, used a model and analysis procedure that corresponds to the statistical description of the double-blind crossover with repeated measures design, the HSRB finds the study to be scientifically sound.

- The study is adequate for use in a weight-of-evidence analysis in support of an inhalation risk assessment for the use of nitrogen dioxide as a medical equipment sterilant, pending resolution of the statistical issues noted in the enclosed report.

Ethics

- The Board concluded that the published report by Ezratty *et al*. (2014) submitted for review meets the applicable requirements of 40 CFR part 26 subpart Q.

A published report: Spak, C.J. *et al*. (1989) Tissue Response of Gastric Mucosa after Ingestion of Fluoride. Karolinska Institute, Huddinge University Hospital, Huddinge, Sweden

Science

- The study by Spak *et al*. (1989) is scientifically sound, providing reliable data.

- This study is adequate for point of departure use in support of an acute dietary risk assessment for fluoride.

Ethics

- Considering the time the study was conducted and based on the information provided, the Board found that no children or obviously pregnant or nursing women were included and the HSRB did not find convincing evidence that the study was conducted in a way that placed participants at increased harm or impaired their informed consent. Therefore, this study meets the ethical standards of 40 CFR part 26 subpart Q.

A published report: Hansson, T. and Roos, B. (1987). The Effect of Fluoride and Calcium on Spinal Bone Mineral Content: A Controlled, Prospective (3 years) study. Sahlgren's Hospital, University of Gothenberg, Sweden

Science

- While the lack of numerous details in the article reporting this study leads to the uncertainties noted separately by the Agency and the Board, the study appears to be scientifically sound and provides reliable data.

- Because the authors report that "some mildly adverse side effects" may have occurred below 30 mg sodium fluoride (NaF)/day (Hansson and Roos, 1987, p. 317) and adverse effects may have occurred below that dose that were not reported by subjects, this study cannot be used in support a LOAEL or NOAEL, but it may be used as part of the overall weight-of-evidence for a lower limit in an acute dietary risk assessment for fluoride.

Ethics

- Considering the time the study was conducted and based on the information provided, the HSRB found that no children or pregnant or nursing women were included and the Board did not find convincing evidence that the study was conducted in a way that placed participants at increased harm or impaired their informed consent. Therefore, this study meets the ethical standards of 40 CFR part 26 subpart Q.

Sincerely,

Rebecca T. Parkin, PhD, MPH
Chair
EPA Human Studies Review Board

NOTICE

This report has been written as part of the activities of the EPA Human Studies Review Board, a Federal advisory committee providing advice, information and recommendations on issues related to scientific and ethical aspects of human subjects research. This report has not been reviewed for approval by the Agency and, hence, the contents of this report do not necessarily represent the view and policies of the Environmental Protection Agency, nor of other agencies in the Executive Branch of the Federal government, nor does the mention of trade names or commercial products constitute a recommendation for use. You may obtain further information about the EPA Human Studies Review Board from its website at http://www.epa.gov/osa/hsrb. You may also contact the HSRB Designated Federal Officer, via e-mail at ord-osa-hsrb@epa.gov

In preparing this document, the Board carefully considered all information provided and presented by the Agency presenters, as well as information presented by public commenters. This document addresses the information provided and presented within the structure of the charge by the Agency.

US ENVIRONMENTAL PROTECTION AGENCY
HUMAN STUDIES REVIEW BOARD

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[1] Term ended February 28, 2015.

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Human Studies Review Board Staff

Toby Schonfeld, Ph.D., Human Subjects Research Review Official, Office of the Science Advisor, United States Environmental Protection Agency, Washington, DC

INTRODUCTION

On January 14, 2015, the United States Environmental Protection Agency's (EPA or Agency) Human Studies Review Board (HSRB or Board) met to finish the final report of the Board's November 5, 2014, meeting, and to address the scientific and ethical charge questions related to three agenda items: a recent study of airway inflammation in asthmatics repeatedly exposed to nitrogen dioxide (Ezratty *et al.*, 2014), a pre-Rule publication by Spak *et al.* (1989) of gastric mucosal effects after ingestion of fluoride, and a pre-Rule publication concerning the effects of fluoride and calcium on spinal bone mineral content (Hansson and Roos, 1987).

REVIEW PROCESS

The Board conducted a public meeting using Adobe Connect[2], on January 14, 2015. Advance notice of the meeting was published in the *Federal Register* as "Human Studies Review Board; Notification of a Public Meeting" (EPA, 2014, pp. 78861-78863).

Following welcoming remarks from Agency officials, the Board discussed the draft final report for their November 5, 2014, meeting. One revision was made before the Board voted to accept the report as final. The report has been posted on the HSRB website at http://www.epa.gov/hsrb/.

Then the Board heard presentations from EPA for the other three agenda items in sequence; all three were publications. This Final Report of the meeting describes the HSRB's discussion, recommendations, rationale and consensus in response to each charge question for each of these publications.

For each agenda item, Agency staff first presented their review of the science and the Board asked the Agency presenters clarifying questions. The staff then described their review of the ethical aspects and the Board asked clarifying questions about those. The HSRB solicited public comments and next asked Agency staff to read the Charge Questions for the publication under consideration. The Board discussed the science questions first and then the ethics question. The Chair then called for a vote to confirm concurrence on a summary statement in response to each charge question.

For their evaluation and discussion, the Board considered materials presented at the meeting, oral comments, the original published reports, related materials and published articles, the Agency's science and ethics reviews of the publications, and a public comment. A comprehensive list of background documents is available online at http://www.epa.gov/hsrb/.

[2] Accessed at *http://epa.connectsolutions.com/hsrb*.

CHARGE TO THE BOARD AND BOARD RESPONSE

A published report: Ezratty, Veronique *et al*. (2014) Repeated Nitrogen Dioxide Exposures and Eosinophilic Airway Inflammation in Asthmatics: A Randomized Crossover Study

Overview of the Study

This randomized, double-blind, crossover study of 19 non-smoker participants (14 men and 5 women, ages 20-69) was conducted in France to assess whether repeated short-term exposures to nitrogen dioxide (NO_2) would increase airway inflammation in asthmatics. All of the participants had intermittent asthma and house dust mite allergies (both of which were clinically confirmed), had either gas stoves or unvented combustion appliances in their homes, and had not had an airway infection for at least six weeks before baseline measurements were taken. Each participant served as his/her own control and was instructed to not use their gas appliances during the study period.

The research included three series of three exposures randomly administered while the participants were at rest in a temperature and relative humidity controlled exposure chamber; each of the exposures were to clean air, 200 ppb (380 $\mu g/m^3$) NO_2 or 600 ppb (1130 $\mu g/m^3$) NO_2. For each of the three-day exposure series, the administration of a single exposure level was for 30 minutes on day 1, twice – one hour apart – for 30 minutes each on day 2, and no exposure on day 3. Participants had two weeks free of exposure between each of the series. Methacholine challenge tests were done at baseline. Spirometry (forced expiratory volume in 1 second [FEV_1] and peak expiratory flow [PEF]) were conducted at baseline (10-30 days before exposure) and on each of the three study days in each series. Additionally, sputum induction, differential cell counts and an enzyme assay were used to assess inflammatory markers at baseline and on each of the 9 study days. Furthermore, participants were asked about respiratory symptoms and feelings of discomfort on each study day and, along with questions about medications, during the two-week period between each series of exposures.

Using generalized linear modeling (GLM) for the 18 participants who completed the study, the investigators determined that there were significant increases in eosinophil percentages and eosinophil cationic protein in sputum but only after the repeated exposures to 600 ppb NO_2; there was no effect on lung function at any exposure level.

Science

Charge to the Board

- Is this study scientifically sound, providing reliable data?
- If so, is this study adequate for quantitative use in support of an inhalation risk assessment for the use of nitrogen dioxide as a medical equipment sterilant?

Board Response to the Charge

HSRB Recommendations

- If the statistical analysis (GLM) cited was the methodology used, the Board finds that parts of the results may not be scientifically sound. If the authors, in fact, used a model and analysis procedure that corresponds to the statistical description of the double-blind crossover with repeated measures design, the HSRB finds the study to be scientifically sound.
- The study is adequate for use in a weight-of-evidence analysis in support of an inhalation risk assessment for the use of nitrogen dioxide as a medical equipment sterilant, pending resolution of the statistical issues noted in this report.

HSRB Detailed Recommendations and Rationale

Overall, the Board agreed with the Agency's scientific assessment of this study (Leshin, 2014) and commented that the research was very well documented by Ezratty *et al*. (2014). The clarity of their methods made the HSRB's review during the meeting quite brief.

Is this study scientifically sound, providing reliable data?
Based on their examination of the study methods, the Board determined that the study design and laboratory procedures were sound. The HSRB did not identify any concerns to bring forward to the Agency, thereby concluding that the data gathered in this study are reliable.

If so, is this study adequate for quantitative use in support of an inhalation risk assessment for the use of nitrogen dioxide as a medical equipment sterilant?
The statistical methods relied on well-known methods but may not have been appropriate for the type of data gathered from a crossover mixed model design with repeated measures. In particular, the Board questioned whether a mixed model procedure was used within the GLM analysis.

The experimental design of this study is a mixed model with 19 random subjects, 3 randomly assigned fixed crossover treatments (0, 200, and 600 ppb No2) within each subject and three repeated measurements (Day 1, 2 and 3) recorded from each treatment within each subject. Therefore, valid statistical analysis should include a repeated measures analysis with fixed treatments and random subjects, and a mixed model procedure such as MIXED in SAS software must be used to provide valid conclusions.

The random effects and the correlation among the repeated measures are *not* accounted correctly when using GLM methods. Therefore, the estimated standard errors, confidence intervals for treatment means, and the p-values reported for treatment mean comparisons are incorrect.

As a result of these findings, the Board recommended that the Agency verify from the authors of this study whether a MIXED or GLIMIXED procedure was used within the generalized linear mixed model analysis; if so, the results would provide valid conclusions.[3]

Ethics

Charge to the Board

- Does the study meet the applicable requirements of 40 CFR part 26, subpart Q?

Board Response to the Charge

HSRB Recommendation

- The Board concluded that the published report by Ezratty *et al*. (2014) submitted for review meets the applicable requirements of 40 CFR part 26 subpart Q.

HSRB's Detailed Recommendation and Rationale

The article by Ezratty *et al*. (2014) reports on a medical study that investigated whether exposure to repeated peaks of "realistic" levels of nitrogen dioxide (NO_2) induced changes in airway inflammation in non-smoking male and female adult volunteers with asthma (Ezratty *et al*., 2014, p. 854). The study was carried out beginning in 2007 at the Clinical Center of Investigation at Bichat Hospital, Paris, France, and was funded by the Center for Research and Innovation in Gas and New Energy Sources, CRIGEN, CDF Suez, the Medical Studies Department of EDF, and the Clinical Center of Investigation at Bichat Hospital, Paris, France. EPA identified the work from the published literature as a potential source of data for evaluating NO_2 as a sterilant of medical equipment.

In the Materials and Methods section of the article, the investigators report that the study was approved by the Ethics Committee of the Hôtel-Dieu Hospital in Paris, France, and was registered with the French Ministry of Health under study number DGS 2006/0016. The article states that investigators enrolled 19 participants (5 women, 14 men) ages 20-69 (Ezratty *et al*., 2014, p. 850), and that "All participants signed informed consent forms before enrollment in the study" (Ezratty *et al*., 2014, p. 851).

In response to a request from the Office of Pesticide Programs' (OPP) Human Studies Ethics Review Officer, Ms. Kelly Sherman (Sherman, 2014a, p. 21), Dr. Veronique Ezratty provided copies of the Ethics Committee's approval documents, the approved protocol, and the study's consent form (Aubier *et al.*, 2007, pp. 26-96). Although the article reporting the study's results was written in English, the protocol, consent document, and approval letter are in French. The protocol states that the study was to be carried out following the principles of the World Medical Association's (WMA) Declaration of Helsinki and in keeping with French national regulations

[3] Several weeks after the Board meeting, Ezratty reported to the Agency that a GLM procedure was used, and that a repeated measures analysis was run but not reported because it did not change the researchers' findings. However, the specific repeated measures methods used were not clarified in Ezratty's response.

on the conduct of clinical trials, known as the Huriet Law (Aubier *et al*., 2007, p. 46). It may be assumed that because the protocol was approved in 2007, the protocol follows the 2000 edition and clarifications of the Declaration in place at the time (WMA, 2004). Although the Ethics Committee of the Hôtel-Dieu Hospital does not have a Federal-wide assurance, the laws and ethical standards under which it operates are parallel to those that guide Institutional Review Boards in the U.S. (Berlin & Gorelick, 2003).

Does the study meet the applicable requirements of 40 CFR part 26, subpart Q?

The Board concurred with the conclusions of OPP's Ethics Review (Sherman, 2014a) that the reported research does not rely on data from intentional exposure of any human subject who was a pregnant or nursing woman or a child. There is no evidence that the conduct of the research was fundamentally unethical or deficient relative to prevailing ethical standards at the time. The participants provided written informed consent, and there is no evidence that the research was conducted in a way that placed participants at increased risk of harm based on the knowledge available at the time the study was conducted.

1. Assessment of risks and benefits

The article reports that NO_2 is a "ubiquitous atmospheric pollutant" (Ezratty *et al*., 2014, p. 850) and that many people are exposed to NO_2 as an indoor air pollutant resulting from combustion, including cooking with gas, and outdoor pollution related to traffic. The highest concentration and longest duration of exposure studied were stated in the article to be consistent with those present in French homes where gas stoves are used for cooking meals. The primary benefit of the study was to society, by generating knowledge of the effects of repeated exposures to "realistic" levels of NO_2's on adults with asthma (Ezratty *et al*., 2014, p. 854).

The consent form identifies clearly that the study offered no benefit to participants (Aubier, 2007, p. 58), although the participants were members of a class of persons who may benefit in the future from knowledge gained in the study. There is no statement in the article regarding the balance between risk to participants and benefit to society. However, the approval of this study by the Ethics Committee of the Hôtel-Dieu Hospital can be interpreted as its assessment, under international ethical guidance and French regulatory standards, that the risks to participants did not outweigh the study's anticipated benefits.

2. Voluntary and informed consent of all participants

Participants were recruited from among adult patients at the Pulmonary-Allergy Clinic at the Bichat Hospital in Paris, France. Although vulnerable populations were not targeted for the study, it is not possible to tell whether participants from potentially vulnerable populations were recruited, or whether the investigators employed protections to minimize coercive recruitment of clinic patients.

To participate in the study, subjects were required to be non-pregnant adults with a diagnosis of intermittent asthma and no history of smoking within the previous 10 years. No children were enrolled. All participants signed an informed consent document in which they certified that they were adults, and that they were not pregnant or lactating. The consent document was

supplemented by a six-page document outlining the study's procedures, which also affirmed participants' right to withdraw from the study for any reason and stated that women who did not use contraceptives during the study were excluded from participation.

3. Equitable selection of study participants

Although vulnerable populations were not targeted, it is not possible to determine whether the selection of study participants was equitable or whether individual members of vulnerable populations were enrolled in the study. The final protocol (Aubier, 2007, p. 47) states that participants were to be compensated a total of €1500 (approximately US$2000 in 2007). This amount does not appear to have been disproportionate or coercive for participation in 12 study visits over several weeks.

A published report: Spak, C.J. *et al*. (1989) Tissue Response of Gastric Mucosa after Ingestion of Fluoride. Karolinska Institute, Huddinge University Hospital, Huddinge, Sweden

Overview of the Study

This single-dose study of fluoride (F) effects on the gastric mucosa was conducted in Sweden; it involved twelve voluntary participants (4 men and 8 women, ages 22-45). Each participant underwent two endoscopies after overnight fasts: the first fast occurred before the day of no dose and the first endoscopy (for the baseline control measurement);and the second fast took place before the participant ingested 20 ml sodium fluoride solution, containing 20 mg F (53 mmolls per liter). Endoscopies were done two weeks or more apart; the study endoscopy was conducted two hours after the participant ingested the solution. During both the control and study endoscopy, the mucosal integrity of the antrum and body of the stomach was examined and graded on a 0-4 scale. Additionally, two biopsy specimens were collected from each of these areas and categorized on a histopathological scale (0-3). The gastric mucosal control and response conditions in the stomach and antrum were evaluated first by a gastroenterologist during the procedure and second by a different gastroenterologist using a videotape taken during the endoscopies.

After taking the study dose, 100% of the participants had six or more petechiae or erosions of the stomach and 50% had a range of changes in the antrum. There were no indications of macroscopic changes in the esophagus or the duodenum. Based on the histopathological results, the authors noted that fluoride affected the gastric mucosa; it caused moderate injury to the surface epithelium, gastric pits and superficial stroma. The authors noted that 25% of the participants experienced nausea. Commenting that their study confirmed prior research results, Spak *et al.* (1989) concluded that only one dose of fluoride at the level then used to treat osteoporosis affected the gastric mucosa.

Science

Charge to the Board

- Is this study scientifically sound, providing reliable data?

- If so, is this study adequate for quantitative use in support of an acute dietary risk assessment for fluoride?

Board Response to the Charge

HSRB Recommendations

- The study by Spak *et al*. (1989) is scientifically sound, providing reliable data.
- This study is adequate for point of departure use in support of an acute dietary risk assessment for fluoride.

HSRB Detailed Recommendations and Rationale

Overall, the Board agreed with the Agency's scientific assessment of Spak *et al.* (D'Agostino, 2014a).

Is this study scientifically sound, providing reliable data?

Several issues were discussed to develop the response to this question.

1. Study design

No major concerns were identified in the study design. However, the authors did not provide a rationale for completion of the follow-up endoscopy 2 hours after exposure to the fluoride solution. This issue is potentially significant given that the erosive/irritant effects of fluoride are likely influenced by the duration of exposure. As such, the severity and extent of lesions observed at 2 hours may be increased or decreased as a function of the time chosen for endoscopic evaluation after chemical ingestion.

2. Endoscopy issues

The report did not provide photomicrographs of the lesions, so the Board could not evaluate the types of lesions reported or the appropriateness of the grading system. That said, the scale appears to be consistent with conventional pathological grading systems used at the time of the study.

3. Histopathological results

Spak *et al.* did not specify whether the histopathological measurements were graded blindly or whether "another gastroenterologist" was one or more reviewers (Spak *et al.*, 1989, p. 1686).

4. Control irritants

Citing one reference, the authors attempted to compare their findings to aspirin and other well-known gastric irritants. They noted that repeated dosing might result in mucosal adaptation as occurs with aspirin. However, no control for gastric toxicity was included in the study and no quantitative references to the toxicological effects of control irritants, such as aspirin, were provided.

In spite of these weaknesses, the experimental design, data collection and interpretation of findings were judged to be scientifically sound.

If so, is this study adequate for quantitative use in support of an acute dietary risk assessment for fluoride?

1. Wilcoxon signed rank test

Spak *et al.* (1989) used the Wilcoxon signed rank test for all of their statistical analyses. This test is the non-parametric rank equivalent of the paired t-test. Differences between the treatment and control are calculated and their absolute values are ranked with adjustments made for tied differences. The statistic is the sum of the ranks corresponding to the positive differences. The assumptions underlying the test are that the differences represent a continuous variable whose probability distribution is symmetric about the hypothesized value of the median, which in this application is zero (Hollander and Wolfe, 1973, pp 27-32; Siegel and Castellan, 1988, pp 87-94). Violation of the underlying assumptions calls into question the validity of the statistical conclusions based on the test.

The footnotes to the table (Spak *et al.*, 1989, p 1686) indicate that the scale for the microscopic evaluation data consists of ordered categories. The scale for the macroscopic data consists of categories of grouped counts. All four sets of differences clearly do not satisfy the underlying continuity assumption of the Wilcoxon test and hence, call into question the validity of the p-values on which their conclusions are based.

2. Raw data conclusions

However, an examination of the raw data in the table does suggest that there are clear treatment effects in all but the macroscopic examination of the antrum. For those data, there was no change for five individuals, a reduction in petechiae or erosions for one individual, and an increase in six individuals (50% of the participants), which does not support the implication of the table footnote that there was a highly significant fluoride effect.

In summary, despite the questionable use of the Wilcoxon test, examination of the raw data does indicate that the results would be adequate to support the authors overall conclusions of the existence of fluoride effects.

Ethics

Charge to the Board

- Does the study meet the applicable requirements of 40 CFR part 26 subpart Q?

Board Response to the Charge

<u>HSRB Recommendation</u>

- Considering the time the study was conducted and based on the information provided, the Board found that no children or obviously pregnant or nursing women were included and

the HSRB did not find convincing evidence that the study was conducted in a way that placed participants at increased harm or impaired their informed consent. Therefore, this study meets the ethical standards of 40 CFR part 26 subpart Q.

HSRB's Detailed Recommendation and Rationale

Spak *et al.* (1989) present very little information about the ethical conduct of the study. Overall, the Board concurred with the Agency's review (Sherman, 2014b).

Does the study meet the applicable requirements of 40 CFR part 26 subpart Q?

The HSRB made the following observations about the study.

1. The publication does not indicate whether pregnant or nursing women were included in the study.
2. The age range begins at 22 years, so no children were participants in this research.
3. There is no information in Spak *et al.* (1989) about informed consent, compensation or recruitment, including whether the participants experienced coercion during recruitment.
4. There is no risk-benefit comparison in Spak *et al.* (1989). There was no known risk based on the knowledge available at the time of the study. The results of the study provided great value to society; it showed the need to reduce fluoride exposures to children.
5. The HSRB recognized that Sweden required IRB reviews beginning 1978, which was likely before this study was conducted.

A published report: Hansson, T. and Roos, B. (1987). The Effect of Fluoride and Calcium on Spinal Bone Mineral Content: A Controlled, Prospective (3 years) study. Sahlgren's Hospital, University of Gothenberg, Sweden

Overview of the Study

This controlled, three-year prospective study of 100 postmenopausal women with idiopathic osteoporosis was conducted in Sweden to evaluate the effects on bone mineral content (BMC) of daily doses lower than 30 milligrams of sodium fluoride (NaF). The women were randomly assigned to four groups: 30 mg NaF fluoride and calcium, 10 mg NaF and calcium, calcium only, and placebo. The first two groups were instructed to take the NaF in the morning and the calcium in the evening; however, there is no record of the participants' compliance with the daily dosing regimen over the three-year study period. BMC was measured using two methods: roentgenograms (one month after treatment began and after 3 years) and dual photon absorptiometry (at the start and at 1, 1.5, 2 and 3 years of treatment). Using the data from these instruments, bone profile curves were calculated for each participant. Eighty-eight women completed the study with dropouts occurring in each of the four groups.

The investigators concluded that only the daily doses in Group A (30 mg NaF and 1 g calcium) increased lumbar spine BMC significantly ($p<0.01$), occurring within 1.5 years; this increase was in the lower range for "age-matched normals" (Hansson and Roos, 1987, p. 316). The authors also noted that five of the 24 women in this higher dose group experienced side effects: four had

nausea and gastritis, and one dropped out at 10 months due to peptic ulcers likely unrelated to the treatments. Side effects occurred to lesser extents in the other three study groups.

Science

Charge to the Board

- Is this study scientifically sound, providing reliable data?
- If so, is this study adequate for qualitative use in support of an acute dietary risk assessment for fluoride?

Board Response to the Charge

HSRB Recommendations

- While the lack of numerous details in the article reporting this study leads to the uncertainties noted separately by the Agency and the Board, the study appears to be scientifically sound and provides reliable data.
- Because the authors report that "some mildly adverse side effects" may have occurred below 30 mg sodium fluoride (NaF)/day (Hansson and Roos, 1987, p. 317) and adverse effects may have occurred below that dose that were not reported by subjects, this study cannot be used in support a LOAEL or NOAEL, but it may be used as part of the overall weight-of-evidence for a lower limit in an acute dietary risk assessment for fluoride.

HSRB Detailed Recommendations and Rationale

This article presents a seemingly simple, scientifically sound study in a clear and concise manner but with a lot of missing details leading to the uncertainties noted both within the Agency science review (D'Agostino, 2014b) and by the Board. In addition, four further weaknesses suggest that the study (as presented) has limitations in its qualitative use in support of an acute dietary risk assessment for fluoride (F).

Is this study scientifically sound, providing reliable data?

1. Missing information

The Board identified the following six missing details that add uncertainty to either the quality or/and significance of the study:

a. No information was provided about recruitment or tracking of participants that could have an impact on compliance. The degree of noncompliance was mentioned by the authors as one explanation for the number of non-responders, and compliance could have had an impact on the incidence of side effects.
b. No information was provided about the level of blindedness within the study. For instance, differences in the nature and/or the number of capsules implied by the "treatment" column in Table 1 (Hansson and Roos, 1987, p. 316) may have been evident to the patients. Whether either individual patients or the researchers who interacted with

the patients knew (or could determine) which group a patient was in could also have affected compliance and the incidence of side effects.

c. Insufficient information was provided about the statistical approaches taken to analyze the data. It is not clear where the p-values came from.
d. Given the somewhat large number of patients who did not complete the study (12% overall) and their unequal distribution among the study groups, more details about the missing data could be helpful for understanding their implications on both BMC and side effects.
e. No data points are presented in Figure 1 (a plot of individual bone mineral content [BMC] over time) (Hansson and Roos, 1987, p. 316). Most of the lines look eye-ball straight, but some are slightly curved; however, the degree of line-smoothing is unknown. Thus, the degree of variance from the apparent smooth trends of BMC over time is unknown.
f. Insufficient information is presented about the distribution of side effects. Despite the reduction of side effects being a secondary goal of this study, actual data are only provided for group A (see below). The existence and number of side effects that happened in other groups is particularly relevant to the intended use by the Agency.

Despite these uncertainties, the Board agreed with the Agency that "The deficiencies do not change the conclusion of the study that gastrointestinal symptoms were observed following treatment with sodium fluoride. However, they do present some uncertainties which must be considered when interpreting the results" (D'Agostino, 2014b, p. 9).

If so, is this study adequate for qualitative use in support of an acute dietary risk assessment for fluoride?

2. Weaknesses

In addition to the above uncertainties, four weaknesses in the study were identified (a-d below).

a. The first pertains to the statistical analytic methods employed by the authors. This is a controlled, prospective study that was meant to address an interesting, practical question for women's health. It appears from the way the results are presented that changes over time were analyzed separately for each of the four treatment groups (including the controls). However, a repeated measures analysis seems to be in order.

The following three weaknesses have both an individual and collective bearing on the proposed use of this study by the Agency to establish a lowest-observed-adverse-effect level (LOAEL) and a no-observed-adverse-effects level (NOAEL).

b. None of the lines in Figure 1 (Hansson and Roos, 1987, p. 316) appear to approach a plateau indicative of a new steady state if NaF dosing were to be continued beyond three years. While such long-term exposures are clearly a chronic risk (rather than the acute toxicity as stated within the charge question) and the reported increases in BMC are beneficial, the existence of a steady state is essential to assure a benign long-term end point.
c. The next weakness results from the lack of information regarding the incidence of side effects at doses lower than 30 mg/day. Although the lack of data provides some grounds

for the statement in the Agency review that "No effects were observed in the lower fluoride treatment group, calcium only group, and placebo group" (D'Agostino, 2014b, p. 9), Hansson and Roos (1987, p. 317) state: "As in these earlier studies, however, not all individuals respond to fluoride treatment and there are some mildly adverse side effects even at low doses." This sentence suggests that such side effects occurred in this study as well, specifically to patients who received less than 30 mg NaF/day. Imbedded within this weakness is the uncertainty of whether any difference was really intended by the authors between the phrases "mild gastrointestinal symptoms" experienced by four of the patients in Group A (Hansson and Roos, 1987, p. 316) and "mildly adverse side effects" (Hansson and Roos, 1987, p. 317).

d. The last of these weaknesses refers back to the Spak *et al*. (1989) paper reviewed above. The fact that all twelve of their subjects tested at 20 mg F showed mucosal damage but only four (25%) developed nausea led those authors to conclude that "using nausea as the first sign of fluoride toxicity might not be valid" (Spak *et al.*, 1989, p. 1686). While the lowest dose of fluoride given by Hansson and Roos was 4.5 mg F (D'Agostino, 2014b, p. 10), those 1989 findings may have a bearing on the argument that an NOAEL can be based only on symptoms in this 1987 study.

Because a) Hansson and Roos (1987, p. 317) reported that "there are some mildly adverse side effects even at low doses," they imply that effects may have occurred at 10 mg NaF/day and b) Spak *et al.* (1989, p. 1686) implied that side effects may have occurred that were not reported by subjects, the Hansson and Roos (1987) study (as reported) cannot justify the two key statements in the Agency's science review; viz., "The LOAEL is 30 mg sodium fluoride/day (13.6 mg F/dose), based on gastrointestinal symptoms (nausea and gastritis)" and "The NOAEL is 10 mg sodium fluoride/day (4.5 mg F/dose)" (D'Agostino, 2014b, p. 6).

Ethics

Charge to the Board

- Does the study meet the applicable requirements of 40 CFR part 26 subpart Q?

Board Response to the Charge

HSRB Recommendation

- Considering the time the study was conducted and based on the information provided, the HSRB found that no children or pregnant or nursing women were included and the Board did not find convincing evidence that the study was conducted in a way that placed participants at increased harm or impaired their informed consent. Therefore, this study meets the ethical standards of 40 CFR part 26 subpart Q.

HSRB's Detailed Recommendation and Rationale

The Board generally agreed with the Agency's ethics assessment (Sherman, 2014c).

Does the study meet the applicable requirements of 40 CFR part 26 subpart Q?

The published article provided no information about whether any ethical oversight was provided, how the participants were recruited or compensated, whether informed consent of participants was obtained, whether they were told they could withdraw from the study, to what degree (if any) subjects and/or scientists were blinded to the treatment groups, or how risks to participants were minimized.

Given that the study was conducted on post-menopausal women, there were no children, pregnant or nursing women among the participants.

With very limited information provided about the conduct of the study, the Board determined that it was not possible to affirm whether the research, which was conducted in a foreign country, was done under procedures at least as protective as those in subparts A through L of Subpart Q (required by 26.1705).

However, the HSRB did not find clear and convincing evidence that the conduct of the research was fundamentally unethical (26.1704).

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