January 20, 2015

EPA-HSRB-14-03

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

Subject: November 5, 2014 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 two items: a new open pour protocol proposed by the Agricultural Handler Exposure Task Force, LLC (AHETF) and a pre-Rule publication by Frampton *et al.* (2002) concerning human exposure to nitrogen dioxide. The Board's key responses to the charge questions are summarized in this letter and are detailed in the enclosed final meeting report.

AHETF Protocol (AHE 170) – Open Pour Loading of Granules

Science

- The Board concludes that given the clearly defined boundaries of the protocol, if performed as described and as recommended by the HSRB, the AHE170 protocol is likely to generate scientifically reliable data that will be useful for assessing the exposure of those who perform open pour loading of granular pesticide products.

Ethics

- If the research is performed as described, it is likely to meet the applicable requirements of 40 CFR part 26, subparts K and L.

A Published Report by Frampton *et al.* (2002) on Nitrogen Dioxide Effects on Airway and Blood Cells

Science

- The sample collection and laboratory analysis parts of this study are scientifically sound. However, the chamber exposure data are not reliable without more information regarding the actual exposure levels to nitrogen dioxide.

- The statistical analysis is adequate to justify the significant differences that the authors identified, but is not adequate to detect all of the differences that may have occurred. This study may be used in a quantitative way as part of a weight-of-evidence analysis to support effects that might occur at the exposure levels reported (0.6 and 1.5 ppm), but this study (as published) is not relevant to support the existence of no effects at the claimed levels of exposure.

Ethics

- The Board concluded that the published report by Frampton *et al.* (2002) submitted for review meets the applicable requirements of 40 CFR part 26 subpart Q, and that the data within this article may be considered acceptable for EPA's reliance, contingent upon the determination of their scientific validity.

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
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INTRODUCTION

On November 5, 2014, the United States Environmental Protection Agency's (EPA or Agency) Human Studies Review Board (HSRB or Board) met to address the scientific and ethical charge questions related to two agenda items: a new protocol proposed by the Agricultural Handler Exposure Task Force, LLC on open pouring of granules (AHE170), and a published article by Frampton *et al.* (2002) regarding airway and blood cell responses to nitrogen dioxide exposures.

REVIEW PROCESS

The Board conducted a public meeting using Adobe Connect[1], on November 5, 2014. Advance notice of the meeting was published in the *Federal Register* as "Human Studies Review Board; Notification of a Public Meeting" (EPA, 2014, pp. 62437-62439).

Following welcoming remarks from Agency officials, the Board heard presentations from EPA for the two agenda items in sequence. This Final Report of the meeting describes the HSRB's discussion, recommendations, rationale and consensus in response to each charge question.

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 Board solicited public comments and next asked Agency staff to read the Charge Questions for the document 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 protocol and published report, related materials and published articles, and the Agency's science and ethics reviews. There were no public comments presented at this meeting. A comprehensive list of background documents is available online at http://www.epa.gov/hsrb/.

CHARGE TO THE BOARD AND BOARD RESPONSE

AHETF Protocol (AHE 170) – Open Pour Loading of Granular Pesticide Products

Overview of the Study

The Agricultural Handler Exposure Task Force, LLC (AHETF) designs and implements study protocols to assess workers' exposures in a variety of workplace scenarios. In this proposal, AHETF plans to evaluate experienced handlers' dermal and inhalation exposures to conventional pesticide formulations in granular form (e.g., passable through 4-79 mesh sieves). The study will

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

focus on open pour loading of pesticides from non-bulk containers into typical agricultural application equipment; application of these pesticides will not be evaluated in this study. EPA plans to use the results to estimate exposures to a variety of pesticides loaded in comparable conditions.

A 7x3 study design has been selected. Seven monitoring areas (MA) across the U.S. have been identified to represent the various climatic, crop and geographic conditions under which the test substances are applied. Within each area, three monitoring units (MU) will be determined; one handler will comprise each MU. The sample of handlers will be identified using a two-step process: screening for appropriate and willing commercial growers, who have appropriately experienced and interested workers; potential participants will be recruited through notices and/or meetings. Similarity restrictions will be used to ensure purposive diversity of the study. Eligible participants will be provided with written informed consent materials and time to review them before deciding whether they will take part in the study. Participants will be given an opportunity in the consent process to request their individual research results.

Growers will select one pesticide from a list of 10, known to be stable and having well-known analytical methods, for use on their property. During regular work days, participants will conduct typical but partially scripted tasks; e.g., transferring 50-lb. bags into spreaders, stacking bags, loading and unloading bags, and/or cleaning up equipment. One of the three ranges of the amount of the active ingredient handled (AaiH), from 5 to 400 lbs. AaiH, will be randomly assigned to the 4+ hour monitoring period for each MU. At least three loads will be poured per monitoring period. The workers will wear two layers of clothing: full-body work clothing plus a long-sleeved, long-legged whole body dosimeter (WBD) underneath. The under layer will be used to assess whole-body dermal exposures. Face/neck wipe and hand wash samples will be collected before exposure, before breaks and at the end of the monitoring period. Breathing zone air samples will be collected using standard personal air monitoring equipment (either fiber filters in cartridges or Occupational Safety and Health Agency (OSHA) Versatile Sampler (OVS) tubes with personal air sampling pumps). Fortified field samples will be used in triplicate to assess the stability of the active ingredient (AI) in the field, during transport and storage, and on sampling materials, such as face/neck wipes and the dosimeter. The study team will observe participants during their monitoring periods and note environmental conditions, personal protective equipment used, and participant activities.

The primary statistical objective is to estimate exposures within three-fold of the actual population values. The secondary objective is to ensure that the study has at least 80% power to distinguish proportionality from independence between exposure and AaiH.

Science

Charge to the Board

- Is this research likely to generate scientifically reliable data, useful for assessing the exposure of those who perform open pour loading of granular pesticide products?

Board Response to the Charge

HSRB Recommendations

- The Board concludes that given the clearly defined boundaries of the protocol, if performed as described and as recommended by the HSRB, the AHE170 protocol is likely to generate scientifically reliable data that will be useful for assessing the exposure of those who perform open pour loading of granular pesticide products.

HSRB Detailed Recommendations and Rationale

Overall, the Board agreed with the Agency's scientific assessment that the AHE170 protocol "addresses the technical aspects of applicable exposure monitoring guidelines and is likely to produce scientifically valid and useful data" (Evans and Sherman, 2014, p. 2) for assessing the exposure of those who perform open pour loading of granular pesticide products. Some aspects of the study are commented on below; these provide opportunities to improve the design or identify points that should be considered by the Agency when interpreting and using the data.

1. Surrogate Related Issues

The AHETF has identified a range of pesticides to use as surrogates for all active ingredients in granular formulations. The use of multiple pesticides as surrogates increases the chances of successfully identifying representative MUs for each AaiH level in each MA. The protocol states that "any of the AHETF surrogates can be used for generating exposure data for this scenario" (Collier, 2014, p. 35). However, the validity of this assertion for a given surrogate pesticide depends on the persistence of that pesticide in the granular formulation over the period of time between the start of exposure and the collection of the dosimeter or skin washes. If a given surrogate pesticide is lost from the formulation by volatilization, degradation, or by irrecoverable sorption into the skin during the exposure period, then the resulting exposure measurement for that specific pesticide will be biased low and result in an underestimate of exposure.

Differences in volatilization for each surrogate pesticide can be evaluated in part by comparing results of the measured vapor-phase inhalation exposure (measured with the OVS tubes) relative to the particle bound formulation (measured on the OVS filter). Differences in the degradation rate on the dosimeter and air samplers for the surrogates can be determined in part by the recovery of spiked pesticide from the fortified samplers if volatilization is found to be negligible. The data analysis for the protocol should include, at a minimum, a qualitative assessment of the relative persistence of the selected surrogate pesticides over the duration of the exposure. For those surrogates that are not completely conserved in/on the granular formulation and/or on the WBD, the data analysis should include a quantitative assessment of chemical loss and how that loss impacts the measured exposure for the open pour loading of granules.

Quantifying the loss of surrogate pesticide due to irrecoverable dermal sorption is more difficult in the absence of absorbed dose (AD) measurements, which are beyond the scope of the protocol. Clearly the loss of surrogate pesticide during the monitoring period by sorption into the dermal layer will bias the exposure measurements low, but it also has implications for the safety of the study and the calculation of margins of exposure (MOEs) as discussed below. The archived literature is rich with information about the performance of WBD (cotton long

underwear) as dosimeters for dermal exposure, particularly as a primary outer garment (i.e., as used in the Jazzercise™ exposure assessment scenario[2]). The efficiency of WBDs is affected by the efficiency of the fabric in collecting and retaining particles for the duration of the scenario-based activity, and retaining AI in the light of volatilization and loss via transfer to the skin. It is not clear whether there is good information on the relationship between the WBD-based exposure data and absorbed dose during field studies. For example, the protocol did not include information about how factors such as temperature and sweat saturation of the WBD garment would influence the estimation of absorbed dose from a whole body dosimeter.

2. Non-conservatism of the protocol

Non-conservatism of study results can be produced from uncertainty in the relationship between 1) exposure estimates based on face/neck wipe and hand wash and 2) the sorption of active ingredients from granules contacting the skin. For example, hands and exposed skin are to be washed to assess exposure. It is well-established that wash recovery efficiency declines with delay time (e.g., Fenske & Lu, 1994; Fenske *et al*., 1996). Granular formulations are typically about 10% AI (i.e., 100,000 ppm); this may be greater than saturation for most or all compound/carrier combinations. Therefore, some fraction of the AI on the outer surfaces of the granules will be readily available for transfer to skin and may behave, upon contact with skin, as pure compound. Recovery of this material would likely be incomplete even with rapid washing, so results will not be conservative. In addition, delay until washing will be variable and will introduce more variability in recovery efficiency. Particles are going to be very large so they will likely fall from the skin prior to washing, and some AI will be absorbed into the skin beyond the point of recovery; therefore, using either process as a basis would underestimate the exposure. Furthermore, the protocol provides for use of new gloves for each worker. This avoids exposure to residues in gloves from activities unrelated to the study but is non-conservative in that gloves can be contaminated under normal working conditions and workers are very unlikely to use fresh gloves every day. The Board recommends that these issues be considered and addressed in interpretation of study results.

3. Non-conservatism of the estimate of MOEs

The uncertainties in absorbed dose relative to the measured exposure can also lead to non-conservatism of stated margins of exposure and consequently of the study's safety. For example, four of the proposed surrogates (chlorpyrifos, imidacloprid, pendamethalin, and tefluthrin) have MOEs that reflect assumed dermal bioavailabilities that are less than 100%. Those assumed dermal bioavailabilities are probably taken from (non-specified) studies that are or may be very dissimilar to conditions relevant to granular pour operations (i.e., dry WBDs versus sweat soaked WBDs). Dermal bioavailability is not independent of loading conditions. Therefore, the assumed bioavailabilities cannot be presumed to be transferable. The Agency should review and document the loading conditions used to develop the dermal absorption factors for these four

[2] Around 1999, EPA's Office of Pesticide Programs decided to use transfer coefficients derived from Jazzercise™ to represent one hour of extreme exercise or four hours of typical activities. For more information on this, see "Overview of Issues Related to the Standard Operating Procedures for Residential Exposure Assessment" presented at the FIFRA Scientific Advisory Panel meeting on September 21, 1999 (pp. 2, 41, and 45-50). For technical information, see Ross, J. *et al.* (1990). Measuring potential dermal transfer of surface pesticide residue generated from indoor fogger use. *Chemosphere*. 20:349-360.

surrogates for their relevance to the field conditions to be tested within this protocol to assure that the MOEs are indeed adequate.

4. Statistical and sample design issues

The chosen sampling design that involves stratifying a large portion of the country into monitoring areas and selecting monitoring units within each area is an appropriate sampling design based on the nature of this study. The justification of the selection of monitoring areas is reasonable, given practical and cost constraints.

The limited randomness in the selection of monitoring units that would likely be achieved by the recruitment process as described in the documents was discussed at some length. Although the Board felt the proposed approach was acceptable given practical constraints, it recommends that, if feasible, some form of classification system be developed that would identify the mechanism by which each MU was recruited and in particular would identify those, if any, that might need to be recruited by "traditional recruitment" in order to provide an after-the-fact (future) indicator of the randomness of the MU selection process.

The objective of creating a database of exposures over a range of exposure scenarios may be achieved with the proposed sampling design for three types of agricultural pesticides, viz.: herbicides, insecticides, and fungicides. The design attempts to take into account climate/environment (7 MAs), usage (11 crops), pesticides (10), and amount of AI handled (3 AaiH ranges). In addition, the documents indicate that the best case scenario would be to have each MU within an MA be classified in a different AI range. Having at least one MU in every one of these combinations clearly is not possible with the chosen sample size.

It has been stated that no data currently exist for this particular exposure issue (Collier, 2014, p. 11; Evans and Sherman, 2014, p. 3). Each MU in the study is expected to perform a variety of tasks, but it is not clear if all or some or only one the 10 pesticides will be handled during the monitoring. Since the goal is to have each MU in an area have a different AaiH level, with three MUs and three AaiH levels, it appears that there are no replications of AaiH levels within an area. As a result, the variability among humans who would be exposed to a particular scenario cannot be addressed using only the information from this study.

Implementation of this protocol will provide quantitative information on a variety of exposure situations related to open pour or granule formulations. While statistical analyses are not the goal of this study, the information will surely be used in some way by someone for that purpose eventually. Lack of replication of the human variability would make it difficult or likely impossible for any user of the database to conduct further statistical analyses.

Ethics

Charge to the Board

- Is the research likely to meet the applicable requirements of 40 CFR part 26, subparts K and L?

Board Response to the Charge

HSRB Recommendation

- If the research is performed as described, it is likely to meet the applicable requirements of 40 CFR part 26, subparts K and L.

HSRB's Detailed Recommendation and Rationale

Overall, the Board agreed with the agency's assessment that this study is ethically acceptable. The Board's detailed reasoning is given below.

1. Risks to subjects

The risks to study participants are outlined in section 2.3 of the protocol (Collier, 2014, pp. 62-67), as follows: a) risk of heat-related illness, b) risk associated with scripting of field activities; c) psychological risks; d) risk of exposure to surfactants and e) risk of exposure to surrogate chemicals. Pregnant and nursing women will be excluded.

a. Regarding heat-related illness, the protocol states that this risk may be increased due to the fact that the subjects must wear an extra layer of clothing (long underwear (or WBD) for exposure monitoring during the manual pouring procedure). Likely, the more significant factors for heat-related illness would be temperature, humidity and level of activity of the subject. In other words, the addition of a single layer of clothing may not be significant in the overall risk of heat-related illness. Therefore, the statement that the use of the extra clothing constitutes greater than minimal risk in this study might be an overstatement.

   To minimize risk of heat-related illness, the protocol appropriately outlines steps for monitoring temperature and if necessary, using cycles of work and rest, moving the worker to a cooler environment, or conducting the testing during early morning or night when temperatures are lower. Given that the subjects will be individuals who are familiar with the manual pouring procedures and will be in good health, the risks are reasonable.

b. The risk of scripted activities is likely to be negligible, since it involves reducing the load size of the material to achieve three load/apply cycles per MU.

c. Likewise, the psychological risks appear to be minimal; they relate to changing clothes in the presence of a researcher, and for women, taking a pregnancy test, which is done in a private room without disclosure of test results to anyone outside the research team.

d. The protocol describes the risk from use of a surfactant (0.01% v/v Aerosol® OT in water). The surfactant is used as a very dilute solution and risks of skin irritation are likely to be negligible. An eye wash is available in case of accidental exposure to the eyes (Collier, 2014, pp. 64-65).

e. However, the handling of surrogate materials itself could, in theory, constitute greater than minimal risk. The standard of minimal risk relates to the probability and magnitude of harm

experienced in daily life[3] and this is generally interpreted as the daily life of *healthy individuals*—which could reasonably be taken to mean individuals in the general (not occupational) population who are not exposed to pesticides on a daily basis. In other words, the fact that agricultural workers are exposed to pesticides in their daily work does not necessarily mean this exposure would *a priori* be considered minimal risk using a general healthy person standard.

The risks of exposure to surrogate materials are described in section 2.3.5 (Collier, 2014, pp. 65-67). Since the study involves one day of exposure, the risks are those associated with acute toxicity, rather than chronic exposure. Table 1 of the protocol shows the dermal MOE for each surrogate (Collier, 2014, p. 66). The calculated MOEs meet or exceed the required minimum MOE in each case. Therefore, the risks are considered acceptable according to EPA standards for exposure to organophosphate pesticides (Collier, 2014, pp. 65-66). Whether or not the overall study constitutes minimal risk, or greater than minimal risk, is difficult to determine, but the risks are reasonably low and are well managed as described in the protocol.

Workers engaged in the pouring procedures for the study will be experienced pesticides handlers, will be using appropriate personal protective equipment (PPE) and will be reminded of safe handling practices.

2. Selection of subjects

The subjects will be offered participation in the study after their employer has agreed to allow recruitment of his/her workers (Collier, 2014, p. 32). Workers will be informed of the study and have an opportunity to learn about the study without their supervisors being present. Employers will sign consent documents stating that they will not influence or coerce their employees to participate in the study. It is not clear whether employees might be prohibited or discouraged from joining the study if employers did not feel it was in their (the employer's) interest to allow participation. Pregnant and nursing women will be excluded. Some workers in agricultural settings have low levels of literacy; these individuals will not be excluded from participation.

3. Informed consent

Informed consent will be conducted in individual sessions using prepared materials in English or in Spanish. For workers who cannot read or who are not comfortable reading consent materials, the materials will be read aloud by the study staff in the presence of a witness, per SOP AHETF 11.I.3 (Collier, date, pp. 261-266). These informed consent arrangements are acceptable.

[3] *Minimal risk* means that the probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests. The definition is in the Code of Federal Regulations at 40 CFR 26.102(i); it is available at http://www.gpo.gov/fdsys/granule/CFR-1999-title40-vol1/CFR-1999-title40-vol1-sec26-102/content-detail.html

4. Privacy and confidentiality

The main privacy issues relate to possible photographs or videos during the study activities, changing of clothing in the presence of researchers, and pregnancy testing. These risks are appropriately managed as described in section 2.3.3 of the protocol (Collier, 2014, pp. 64).

5. Monitoring of data

The protocol describes monitoring for heat-related illness in Section 2.3.1 (Collier, 2014, p. 62). Workers will be observed and reminded to use appropriate PPE during handling of the product. Nearby medical facilities will be identified and transportation and costs for any needed medical care will be provided, per section 2.3.5 (Collier, 2014, pp. 65-67).

6. Vulnerable or disadvantaged subjects

Given that some agricultural workers are economically disadvantaged, or may have low literacy levels, these individuals are potentially vulnerable. It is acceptable to enroll these subjects, given that the study involves low levels of risk, participants are appropriately compensated for their time, and consent procedures require adequate oral explanation of the study for those who cannot read. It is important that language issues are adequately addressed—for example, that bilingual or Spanish speaking witnesses are available if needed. For other workers who are not fluent in English, or who do not read English and are not Spanish-speakers, they would need to be excluded from the study due to the lack of translated forms and materials.

In summary, the study has appropriate procedures and protections in place for human subjects. The level of risk is low and the procedures to minimize risk are reasonable. The social value of conducting the research is significant and the overall risk/benefit ratio is acceptable.

A published report by Frampton *et al.* (2002) of intentional human exposures to nitrogen dioxide and their effects on airway and blood cells[4] [5]

Overview of the Study

This standard, three-period cross-over inhalation toxicity study was conducted to assess the "effects of nitrogen dioxide (NO_2) on airway inflammation, blood lymphocyte recruitment to the lung, and susceptibility of airway cells to infection with influenza virus and RSV [respiratory syncytial virus]" (Frampton *et al.*, 2002, p. L161). Twenty-one (12 male, 9 female) adult subjects participated in this study; they were recruited, from the population of students and community members around the University of Rochester. Eligible participants were evaluated individually in an environmental chamber during 1995-1996. The participants averaged 27 years old, were non-smokers, had no history of cardiac or respiratory diseases, and were free of respiratory infections for at least six weeks. They were exposed three times (to air and to 0.6 ppm and 0.15 ppm NO_2) for three hours each, during which they exercised intermittently several times. The

[4] Chair's note: Dr. Gary Chadwick recused himself from Board decisions and votes related to this study.
[5] Following this meeting, the EPA decided to not rely on this study.

exposure levels typified those found in indoor environments with unvented combustion sources (Frampton *et al.*, 2002, p. L155). The three exposure days were separated by at least three weeks.

Airway exposures were measured using standard spirometry methods, fiberoptic bronchoscopy, and airway brush biopsies. Blood cell counts were performed on the bronchoscopy and brush biopsy samples. Flow cytometry was used to assess cell differential counts, blood lymphocytes and other metrics. Cell cultures were used to determine the viability of respiratory and epithelial cells in the presence of influenza and RSV strains.

Analysis of variance (ANOVA) methods were used to evaluate the effects related to NO_2 exposures. Period, carryover, treatment effect and gender differences were examined. Residuals were assessed to determine whether the assumption of normality had been violated.

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

- The sample collection and laboratory analysis parts of this study are scientifically sound. However, the chamber exposure data are not reliable without more information regarding the actual exposure levels to nitrogen dioxide.

- The statistical analysis is adequate to justify the significant differences that the authors identified, but is not adequate to detect all of the differences that may have occurred. This study may be used in a quantitative way as part of a weight-of-evidence analysis to support effects that might occur at the exposure levels reported (0.6 and 1.5 ppm), but this study (as published) is not relevant to support the existence of no effects at the claimed levels of exposure.

HSRB Detailed Recommendations and Rationale

At its core, this paper describes some very detailed and scientifically sound biological sample collection and analytical methods. However, the Board noted issues that add to the Agency's review (Leshin, 2014). Specifically, the HSRB has four concerns related to the operation of the exposure chamber that greatly weaken the Board's confidence in the reliability of the study's data and two concerns about statistical methods (two concerns) that weaken the study's reliability and constrain its potential power.

1. **Exposure chamber**
   a. In regard to the chamber, on p. L156 Frampton *et al.* (2002) state that "For comfort, temperature and relative humidity were maintained at 37.1 ± 3.0°C and 21.2 ± 0.92%

(mean ± SD), respectively." This temperature (corresponding to 98.8°F) is outside of the normal comfort range and comprises a potential heat stress setting, especially when combined with an exercise regime "sufficient to increase the minute ventilation to 40 L/min" for 10 of each 30 minutes for three hours (Frampton *et al*., 2002, p. L156). No recognition of this risk is mentioned within the paper (or its associated consent information).

b. The authors state "The capabilities for generating and maintaining pollutant levels and constant temperature and humidity have been described previously (48)" (Frampton *et al.*, 2002, p. L156). This citation (ref. 48) is probably in error and should be ref. 49 (and likely reference 49 should be reference 48).[6] Reference 49 is a three-page extended conference abstract that describes the designed operating capabilities of this chamber: "This system [(S), (DX), (R) and (H)] was designed to vary the chamber temperature from about 10°C to 31.5°C and relative humidities [sic] from about 25 to 85%" (Utell *et al*., 1984, p. 220); these limits are repeated on page 221 in Table 1. Thus, the reported research by Frampton *et al.* (2002) seems to have been conducted outside of both the temperature and humidity ranges for which the chamber was designed. No comment regarding exceeding these limits was found within the paper.

c. Two of the Frampton *et al.* (2002) claims regarding NO_2 on page L156 are without any supporting methods, data, or references but are virtually word-for-word from the aerosols reported in reference 49 (Utell *et al*., 1984, p. 220). The first claim, "This [referring to ~0.3 atmospheric changes/min] enabled NO_2 levels to reach >90% of target levels within 4 min" (Frampton *et al*., 2002, p. L156), is technically incorrect. Comments elsewhere in reference 49 read, "A volumetric control is incorporated into the chamber air supply to stabilize the air flow at 10 m^3/min" (the same flow rate as in Frampton *et al*., 2002) and "the effective chamber volume (less instrumentation furniture, etc.) is approximately 40 m^3" (both quotes are from Utell *et al*., 1984, p. 220). Thus, the ideal (best case) air exchange time is 40 m^3 divided by 10 m^3/min or the four minutes referred to by the authors.[7] However, 2.3 air changes (at least 9 minutes) are required in order for any injected air component (like NO_2) to reach >90% of target levels (derived from: $-\ln(1 - C/C_{target}) = -\ln(1 - 0.9) = -\ln(0.1) = 2.3$). Less than ideal mixing would have extended the room's actual air exchange rate by 10% to 50% of its ideal value, and thus the time to reach 90% of its target level could have been 10 to 15 minutes. Because the authors do not describe the startup sequence of the exposure chamber, the Board could not determine how long in advance NO_2 might have been injected into the chamber and the magnitude of the effect of this delay on the exposure conditions experienced by the test subjects.

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[6] Their reference 48 is "Utell MJ, Frampton MW, Roberts NJ Jr, Finkelstein JN, Cox C, and Morrow PE. Mechanisms of nitrogen dioxide toxicity in humans. *Health Effects Institute Research Report* 43:1–44, 1991" (Frampton *et al*., 2002, p. L165). It appears that this reference to 48 should be to reference 49 that is "Utell MJ, Morrow PE, Hyde RW, and Schreck RM. Exposure chamber for studies of pollutant gases and aerosols in human subjects: design considerations. *J Aerosol Sci* 15: 219–221, 1984" (Frampton *et al*., 2002, p. L165). Reference 49 was probably also mistakenly cited on p. L155 of the Frampton *et al*. (2002) paper in reference to toxicity mechanisms "Because of its oxidative potential and limited solubility, NO_2 is a deep lung irritant, and accidental exposures to high concentrations can cause acute lung injury and death (13, 49)."

[7] Less than ideal mixing will extend this time to more than four minutes. On p. 221 of reference 49, the authors mention "a wall-mounted air circulation unit which is normally kept in operation to assist in chamber mixing" (Utell *et al*., 1984). Without more specifics, such a unit would probably keep the room's actual air exchange rate within 10% to 50% of its ideal value.

However, even a 15-minute transient is a small fraction of subjects' three-hour exposure periods.

d. Another claim is potentially of much greater concern. In their Methods section, the authors state that "The concentrations of NO_2 at the 3- and 6-ft levels within the chamber varied by no more than 5% of the mean" (Frampton *et al*., p. L156). Furthermore, in the Results, the authors claim that "Actual achieved NO_2 concentrations were 0.61 ± 0.02 and 1.50 ± 0.02 (SD) ppm" (Frampton *et al*., 2002, p. L157). However, no method of measuring the NO_2 was mentioned; measuring NO_2 concentrations is not a trivial matter, and its method is worthy of a description. While these oversights might just reflect the authors' focus on biologic responses and these results may be a repeatable measure of chamber performance, it is also possible that this claim of uniformity was simply copied from reference 49 that states "Sampling of test aerosol concentrations at 3' and 6' levels (3 x 3 matrix) within the chamber has revealed that the aerosol concentrations vary by no more than ± 5% of the mean" (Utell *et al.*, 1984, p. 220). In contrast to those test aerosol concentrations, Figure 1 of reference 49 (Utell *et al*., 1984, p. 220) indicates only one location for environmental monitoring ["EM"]. In fact, the authors state later that "Chamber monitoring is based on environmental factors, *e.g*. temperature, air flow, relative humidity, and the pollutant per se" (Utell *et al*., 1984, p. 220). This statement referenced by Frampton *et al.* to that citation "for generating and maintaining pollutant levels," and the lack of a method to measure NO_2 imply that the authors of Frampton *et al.* may have based their exposure values on the rate at which the 5000 ppm NO_2 compressed gas was emitted into the venturi mixer and diluted into the 10 m^3/min air supplied to the exposure chamber; *i.e.*, the reported concentrations of NO_2 may be merely calculated but unverified assumptions. Neither the length nor the material of the ducting that carried the air from the control and mixing equipment located in an adjacent engineering space to the exposure chamber was described within either paper. Since NO_2 is fairly reactive, it is at least possible - if not likely - that some of the supplied NO_2 was lost *en route* to (or within) the exposure chamber. If the test concentrations were calculated rather than measured, they were probably not as high as expected and reported. This uncertainty makes the exposure side of this study seem unreliable.

Although there is one author (Utell) common to both Frampton *et al*. (2002) and reference 49 (Utell *et al*., 1984), the four concerns noted above suggest that the authors of the former paper may have had a limited understanding of the exposure chamber itself.

2. Statistical issues

The statistical design and analysis procedures are clearly stated on page L157 of Frampton *et al.* (2002). The study's three-period cross-over design with washout periods was commonly used in this type of situation. Based on a document containing background material, an undescribed pre-study power analysis apparently indicated that four subjects for each of the six treatment sequences would provide adequate power (Sherman, 2014, p. 45). Additional subjects were recruited to allow for dropouts but data from only 21 subjects are reported in the paper; this number of actual participants does have an effect on the study's power. The analyses of variance included period and cross-over effects. Checks on model assumptions were carried out. These analysis procedures are standard and strengthen the acceptability of the numerical results.

a. The first statistical concern relates to the treatment of outliers. Frampton *et al.* (2002) state that "Data means shown in RESULTS include all study subjects, even though statistical outliers were excluded for the ANOVA" (p. L157). Deletion of outliers is an acceptable practice as long as it is reported as part of the methods. However, no indication was given as to the criteria by which an observation was identified as an outlier. More importantly, there was neither mention of which variables were affected nor the severity of the problem. As a consequence, in the Results, parts or all of Figures 1 through 6 do not accurately reflect the numerical results of the statistical analysis (pp. L159-L161). Moreover, the extent of the inaccuracies cannot be determined; Figure 4A (p. L160) is a good example of this issue. The authors indicate that the only significant effect is the NO_2 main effect. However, the figure shows a very large difference between male and female responses at 1.5 ppm which would be indicative of a potential gender × NO_2 level interaction. At best, this leads to potential confusion as to which effects may actually be significant.
b. The level of the second statistical concern depends upon the intended use of the Frampton *et al.* (2002) data. The ANOVA tests the authors used were adequate to support their conclusions that pulmonary responses were observed. However, in some cases the paper could have used more powerful statistical tests; these might have detected other significant responses that were not recognized. A good example is the respiratory ventilation data that comprise the first two data columns of Table 2 (at rest and while exercising) (p. L158). In both columns, the mean respiratory rates progress with increasing dose of NO_2, but the only test mentioned was Student's t-test for total NO_2 intake. The differences in ventilation at the two doses subjectively look rather large (*i.e.*, the increase in rates for all but the females while exercising are in the range of +4 to +9%); these could have been significant if the results had been analyzed using paired t-tests. Another statistical option might have been some form of regression analysis, but the authors only provided data for 0.6 to 1.5 ppm exposures, instead for the three sets of data (including 0 ppm) as was done for all other comparisons. The existence of such increases in ventilation rate would be consistent with the effects of such a respiratory irritant and with the decreases in hematocrit that were observed and presented in Figure 1 (p. L159) for all three test levels (0, 0.6, and 1.5 ppm).

In summary, the statistical methods used in the study do not detract from the significant responses that the authors identified nor from their conclusions, but the limitations to these tests imply that the authors may not have identified all of the statistically significant responses to these exposures that may be of interest to the Agency.

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 Frampton *et al.* (2002) submitted for review meets the applicable requirements of 40 CFR part 26 subpart Q, and that the data within this article may be considered acceptable for EPA's reliance, contingent upon the determination of their scientific validity.

HSRB's Detailed Recommendation and Rationale

The Board agreed overall with the Agency's assessment of the ethics of the study (Sherman, 2014). The Frampton *et al.* (2002) article, published in the *American Journal of Physiology of Lung Cellular and Molecular Physiology*, is the report of a medical study intended to test the effects of nitrogen dioxide (NO_2) on airway inflammation, blood cells, and resistance to respiratory viral infection in 21 normal, non-smoking adult male and female volunteers. The investigation was conducted at the University of Rochester, School of Medicine and was funded by the National Institutes of Health and the Center for Indoor Air Research. It was designed as a study of intentional exposure to NO_2 as an environmental pollutant, but EPA identified the work as a potential source of data for evaluating NO_2 as a sterilant of medical equipment.

In the Methods and Study Design section of the article, the investigators report that the study was approved by the University of Rochester's Research Subjects Review Board, the university's institutional review board (IRB) for protection of human subjects in research (Frampton *et al.*, 2002, pp. L156). At that time the IRB would have been operating under regulatory standards of the 1996 version of 45 CFR 46[8] and the ethical guidance of the Belmont Report[9].

The article states that investigators enrolled 21 subjects (9 females, 12 males) ages 18-40 and that "Informed consent was obtained" (Frampton *et al.*, 2002, p. L156). When interviewed by Agency staff in August 2014 and later contacted by email, the article's first author, Dr. Mark Frampton, reported that none of the participants was pregnant or nursing, and that subjects were tested for pregnancy before each exposure (Sherman, 2014, p.30).

In response to a request from EPA staff, the University of Rochester's IRB provided electronic copies of available records on this study, which included the study's protocol and consent document, and the IRB's approval letter. It may be assumed that the IRB approved the protocol following the above regulatory requirements in place at the time the study was reviewed.

The Board concurred with the conclusions of the OPP's Ethics Review (Sherman, 2014) 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. There is no evidence that 1) 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) or 2) vulnerable populations were targeted. However, because

[8] Protection of Human Subjects in the U.S. Code of Federal Regulations, 45 CFR part 46. For a timeline of these federal regulations, go to http://history.nih.gov/about/timelines_laws_human.html
[9] Available at http://www.hhs.gov/ohrp/humansubjects/guidance/belmont.html

recruitment processes focused on the University community where the study was conducted, and the protocol does not address additional protections for students or employees, there is a possibility that some participants' ability to give informed consent may have been impaired by coercion.

1. Assessment of risks and benefits

The article reports that many Americans are exposed to levels of NO_2 as an indoor air pollutant resulting from combustion, such as when natural gas-fueled ice resurfacing machines are used in enclosed ice rinks (Frampton *et al*., 202, p. L155). The primary benefit of the study was to society, by generating knowledge of NO_2's health effects. The highest concentration studied was a level stated in the consent form to be a level observed in homes where gas stoves are used. Risks of exposure to NO_2 are not clearly spelled out in the consent document, although the short-term risks of the bronchoscopy are identified. Neither the article nor the consent form addresses any potential benefit to participants or the balance of risks and benefits. The University of Rochester IRB's approval of this study can be interpreted as its assessment that the risks to participants did not outweigh the study's anticipated benefits under ethical and regulatory standards in place at the time of its review.

2. Equitable selection of study participants

Although vulnerable populations were not targeted, it is not possible to determine whether selection of study participants was equitable or whether individual members of vulnerable populations were enrolled in the study. According to the protocol and consent document, participants were compensated a total of $550 for taking part in all three phases of the study, with progressively increased exposure to NO_2, a blood draw, and bronchoscopy at each stage. Participants received $50 for completion of the first phase, an additional $50 for completion of the second phase, and additional $450 for completion of the third phase. Although the compensation schedule was structured to promote participants' completion of all three phases, the amount and schedule appear do not appear to have been disproportionate or coercive.

3. Voluntary and informed consent of all participants

Participants were recruited from among members of the University of Rochester community in Rochester, New York, including students and employees of the University. Although vulnerable populations were not targeted for the study, it is not possible to tell whether participants from potentially vulnerable populations were recruited. Similarly, there is no indication that the study protocol included mechanisms designed to minimize coercive recruitment and enrollment of students and employees who may have been in a subordinate position to the researchers.

To participate in the study, subjects were required to be healthy, non-pregnant adults with no history of smoking. No children were enrolled. After an initial conversation with the study coordinator by phone, potential participants met with one of the investigators to discuss the study. Potential participants received a copy of the consent document to take home for further review before deciding whether to enroll. In his communication with EPA staff, Dr. Frampton reported that participants signed consent documents, and documents from the IRB call for

signatures from the participant, an investigator, and a witness. Participants were informed in the consent document that they were able to withdraw from the study at any time.

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