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EPA-HSRB-11-02

Paul Anastas, PhD
EPA Science Advisor
Office of the Science Advisor
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Subject: April 13-14, 2011 EPA Human Studies Review Board Meeting Report

Dear Dr. Anastas,

The United States Environmental Protection Agency (EPA or Agency) requested that the Human Studies Review Board (HSRB) provide scientific and ethics reviews of several completed studies and scenarios involving intentional exposure of human subjects to pesticides or other EPA regulated compounds.

The first of these scenarios, a set of three interrelated studies measuring dermal and inhalation exposure of professional agricultural workers who spray pesticides using open-cab airblast equipment, was conducted by the Agricultural Handler Exposure Task Force, LLC (AHETF). These studies (AHE62, AHE63 and AHE64) were conducted after publication of the EPA's expanded final rule for protection of subjects in human research (40 CFR 26) on February 6, 2006 (71 Federal Register 24, 6137). These studies were conducted under a single scenario monograph (MRID 48326701), which incorporates the data from these three studies into a single dataset. An additional set of data, collected as part of a third-party sponsored protocol (AHE07) conducted prior to publication of the EPA's expanded final rule for protection of subjects in human research, will also be included in this data set. This data set will be posted to the Agricultural Handlers Exposure Database (AHED®), and used generically to estimate daily dermal and inhalation exposures of workers who treat agricultural crops with conventional pesticides using open-cab airblast equipment.

In addition, the Board reviewed a completed study of dermal and inhalation exposure of professional janitorial workers who clean indoor surfaces with an antimicrobial pesticide product using ready-to-use wipes or a trigger spray bottle and wipe, conducted by the Antimicrobial Exposure Assessment Task Force II (AEATF II). This study (AEA-02) was also conducted after publication of the EPA's expanded final rule for protection of subjects in human research. The data will be posted to the Biocide Handlers Exposure Database (BHED®), and used generically to estimate daily dermal and inhalation exposures of those who wipe indoor surfaces with antimicrobial pesticides.

The Board also reviewed a published study involving intentional human exposure to the zinc oxide contained in sunscreens. The Agency proposes to rely on this study, conducted after to publication of the EPA's expanded final rule for protection of subjects in human research, for regulatory actions.

Finally, the Board considered additional sponsor-provided information regarding the Kisicki *et al*. (1999) study on chlorpyrifos, which was originally discussed by the HSRB in June 2009 (EPA HSRB 2009). As the review and discussion of this additional information was conducted independently rather than at the request of the Agency, the Board's conclusions are attached to this meeting report as Appendix 1.

The enclosed report provides the Board's response to EPA charge questions presented at the April 13-14, 2011 meeting.

Assessment of Completed AHETF Research Studies AHE62, AHE63 and AHE64: Determination of Dermal and Inhalation Exposure to Workers During Airblast Applications of Liquid Sprays Using Open Cab Equipment (MRID 48289611, 48289612, 48289613, 48289614, 48289615, 48289616 and 48326701).

Science

The Board concluded that the research reported in the completed monograph, associated field study reports, and associated supplemental documents was conducted in a manner that was reasonably faithful to the design and objectives of the protocol and governing documents of the AHETF.

The Board also concluded that the Agency has adequately, but not completely, considered the limitations on these data that should be considered when using the data in estimating the dermal and inhalation exposure of those who apply conventional pesticides with open-cab airblast equipment. Additional limitations and concerns have been identified by the Board, and the conclusion as to the generalizability of these data requires further consideration and analysis.

Ethics

The Board concluded that the study was conducted in substantial compliance with subparts K and L 40 CFR 26.

Assessment of Completed AEATF II Research Study AEA02: Measurement of Potential Dermal and Inhalation Exposure during Application of a Liquid Antimicrobial Pesticide Product Using Trigger Spray and Wipe or Ready-to-Use Wipes for Cleaning Indoor Surfaces (MRID 48375601).

Science

The Board concluded that the research reported in the completed monograph, associated field study reports, and associated supplemental documents was conducted in a manner that was reasonably faithful to the design and objectives of the protocol and governing documents of the AEATF II.

The Board also concluded that the Agency has adequately, but not completely, considered the limitations on these data that should be considered when using the data in estimating dermal and inhalation exposures of handlers who are applying antimicrobial pesticides using a trigger spray or ready-to-use wipes.

Ethics

The Board concluded that the study was conducted in substantial compliance with subparts K and L 40 CFR 26.

Assessment of Published Research Study MRID 48387301: Gulson *et al.* (2010) Small Amounts of Zinc from Zinc Oxide Particles in Sunscreens Applied Outdoors Are Absorbed through Human Skin.

Science

The Board concurred with the Agency's assessment that the Gulson *et al.* (2010) study provides some potentially useful data on the dermal absorption of zinc from zinc oxide nanoparticles in sunscreen applied to human skin, despite the multiple limitations identified by the HSRB. However, the Board advised the Agency to proceed with caution when using these data in risk assessment, as these data cannot be used as a stand-alone set to assess dermal absorption of zinc oxide.

Ethics

The Board concluded that there was insufficient information available at the time of the April meeting to determine whether the Gulson *et al*. (2010) study was conducted in substantial compliance with procedures at least as protective as those in subparts A - L of EPA's regulation at 40 CFR Part 26. The Board recommended that EPA seek additional information from Macquarie University or the study investigator (including a copy of the research protocol and unsigned informed consent form) and that information be provided to the HSRB for reconsideration.

Sincerely,

Sean Philpott, PhD, MSBioethics
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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* Not in attendance at the April 13-14, 2011 Public Meeting

INTRODUCTION

On April 13-14, 2011, the United States Environmental Protection Agency's (EPA or Agency) Human Studies Review Board (HSRB) met to address scientific and ethical issues concerning two completed study monographs. One study measured levels of dermal and inhalation exposure received by janitorial workers who apply antimicrobial pesticides using trigger spray or ready-to-use wipes. The second was comprised of three completed, interrelated studies measuring levels of dermal and inhalation exposure received by pesticide applicators who spray pesticides with open-cab airblast equipment. These studies, along with a fourth study conducted prior to publication of the EPA's expanded final rule for protection of subjects in human research, were compiled into a single scenario monograph (MRID 48326701), which incorporates the data from these studies into a single data set. In accordance with 40 CFR 26.1602, EPA sought HSRB review of these two completed study monographs. The completed studies are discussed more fully below.

In addition, the Agency sought HSRB review of one published study of dermal absorption of zinc (Zn) from zinc oxide (ZnO) nanoparticles in sunscreen applied to human skin. This study, conducted after publication of the EPA's expanded final rule for protection of subjects in human research, was identified by Agency scientists from the peer reviewed literature. This study, which the Agency proposes to rely upon for regulatory actions, is discussed in detail below.

Finally, the HSRB considered additional sponsor-provided information regarding a pre-Rule intentional exposure study of chlorpyrifos, originally discussed by the Board in June 2009. The Board's review and discussion of this additional information was conducted independently rather than at the request of the EPA. The Board's discussion and conclusions are attached to this meeting report as Appendix 1.

REVIEW PROCESS

On April 13-14, 2011, the Board conducted a public face-to-face meeting in Arlington, Virginia. Advance notice of the meeting was published in the Federal Register as "Human Studies Review Board; Notice of Public Meeting" (76 Federal Register 59, 17122).

Following welcoming remarks from Agency officials, the Board heard presentations from EPA on the following topics: three completed studies (compiled into a single monograph) measuring dermal and inhalation exposure received by pesticide applicators who spray pesticides with open-cab airblast equipment, one completed study measuring levels of exposure received by janitorial workers when applying antimicrobial pesticides using a trigger spray or ready-to-use wipes, and one published study measuring dermal absorption of zinc (Zn) from zinc oxide (ZnO) nanoparticles in sunscreen applied to human skin.

The Board also asked clarifying questions of several study sponsors and/or research investigators, including:

Ms. Megan Boatwright, Analytical Coordinator, Golden Pacific Laboratories
Dr. Victor Cañez, Technical Chair, Agricultural Handler Exposure Task Force, LLC (AHETF).
Dr. Richard Collier, Administrative Committee Chair, AHETF.
Dr. Has Shah, Manager, Antimicrobial Exposure Assessment Task Force II (AEATF II).
Mr. Robert Testman, Vice-President, Golden Pacific Laboratories.

Public oral comments were provided by:

Dr. Victor Cañez, Technical Chair, AHETF.
Dr. Richard Collier, Administrative Committee Chair, AHETF.
Dr. Has Shah, Manager, AEATF II.

One written public comment was submitted by Ms. Carol Dansereau, Executive Director of the Farm Worker Pesticide Project, Seattle, WA.

For their deliberations, the Board considered the materials presented at the meeting, oral comments, and Agency background documents (e.g., published literature, sponsor and investigator research reports, study protocols, data evaluation records, and Agency science and ethics reviews of proposed protocols and completed studies). A comprehensive list of background documents is available online at http://www.regulations.gov.

CHARGE TO THE BOARD AND BOARD RESPONSE

Assessment of Completed AHETF Research Studies AHE62, AHE63 and AHE64: Determination of Dermal and Inhalation Exposure to Workers During Airblast Applications of Liquid Sprays Using Open Cab Equipment.

Overview of the Study

Three separate field studies were conducted, each monitoring dermal and inhalation exposure of workers to commercially available pesticides while spraying tree or trellis crops in three different U.S. states where open-cab airblast equipment is commonly used in production agriculture. A total of 13 professional agricultural handlers were monitored as they applied pesticides using open-cab airblast equipment: three adult men applying pesticides to grape vines in California (AHE62), five adult men applying pesticides to grape vines in New York (AHE63), and five adult men applying pesticides to pecan trees in Oklahoma (AHE64). The scenario design, protocols for the three studies, SOPs and governing documents were reviewed favorably by the HSRB at its October 21-22, 2008 (EPA HSRB 2008b).

Monitored on actual days of work, study participants handled from 7 to 90 lbs of active ingredient (carbaryl or malathion), spraying 3 to 24 acres in 1.4 to 10.6 hours. Dermal exposure was measured using hand washes, face/neck wipes, and whole body dosimeters (100% cotton union suits) for the remainder of the body (torso, arms, and legs). Inhalation exposure was measured using personal air sampling pumps and OVS mounted on the shirt collar. Results represent

dermal exposure with and without chemical-resistant hats while wearing a long-sleeved shirt, pants, shoes/socks and chemical-resistant gloves, and inhalation exposure without respiratory protection.

The Agency proposes to use data from these three studies, along with a previous open-cab airblast study involving 15 male participants applying pesticides to apple, orange, peach and pear trees in Florida, Georgia and Idaho (AHE07; Smith 2005), to estimate generically daily dermal and inhalation exposures of workers who treat agricultural crops with conventional pesticides using open-cab airblast equipment.

Science

Charge(s) to the Board

1. Was the research reported in the AHETF completed monograph report and associated field study reports faithful to the design and objectives of the protocol, SOPs and governing documents?

2. Has EPA adequately characterized, from a scientific perspective, the limitations on these data that should be considered when using the data in estimating exposure of those who apply pesticides with open-cab airblast equipment?

Board Response to the Charge(s)

HSRB Recommendation

The Board concluded that the research reported in the completed monograph, associated field study reports, and associated supplemental documents was conducted in a manner that was reasonably faithful to the design and objectives of the protocol and governing documents of the AHETF.

The Board also concluded that the Agency has adequately, but not completely, considered the limitations on these data that should be considered when using the data in estimating the dermal and inhalation exposure of those who apply conventional pesticides with open-cab airblast equipment. Additional limitations and concerns have been identified by the Board, and the conclusion as to the generalizability of these data requires further consideration and analysis.

HSRB Detailed Recommendations and Rationale

The HSRB concluded that the research reported in the completed monograph report and the associated field study reports of Open Cab Airblast Applicator Exposures (Bruce 2010a; Bruce 2010b; Bruce 2010c; Klonne and Holden 2010, Smith 2005) was faithful to the design and objectives of the protocol, SOPs, and governing documents. Four limitations were suggested as potential additions to those outlined in the Agency's Reviews of the monograph and associated field study reports (Crowley 2011; Crowley and Sarkar 2011). The magnitude of one of these

limitations cannot be determined but could significantly impact the accuracy of the measured exposures in handlers who are not wearing a chemical resistant (CR) hat.

The AHETF seems to have responded well to most of the concerns expressed by the HSRB during their prior reviews of the protocol (EPA HSRB 2006; EPA HSRB 2008b). For instance, the use of an informal survey/poll of local experts that identified the absence of the "Kinkelder" airblast sprayer, the use of a refined and better defined recruitment process, and the inclusion of descriptive records of applicator behavior. In the end, the analytical chemistry methods seemed appropriate and well chosen, the quality assurance (QA) considerations appeared to be in place, and the desired study conditions were largely obtained.

Although no formal statistical test for differences among crops or clusters was provided, the visual evidence in the Science Review (Figure 1; Crowley 2011, 12) is strong support for their similarity. The Board supports the Agency's plan to combine the datasets and to use a mixed-model approach for statistical analysis to account for potential data clustering for both the existing data and new monitoring. The Board also agrees with a similar approach and conclusion regarding the negligible effect of canopies used by six of the 28 monitoring units (MUs).

The use of head patches inside and outside CR hats was innovative. Its use obviated the need for an MEA for the portion of the head covered by the hat, probably increased the accuracy of measuring exposure with a hat, and yielded potentially useful estimates of the protection from exposure that the hat provided. At the same time, the use of this technique presents two limitations in estimating exposures without such a hat.

First, it could be argued that when estimating exposure without such a CR hat that the amount reaching the inner patch should not be counted. This argument would apply if the pathway by which the active ingredient that was deposited onto the inner patch was via a micro-scale form of drift under the hatband ("penetration") and not directly through the hat's material ("permeation"). The effect on the overall dermal exposure of this sort-of double counting the patch data is insignificantly small (on the order of 0.1%) due to the very small deposition measured on the inner patch.

Second, the use of this innovation may have reduced the accuracy of measuring exposure without a hat because the presence of a wide-brimmed hat probably also reduced deposition onto the exposed face and neck from what it would have been had the hat not been there at all. The magnitude of this reduction is undetermined and seems undeterminable with the information available. The brim is likely to have acted somewhat like a canopy, only much closer. Its effect is likely to have been greater on the portions of the face and neck closest to the underside of the hatband and to decrease on the portions further away, such as the tip of the nose. The effect of the brim's protection is probably clear but not overwhelming.

The Agency might reconsider applying a 1/2 limit of quantitation (LOQ) factor when no active ingredient is found in the backup portion of the OVS air samples. A proper sample should have no airborne chemical in that portion. In fact, finding active ingredient there implies some degree of overloading of the front portion of the sampling media. Consistently assigning a non-

detect measurement in the backup portion of a value of 1/2 limit of detection (LOD) results in a biased result.

Four additional limitations were suggested. The first is to include such qualitative data as the absence of the "Kinkelder" airblast sprayer from the array of equipment included within the study. This sprayer is an older model noted by the local experts to be prone to spray drift. The implications of higher drift on handler exposures depend on a combination of environmental conditions and work practices. However, the broader point is that such qualitative information could become a valuable resource to future users of the exposure database.

The second limitation is the potential underestimation of spray actually deposited onto the wiped portion of the subjects' head due to shielding by the brim of the CR hats that were always worn.

The third limitation is the possible exceptional status of one monitoring unit involved in exposure monitoring for open-cab spraying of Oklahoma pecans (Study AHE64; Monitoring Unit A5). This MU used nozzle pressure that was twice as high as that recommended for the type of nozzle (and three to four times as high as reported by any other MU using this type of nozzle). The Board recognizes that increasing nozzle pressure will cause a decrease in droplet size and an increase in spray drift. This MU also changed from spraying in both directions to spraying in only one direction for the last third of his application time. This was the only MU for which such a comment was made. Two reasons were suggested by the Board for why an applicator might make such a change in spraying direction: either to navigate sloping terrain or/and to avoid windblown drift. No information is available regarding the terrain, but the initial one way pattern resulted in this MU spraying only when driving upwind. Both of these reasons could cause this MU's exposures to be low. Whether these differences would otherwise disqualify this MU is unknown.

The fourth limitation is the need to rely on model accuracy for estimates to be interpretable. The primary objective is to estimate the geometric mean, the arithmetic mean, and the 95th percentile of normalized exposures (exposure per pound of active ingredient handled [AaiH]) within 3-fold with 95% confidence. The secondary objective is to assess proportionality (ln(*exposure*) = ln(*AaiH*)) against the alternative of independence in ln(*exposure*) and ln(*AaiH*) with 80% power. It is critical to carefully evaluate the validity of the model to ensure that the mean of the normalized exposures on the log scale (and the geometric mean on the data scale) are interpretable. Because clusters are part of the study's design, a random cluster effect should be included in the model. How the presence of a cluster effect impacts interpretation should be considered carefully.

In the Agency's Review (Crowley and Sarkar 2011), an alternative approach was taken in which the normalized exposures were modeled using a lognormal distribution. The validity of this approach depends on two assumptions. First, the expectation of ln(*exposure/AaiH*) must be linear. It is important to assess the validity of the linear relationship between ln(*exposure*) and ln(*AaiH*). Second, for the approach to be valid, there cannot be a cluster effect. Otherwise, the mean of the distribution would change with cluster so that a single lognormal distribution would not describe the normalized exposures. Perhaps this is why some of the Q-Q plots for the log-

normal distribution (Appendix B; Crowley and Sarkar 2011, 24-5) indicate some lack of fit, especially in the tails. (It is not possible to be sure that these correspond to the inhalation data for which a significant cluster effect was present because the graphs are not labeled.)

The Agency would benefit from carefully considering all assumptions underlying a proposed statistical approach and then checking the validity of each. Care should be taken to ensure that the results can be meaningfully interpreted. The term "arithmetic mean" should be changed to "mean," especially when a model is used to estimate the population mean. It would be helpful for the axes of all graphs to be labeled. Lastly, in the write-up of results, implying that the failure to reject a null hypothesis indicates that the null hypothesis is true, should be avoided.

The Agency should note that more detailed suggestions and analyses may be provided by some Board members independent of this consensus Board report.

Ethics

Charge to the Board

Does the available information support a determination that the studies were conducted in substantial compliance with subparts K and L of 40 CFR Part 26?

Board Response to the Charge

HSRB Recommendation

The Board concurred with the Agency's assessment (Sherman 2011a) that the study was conducted in substantial compliance with subparts K and L 40 CFR 26.

HSRB Detailed Recommendation and Rationale

The documents provided include reports of each of three field studies conducted on behalf of the AHETF (Bruce 2010a; Bruce 2010b; Bruce 2010c). The submitted study documents state that the study was conducted in substantial compliance with the ethical and regulatory standards of 40 CFR 26, Subparts K and L. The requirements of FIFRA §12(a)(2)(P) and Title 3, § 6710 of the California Code of Regulations also apply.

The three protocols were each reviewed and approved pursuant to the standards of the Common Rule (45 CFR Part 46, Subpart A) by an independent human subjects review committee, Independent Institutional Review Board, Inc. (IIRB, Inc.) of Plantation, FL. Minutes of IIRB, Inc. meetings and a copy of IIRB, Inc. policies and procedures were provided (Bruce 2010d; Bruce 2010e; Bruce 2010f; IIRB 2010a; IIRB 2010b; Sherman 2011a). IIRB, Inc. is registered with the US Office for Human Research Protections (OHRP; Reg. # IORG0002954) and is fully accredited by the Association for the Accreditation of Human Research Protection Programs, Inc. (AAHRPP).

1. The Board concurred with the conclusions and factual observations relating to the study, as detailed in the EPA's Ethics Review (Sherman 2011a) and summarized briefly below.

a. Prior HSRB and Agency Review.

Because each of these three studies was initiated after April 7, 2006, prior submission of the protocol and supporting materials to EPA was required by 40 CFR §26.1125. The requirements of 40 CFR §26.1125 for prior submission of the protocol to EPA and of §26.1601 for HSRB review of the protocol were satisfied. The scenario designs and study protocols were initially approved by IIRB, Inc. in July 2008. The HSRB discussed these three protocols at its October 2008 meeting, concurring with the Agency's assessment that these three proposed open cab airblast field study protocols, if revised as suggested by the Agency and the HSRB, would meet the applicable requirements of 40 CFR Part 26, subparts K and L (EPA HSRB 2008b).

b. Responsiveness to HSRB and Agency Recommendations.

The initial ethics reviews by the Agency (Carley and Evans 2008a; Carley and Evans 2008b; Carley and Evans 2008c) and by the HSRB (EPA HSRB 2008b) provided nine recommendations with regard to these three protocols. All of those recommendations, and the responses made with regard to them, are detailed in the submitted documents (Sherman 2011, 25). The HSRB agreed with the Agency that the comments by the Agency and HSRB were addressed satisfactorily.

2. The Board concluded that this study, as conducted, met all applicable ethical requirements for research involving human participants, in accordance with the following criteria:

a. Acceptable risk-benefit ratio.

The risks to study participants were minimized appropriately and were justified by the potential societal benefits, particularly data on the dermal and inhalation exposure of professional pesticide applicators to the liquid pesticides they apply to orchard and trellis crops using an airblast sprayer drawn by a vehicle with no cab. These data could be used to develop mechanisms to protect future persons who apply these liquid pesticides.

Minors and pregnant or lactating women were excluded from participation, with pregnancy confirmed by over-the-counter pregnancy testing on the day of study or by opt-out. The potential of stigma resulting from study exclusion was also appropriately minimized.

Clear stopping rules and medical management procedures were in place, and no adverse events or other incidents of concern related to product exposure were reported.

The study was designed to minimize the risks of exposure to the test compounds, subject to being able to accomplish the purposes of the study.

b. Voluntary and informed consent of all participants.

The study protocol included several mechanisms designed to minimize coercive recruitment and enrollment.

Monetary compensation was not so high as to unduly influence participation.

In addition, it is worth noting that twelve of the thirteen subjects were owner-operators, and thus less likely to be as vulnerable to coercion or undue influence as an employee might be.

3. Eight minor protocol deviations were reported. In addition, there was one unreported but minor protocol deviation. These are documented in detail in the EPA Ethics Review (Sherman 2011a, 8-10). The Board agrees with the Agency's assessment that these deviations did not compromise the informed consent process or put the study participants at increased risk.

4. The Board agrees with the observations in the EPA Ethics Review (Sherman 2011a) with regard to heat index thresholds. In particular, in November 2009—after this research had been conducted—the SOP for these types of studies was revised to lower the acceptable heat index threshold from 120º F to 105º F. However, as noted in Table 4 of the EPA Ethics Review (Sherman 2011a, 15), the heat index values for the subjects enrolled in this study never exceeded 105º F, so this was not an issue for this research.

Assessment of Completed AEATF II Research Study AEA02: Measurement of Potential Dermal and Inhalation Exposure during Application of a Liquid Antimicrobial Pesticide Product Using Trigger Spray and Wipe or Ready-to-Use Wipes for Cleaning Indoor Surfaces.

Overview of the Study

The objective of this study was to measure individual exposures to a surrogate antimicrobial pesticide (didecyl dimethyl ammonium chloride; DDAC) while cleaning surfaces with a trigger spray and wipe or with ready-to-use wipes. A total of 36 volunteers participated in the study, 18 cleaning surfaces using a trigger spray and wipe and 18 clearing surfaces using ready-to-use wipes in one of three building types (an office building, a Rite Aid pharmacy building, or a retired teacher's memorial building in Fresno, CA). Participants cleaned surfaces for one of six pre-determined wiping times (30-60, 60-90, 90-120, 120-150, 150-180 and 180-210 minutes total cleaning time, respectively). The study protocol, SOPs and governing documents were reviewed favorably by the HSRB at its April 9-10, 2008 meeting (EPA HSRB 2008a).

Dermal and inhalation exposure monitoring was conducted while study participants cleaned surfaces. Dermal exposure was measured by inner and outer body dosimeters. Airborne concentrations of the surrogate compound were monitored in the participant's breathing zone using an OVS tube sample collector connected to a personal sampling pump. Environmental conditions were also recorded at the time of monitoring, and observers made notes, photographs and videos of participant activity throughout the wiping period.

These exposure data will be used to populate a database representing inhalation and dermal exposure during a number of antimicrobial handler scenarios. A scenario is defined as a pesticide handling task based on activity (e.g., application) and equipment type (e.g., mop & bucket, ready-to-use wipes, pressure treatment of wood facilities, painting). These data will be used by the Agency to estimate dermal and inhalation exposures of antimicrobial handlers who are applying pesticides using a trigger spray and wipe or ready-to-use wipe under a variety of scenarios.

Science

Charge(s) to the Board

1. Was the research reported in the AEATF II completed wipe study report faithful to the design and objectives of the protocol and governing documents of the AEATF II?

2. Has EPA adequately characterized, from a scientific perspective, the limitations on these data that should be considered when using the data in estimating exposure of those who clean indoor surfaces with antimicrobial pesticides using a trigger-bottle and wipes or ready-to-use wipes?

Board Response to the Charge(s)

HSRB Recommendation

The Board concluded that the research reported in the completed monograph, associated field study reports, and associated supplemental documents was conducted in a manner that was reasonably faithful to the design and objectives of the protocol and governing documents of the AEATF II.

The Board also concluded that the Agency has adequately, but not completely, considered the limitations on these data that should be considered when using the data in estimating dermal and inhalation exposures of antimicrobial handlers who are applying pesticides using a trigger spray or ready-to-use wipes.

HSRB Detailed Recommendations and Rationale

The Board concurred with the EPA's assessment (Leighton 2011) that the AEATF II made the appropriate changes to the protocol proposed by the EPA and HSRB and has executed the study in a way that is reasonably faithful to the design and objectives of the protocol and governing documents of the AEATF II. Although several protocol deviations occurred, they were unlikely to have adversely affected the reliability of the data. The Agency's analysis of the data, especially their use of adjustments for non-detects in the fortified samples (of which there were more than a few) was an improvement.

There was one potentially important shortcoming of the study. The calculated rates at which the active ingredient was applied to the treated surfaces did not achieve the stated target application rate of 7 mg of active ingredient per m^2 of treated surface. Only one of the partici-

pants using ready-to-use wipes and only two of the participants using a trigger spray achieved the 7 mg/m^2 target. Neither the origin nor the significance of not meeting this target is known. While the protocol failed to achieve this technical target, this target was not recognized heretofore to potentially be in conflict with achieving the important goal of letting the subjects "wipe surfaces as they would normally work" (Selim 2011, 43). By all appearances, the study did achieve the latter goal.

The Board believes that the EPA has insufficiently characterized from a scientific perspective the limitations on these data. There were limitations that were not satisfactorily addressed. As an example, there were too many non-detects in the whole body dosimeters, which created the positive regression lines for the dermal exposures by anchoring at the low end. The assumptions of their modeling should be tested. Then, the appropriate regression models should be run on the results of the hand wipes (exposure by AaiH). If this is done, the effects of the non-detects can be evaluated in a step-wise fashion.

The results of the non-parametric bootstrap had narrower confidence limits and should be used instead of the parametric bootstrap results. Interpretations of the results should be re-evaluated, as they are currently too strong given the lack of any statistical significance. The lack of any relationship of inhalation exposure to AaiH should be accepted. The results of evaluating the K-factor, especially for inhalation, show the study to be underpowered to evaluate this association. Conclusions that estimates at the high end of AaiH are overestimates are definitely incorrect in the threshold model, which shows values below the estimated line at high AaiH and higher values in the middle of the range of AaiH that are elevating the curve.

Two potential new limitations to the study were also identified. One is an outgrowth of a search to explain the result that the dermal unit exposure value for the ready-to-use wipe applications is twice as large as that for the trigger spray bottle. The difference in results may be due to the different ways in which the amount of active ingredient handled was calculated in the two studies, and this explanation has implications to the validity of extrapolations based on AaiH. The two methods treated about the same amount of surface area in the same amount of time and resulted in about the same exposure, but the amount of biocide handled by the trigger spray bottle applicators was about twice that handled by ready-to-use wipe applicators. The study was not designed to tell how much active ingredient (ai) actually reached the target surface nor if the two methods were equally effective in treating the surfaces. Wiping has no intrinsic means to control the rate at which the active ingredient is applied. The rate depends completely on the applicator. The methods used to calculate the amount of ai handled may not directly relate to the amount. Neither method accounts for the amount of ai that stayed on the wipe or rag at the time it was discarded, and this unknown may have affected the calculated amount of ai handled. The possibility that the almost two-fold difference in the amounts handled (and in unit exposure values) is attributable to the difference in the amount left on the wipes and rags suggests a limitation to the data that should be considered when using the data. The need to extrapolate via unit exposures may be elevated due to the fact that the test conditions were well less than the target application rates of 7 mg ai/m^2.

The second limitation stems from the predominance of hand exposures. Noting that the hands account for 92% of the dermal exposure for the trigger spray bottle uses (Selim 2011, 107)

and 98% for the ready-to-use wipe users (Selim 2011, 98) brings attention to a potentially important limitation of the recovery correction factor based on the DDAC dermal exposure and recovery study conducted by Boatwright (2007). Although this study investigated the recovery at two dermal application rates, it appears that it did not investigate the effect of retention time on recovery from the skin. Without data one can only conjecture that the behavior of DDAC might mimic the behavior of the few insecticides in which similar tests have included time. In their January 2007 report, the EPA's Scientific Advisory Panel pointed out the importance of a chemical's residence time on the skin in the recovery efficiency (EPA SAP 2007). (This report was also cited by the HSRB in their June 2007 policy on MEAs.) Parts of that discussion implied that, in general, recoveries from skin are (or appear to be) higher soon after deposition and to decrease as either the chemical resides on the skin for longer durations or continuous or repeated applications saturate the ability of the skin to retain as much of the ai deposited later as it could earlier. Both of these scenarios result in what appear to be decreases in the recovery efficiency of those chemicals from skin as exposure time increases.

The Agency should note that more detailed suggestions and analyses may be provided by some Board members independent of this consensus Board report.

Ethics

Charge to the Board

Does the available information support a determination that the study was conducted in substantial compliance with subparts K and L of 40 CFR Part 26?

Board Response to the Charge

HSRB Recommendation

The Board concurred with the Agency's assessment (Sherman 2011b) that the study was conducted in substantial compliance with subparts K and L 40 CFR 26.

HSRB Detailed Recommendation and Rationale

The documents provided include a report of a completed wipe study conducted on behalf of the AEATF II (Selim 2011). The submitted study documents state that the study was conducted in substantial compliance with the ethical and regulatory standards of 40 CFR 26, Subparts K and L. The requirements of FIFRA §12(a)(2)(P) and Title 3, § 6710 of the California Code of Regulations also apply.

The protocol was reviewed and approved pursuant to the standards of the Common Rule (45 CFR Part 46, Subpart A) by an independent human subjects review committee, IIRB, Inc. of Plantation, FL. Minutes of IIRB, Inc. meetings and a copy of IIRB, Inc. policies and procedures were provided (IIRB 2010a; IIRB 2010b; Selim 2011; Sherman 2011b). As stated previously, IIRB, Inc. is registered with OHRP and is fully accredited by AAHRPP.

1. The Board concurred with the conclusions and factual observations relating to the study, as detailed in the EPA's Ethics Review (Sherman 2011b) and summarized briefly below.

   a. Prior HSRB and Agency Review.

      Because this study was initiated after 7 April 2006, prior submission of the protocol and supporting materials to EPA was required by 40 CFR §26.1125. The requirements of 40 CFR §26.1125 for prior submission of the protocol to EPA and of §26.1601 for HSRB review of the protocol were satisfied. The HSRB reviewed the protocol at its April 2008 meeting, concluding that "if the proposed mop and wipe scenario design, protocol, and supporting documentation is revised as suggested in EPA's review, the research would meet the applicable requirements of 40 CFR part 26, subparts K and L" (EPA HSRB 2008a, 2).

   b. Responsiveness to HSRB and Agency Recommendations.

      The HSRB noted two instances where the sponsor did not address comments raised by the HSRB in its April 2008 report. In the first instance, the HSRB recommended that the consent form be revised to explain that the "underlying purpose of the study will be to collect information that will be provided to the EPA, and that the EPA would use that information to determine the appropriate standards for allowable exposures to products such as the test compound" (EPA HSRB 2008a, 25). In the second instance, the Board recommended that the recruitment flyer should explain that the research would measure inhalation as well as dermal exposure (EPA HSRB 2008a, 26). The sponsors could not explain the lack of responsiveness to these two recommendations. The Board concluded that the lack of responsiveness to these two HSRB recommendations did not compromise the ethical conduct of the study, but recommended that the Agency follow up with the sponsor to determine why these recommendations were not addressed.

2. The Board concluded that this study, as conducted, met all applicable ethical requirements for research involving human participants, in accordance with the following criteria:

   a. Acceptable risk-benefit ratio.

      The risks to study participants were minimized appropriately and were justified by the potential societal benefits, particularly data on the dermal and inhalation exposure of professional janitorial workers to the liquid antimicrobials they apply. These data could be used to develop mechanisms to protect future persons who apply these liquid antimicrobials.

      Minors and pregnant or lactating women were excluded from participation. All study participants were at least 18 years old. All females self-administered an over-the-counter pregnancy test on the day of monitoring, and all such tests were negative. No nursing mothers were enrolled in the study.

The study was designed to minimize the risks of exposure to the test compounds, subject to being able to accomplish the purposes of the study. The study involved the use of a commonly used, commercially available cleaning product, in a matter consistent with its labeling.

Clear stopping rules and medical management procedures were in place. There was one report of a participant leaving early because he was feeling ill. Although the observation log does not indicate whether the worker was feeling ill as a result of his participation in the research or whether his illness was unrelated to the study, documentation provided to the Agency and the HSRB subsequent to the April 2011 meeting suggests that this participant was unwell prior to study initiation. However, there was no indication that there had been any follow up of the participant. Although there is little reason to think that this participant would develop a serious and unexpected medical problem related to his participation after leaving the study site, the Board recommended that the Agency work with sponsors to develop a SOP for following up with participants who leave a study early because of illness.

b. Voluntary and informed consent of all participants.

The study protocol included several mechanisms designed to minimize coercive recruitment and enrollment.

Monetary compensation was not so high as to unduly influence participation.

3. Two minor protocol deviations were reported. In addition, there were two unreported but minor protocol deviations. The reported deviations were that: 1) the protocol called for study participants to take 10-minute rest breaks, but most participants declined or took breaks that were less than 10 minutes in length; and 2) a photograph of a participant's face was taken at one monitoring site, although no images were included in the report.

The Agency review noted one unreported deviation (Sherman 2011b). This deviation involved the enrollment of three participants (one who was monitored and two who were alternates) who self-reported that their health was only "fair," despite the requirement that all participants be in "good health". In response to a similar protocol deviation that occurred during the related AEATF II mop study discussed at the October 2010 HSRB meeting, the Board recommended the AEATF II develop a SOP to clarify its health status reporting and inclusion criteria (EPA HSRB 2010). The Board noted that this study was conducted prior to the October 2010 HSRB meeting.

The HSRB also discovered an additional unreported deviation, namely the use of an "IRB-unapproved form to collect personal information about volunteers and to sign a 'Worker Health Statement'" (Selim 2011, 3). These forms were stored separately from the field phase data. The sponsors could not explain this deviation from the protocol.

The HSRB concluded that these protocol deviations did not compromise the informed consent process or put the study participants at increased risk.

Assessment of Published Research Study by Gulson *et al.* (2010): Small Amounts of Zinc from Zinc Oxide Particles in Sunscreens Applied Outdoors Are Absorbed through Human Skin.

Overview of the Study

In the Gulson *et al.* (2010) study, 20 human volunteers were exposed to sunscreens containing stable isotopes of zinc oxide (ZnO) for the purpose of measuring dermal absorption of zinc (Zn) from particles of varying size. Such metal oxide nanoparticles are being used more and more frequently in sunscreens because they reflect and absorb ultraviolet (UV) light as transparent coatings, rather than as opaque coatings as with traditional metal oxide sunscreens.

The study was conducted in Australia in March 2009. Ten male and ten female subjects, ranging from 19 to 66 years of age, were enrolled. Study participants wore UV protective upper body clothing with a patch removed to expose a small section of skin on the back. Sunscreen containing stable isotope ^{68}Zn oxide, formulated as "bulk" particles (110 ± 46 nanometers [nm] in size) or as nanoparticles (19 ± 8 nm in size), was applied twice daily for a period of five days to the exposed section of skin on the back. Eleven participants (5 males and 6 females) applied ^{68}Zn oxide formulated as nanoparticles and nine participants (5 males and 4 females) applied ^{68}Zn oxide formulated as bulk particles. Participants were encouraged to wear other non-zinc types of sunscreen on the areas of skin not covered by the UV-resistant clothing. The test material was removed from each subject's back at the end of each day with an alcohol-lanolin wipe.

Urine and blood samples were taken eight days before sunscreen application, immediately before the first application, at the end of each of the five days of application, and six days after the last application. Dermal absorption of Zn was calculated from the changes in the ratios of ^{68}Zn to ^{64}Zn in urine and blood samples during the course of the experiment; Zn was measured using Multi-Collector Inductively Coupled Mass Spectrometry (MC-ICP-MS).

Science

Charge(s) to the Board

1. Is the Gulson *et al*. (2010) study scientifically sound, providing reliable data?

2. If so, is the Gulson *et al*. (2010) study relevant for qualitative use in support of an assessment of the absorption of zinc oxide through the skin?

Board Response to the Charge(s)

HSRB Recommendation

The Board concurred with the Agency's assessment (Ryman and Dole 2011) that the Gulson *et al.* (2010) study provides some potentially useful data on the dermal absorption of zinc

from ZnO nanoparticles in sunscreen applied to human skin, despite the multiple limitations identified by the HSRB. However, the Board advised the Agency to proceed with caution when using these data in risk assessment, as these data cannot be used as a stand-alone set to assess dermal absorption of zinc oxide.

HSRB Detailed Recommendations and Rationale

The Gulson *et al.* (2010) study provides some potentially useful data on the dermal absorption of zinc from zinc oxide nanoparticles in sunscreen applied to human skin. The analytical methods used in the study appeared to be sound. The use of the ^{68}Zn as a tracer for Zn from the sunscreen was an innovative approach for assessing absorption and the presence of a chemical in body fluids (e.g., blood or urine) when the same chemical is also present endogenously. The Board also agreed with the comments of the Agency (Ryman and Dole 2011) that the distinction between bulk and nanoparticles was not as absolute as indicated in the study since the lower range of size for bulk particles is within the range of the size for nanoparticles. This overlap in size between the groups of particles should not affect the outcome of the studies and the interpretation of the results significantly. However, the Board raised a number of caveats, including lack of a proper control group, small sample size, and sample contamination, which limits the utility of this study in risk assessment of zinc oxide-based sunscreen.

While the analytical approaches used in the Gulson *et al.* (2010) study to measure Zn in blood and urine were solid, the study design itself was faulty. For example, there were no untreated controls to verify the consistency of Zn levels in untreated individuals. The presence of increases in ^{68}Zn in the individual who applied the sunscreen (hereafter referred to as "the applicator") creates questions regarding the changes that might occur with time independent of sunscreen application. The design was not a carefully controlled experimental trial, and therefore was subject to a variety of behavioral differences among the subjects as they pursued their beach recreation activities that are likely to have influenced the results.

Despite the fact that the tracer analytical technique that measured Zn in blood and urine did an excellent job in distinguishing endogenous Zn from Zn absorbed from the sunscreen, this procedure did not distinguish between dermal penetration of soluble Zn from penetration of intact nanoparticles, or from absorption via dermal versus oral routes. Therefore, this study has many uncertainties associated with the interpretation of the data, and does not provide information regarding the penetration of nanoparticles through intact, healthy skin. The study thus is of limited value in judging the level of intact nanoparticle absorption through intact skin.

The sunscreen formulation contained isopropyl myristate, which is a known chemical enhancer of skin penetration, while the wash off solution used contained alcohol and lanolin. The impact of these two variables on Zn absorption was not considered, nor did the study report explain how much Zn was removed by the daily alcohol and lanolin wash off procedure. Another source of concern is the potential impact of ethylenediaminetetraacetic acid (EDTA), a commonly used preservative and stabilizer, on the zinc oxide nanoparticle formulation. The authors did not address the possibility that EDTA can chelate ionic Zn released from the nanoparticle formulation. This is an important consideration since chelation of free zinc in the skin by EDTA

can influence the degree of dermal absorption. EDTA is routinely used in this type of sunscreen formulation, but its impact on dermal Zn absorption was not addressed.

The final study design was based on a pilot study in which the zinc oxide active ingredient was 51%, while the ^{68}Zn isotope in the final study was 99% enriched ^{68}Zn. The authors provide insufficient information on the pilot study (e.g., age, gender, or skin type of subjects). The Board concurs with the Agency's assessment of lack of clarity on how the pilot study was used in the design of the final study and subject selection.

Other variables not accounted for include the frequency and time of garment changes and the type of activities the subjects engaged in after the first 30-minute exposure interval. There was no mention either of the total length of the subjects' sun exposure and the timing between the first and second daily exposure. Although UV exposure was estimated to be the same for all subjects, differences in length and intensity of physical activity likely contributed to the degree of sweating and blood flow to the skin. No information was provided on the time of the second application of the sunscreen in relationship to the first application. Description of dosing intervals was missing.

The authors of the Gulson *et al.* (2010) study provided no justification or criteria for selecting subjects 4, 8, 9 and 13 used for complete analysis of all samples collected (Figure 8; 2010, 146). The use of retention rates to address the issue of urine sample contamination was a very good approach; however, there was no mention of how these potential contaminations occurred and the potential for hand contamination to have caused hand-to-mouth dosing of the subjects. The investigators also did not consider the effect of the ZnO that was retained in the applicator's hand(s) on the quantities of the dosing rates reported in Table 1 of the manuscript (Gulson *et al.* 2010, 143).

The investigators speculated that contamination of urine samples could result from subject's hands during urine collection. Six urine samples were allegedly contaminated with ^{68}Zn from sunscreen. In addition, it appears that the authors did not attempt to minimize, control for, or account for the possibility of the contaminated hand-to-mouth route of exposure among their subjects. While the authors provided a rather convincing post-hoc series of arguments to support a hypothesis of contaminated urine samples (Gulson *et al.* 2010, 146-7), they provided no description of a mechanism for how the zinc would have gotten from a subject's back into the urine sample. Since the collection containers were pre-cleaned and the beach towels provided to the subject were renewed daily in order to eliminate any potential source of sample contamination, the reader is left to infer that six of the ten female subjects somehow got a portion of the sunscreen from their backs onto their hands and subsequently from their hands into the urine collection containers. If this transport of sunscreen occurred with these subjects, then it is also plausible that ^{68}Zn from the sunscreen was on the hands of all the subjects but that not all subjects contaminated their urine samples. If that were the mechanism, then it also seems likely that sometime during the day these subjects had one or more occasions to transfer sunscreen into their mouths via food, beverages, mouth wiping and other hand-to mouth activities. Thus, it is plausible, if not likely, that the hands of many or all subjects were contaminated with ^{68}Zn, and that either some (if not most) of the ^{68}Zn measured in blood and urine was the result of the transfer from the subject's back into their hands and then to their mouths, leading to gastrointestinal ab-

Comment [Ryman1]: It was the study investigators, not the Agency, that speculated how this could occur. The Agency merely included it in the review.

sorption of ^{68}Zn. This alternative plausible mechanism could completely negate the validity of the authors' conclusions regarding dermal absorption.

Gulson and his colleagues may have also made significant errors in the quantities of the doses reported in Table 1 (Gulson *et al.* 2010, 143) by not considering the amount of ZnO that was retained on the applicator's hands after dosing each subject. The report does not say whether or not the applicator was wearing gloves or using the same one-finger application technique as described for their pilot study. Gloves would have been important in the pilot study to avoid particulate contamination of the samples being generated for examination by scanning electron microscopy. It is reasonable to expect that a significant amount of sunscreen would have been retained on the applicator's glove or hand after each application. It is also reasonable to expect that a larger fraction of the first application was retained on the applicator's hand than the fraction retained from subsequent applications. Thus, the effect that the retention of sunscreen on the applicator's gloves or hands would have had on individual subjects cannot be determined.

While the source of the $\Delta^{68}Zn\%$ in the blood from the applicator reported in Figure 4B (Gulson *et al.* 2010, 144) is unknown, the similarity of the $\Delta^{68}Zn\%$ in her blood to that reported for the blood of the test subjects indicates that the applicator's absorbed dose was similar to that of the test subjects. Both of the following possible sources of the applicator's dose have serious implications to the results. If the source were via the hand-to-mouth route, contamination due to field practices would seem to be even more widespread than just intra-subject. The source of her ^{68}Zn could be from dermal absorption through her hands, but if the absorption rate through hands is less than through the skin on the back (as has been reported in studies assessing anatomical variations in dermal absorption of OP insecticides), the similarity of the blood and urinary excretion rates for the applicator and the subjects would imply that the amount retained on the applicator's hands could have been several times larger than the dermal doses reported to be applied to the subject's backs in Table 1 (Gulson *et al.* 2010, 143). Thus, such dose values may be inaccurate.

While the authors did not even mention isotope fractionation in their article, the effect of isotope fractionation probably caused the authors to slightly over-estimate their calculated Zn absorption factor. Isotope fractionation refers to the separation of one isotope in comparison to either a lighter or heavier isotope of the same element; in this case, the human body's preferential absorption or retention of ^{68}Zn versus ^{64}Zn. This phenomenon was reported to occur in both of the two "other studies of biological samples" cited by Gulson et al. (2010, 144). Isotopic fractionation may have been the unrecognized cause of the finding by Gulson *et al*. that the mean $^{68}Zn/^{64}Zn$ ratio in their pre-exposure blood samples (0.4158) was 7% higher than the 18.8%/48.6% = 0.3868 natural background referred to in the manuscript (2010, 141). However, the effect of isotope fractionation may only reduce the $^{68}Zn/^{64}Zn$ ratio by a few percent.

The researchers also failed to establish the temporal stability of an individual's $^{68}Zn/^{64}Zn$ ratio upon which their conclusion of an increase of that ratio in blood or urine is based (Gulson *et al.* 2010). The only example of time-series data that was located in the material presented to the Board was the results by Ohno *et al.* (2005), which measured the $^{68}Zn/^{64}Zn$ ratio in blood samples collected once per month from just one person in which the average month-to-month varia-

tion was reported to be ±6.6% (a coefficient of variation of ±6.2%). This natural variation is considerably more than the 0.42% and 0.23% mean post-exposure increases reported by Gulson *et al*. (2010) over 13 and 19 days from day 0 to the end of the exposures and the six-day follow-up, respectively. The magnitude of this natural variation and the lack of an untreated control group suggest that the accuracy of the reported absorption factor could have been erroneous.

Regarding gender-related differences in dosing, the authors provide insufficient information for the reader to tell whether there was any interaction between the subject's individual body weights (not reported) and "a significant difference between doses for males and females with a mean of 4.6 mg/cm^2 for males versus 3.7 mg/cm^2 for females" (Gulson *et al.* 2010, 142). Without any implication of probability, it is possible that the fact that significant differences between genders were found only when comparing their change in $^{68}Zn/^{64}Zn$ ratios without body weight (reported in Figure 5A) but not when comparing their total Zn content in whole blood using the subject's weight (reported in Figure 5B) was a spurious association. However, the author's calculations for $\Delta^{68}Zn\%$ did take into account differences in gender and body weight by adjusting for fat-free body mass via BMI.

Regarding study sample selection, there are two major statistical concerns with the sample's demographic constitution. The report indicates that the data came from 20 subjects split between the nanoparticle group, with 6 females and 5 males and the bulk group with 4 females and 5 males (Table 1; Gulson *et al.* 2010, 143). This table, listing the subjects' demographics, showed greater similarities among the subjects due to family connections. Therefore, the question arises whether the sample of individuals used in the study represents a random sample of the reference population or whether is it a convenience sample. Because the experimental error or within treatment variation could be underestimated in a non-random, convenient, and small sample, the statistical validity of the data used in this study is questionable. The second issue is related to the known outlier (subject 7, enrolled in the nanoparticle group). This subject's observed data point was relatively larger than all other data points (e.g., Figures 4 and 5; Gulson *et al.* 2010, 144). Also, an adverse or allergic reaction was mentioned in the report as the likely reason for the large response observed in the case of subject 7. Therefore, this single observation could be the main cause of the observed skewedness in the experimental data. The researchers performed a log transformation to correct this high degree skewedness, which may be caused by this single outlier. The statistical significance reported in the paper (gender x treatment) may have disappeared if they had excluded this observation from the analysis or performed any robust weighted linear model analysis to adjust for this influential data.

Based on these concerns, the statistical validity of the data used and the conclusion derived may be questionable. Thus, the results reported could be treated as exploratory in nature and further studies are needed to confirm the conclusion.

Ethics

Charge to the Board

Is there adequate information to determine that the Gulson *et al*. (2010) study was conducted in substantial compliance with procedures at least a protective as those in subparts A - L of EPA's regulation at 40 CFR Part 26?

Board Response to the Charge

HSRB Recommendation

The Board concluded that there was insufficient information available at the time of the April meeting to determine whether the Gulson *et al*. (2010) study was conducted in substantial compliance with procedures at least as protective as those in subparts A - L of EPA's regulation at 40 CFR Part 26. The Board recommended that EPA seek additional information from Macquarie University or the study investigator, including a copy of the research protocol and an unsigned informed consent form, and that this information be provided to the HSRB for reconsideration.

HSRB Detailed Recommendation and Rationale

This is the first time EPA has asked the HSRB to review a study from the published literature that was conducted after EPA's amended Rule for the Protection of Human Subjects of Research. The study was conducted in Sydney, Australia and received approval from the ethics committee of Macquarie University, as required by the Australian Code for the Responsible Conduct of Research (2007).

As noted in EPA's ethics review (Parsons 2011), 40 CFR Part 26, subparts A through L do not apply; the research was neither conducted or supported by EPA nor was it conducted by a person with the intention to submit the results to EPA. FIFRA §12(a)(2)(P) does not apply to this research since the compound tested is not a pesticide. Thus, the applicable acceptance standards are as follows:

> 40 CFR §26.1703. Prohibition of reliance on research involving intentional exposure of human subjects who are pregnant women (and therefore their fetuses), nursing women, or children. Except as provided in §26.1706, in actions within the scope of §26.1701 EPA shall not rely on data from any research involving intentional exposure of any human subject who is a pregnant woman (and therefore her fetus), a nursing woman, or a child.
>
> 40 CFR §26.1705 Prohibition of reliance on unethical human research with non-pregnant, non-nursing adults conducted after April 7, 2006. Except as provided in §26.1706, in actions within the scope of §26.1701, EPA shall not rely on data from any research initiated after April 7, 2006, unless EPA has adequate information to determine that the research was conducted in substantial compliance with subparts A through L of this part, or if conducted in a foreign country, under procedures at least as protective as those in subparts A through L of this part. This prohibition is in addition to the prohibition in §26.1703.

The HSRB determined that most of the information that the Board has about the ethical conduct of the Gulson *et al*. (2010) study is without supporting documentation and is thus inferential. Although the Board thought that there is no reason to believe that the conduct of the reported research was fundamentally unethical, in the absence of supporting documentation like the informed consent form and research protocol, the Board concluded that it did not have adequate information to determine that participant selection was voluntary, that information about risks and benefits was sufficiently communicated, that study participants understood the nature of the research or their right to withdraw from the study, or that the female participants were neither pregnant nor nursing. Thus, the Board concluded that it lacked adequate information to determine that the research was conducted under procedures at least as protective as those in subparts A through L of 40 CFR Part 26.

In the absence of the informed consent form, the Board had insufficient information to determine how study risks and benefits may or may not have been characterized, whether participants were informed of their right to withdraw from the study, whether participation involved reasonable or unreasonable compensation or pressure to participate with family members. The Board offers the following reasoning for its recommendation:

1. Acceptable risk-benefit ratio.

   The Board agreed with the EPA review (Parsons 2011) that the main risks to participants were in blood sampling and possible adverse reaction to the sunscreen formulations. According to Gulson *et al*., the sunscreen formulations contained an enriched level of a stable isotope of zinc, which does not pose a higher risk than naturally occurring zinc and "many" commercially available sunscreens contain "nanoparticulate TiO2 and/or ZnO" (2010, 140). Thus, although the risk-benefit balance was not discussed in the study report, the risks appear to be low.

   No minors were enrolled in the study. However, apart from an email from the study investigator to the Agency (Parsons 2011), the Board had no evidence that pregnant or lactating women were excluded from participation.

   The study reports that one participant (Subject 7) had an adverse reaction to the sunscreen. The nature and severity of the adverse reaction was not described.

   According to the published study, the risks were mitigated as follows: 1) risks of sun exposure were mitigated with UV-protective clothing and advice to wear sunscreen; 2) Subject 7 ceased participation in the application portion of the study after an adverse reaction; and 3) a trained phlebotomist drew participants' blood.

   The Board concluded that although the risks of the study were low, the "adequacy of information" standard should not be strictly correlated with risk. That is, the threshold for adequacy of information should not be understood in proportion to the amount of risk posed by the study (if the risk is low, then the threshold for adequacy can be low). The Board determined that such a standard would exclude other requirements of ethical research including voluntary informed consent, respect for participants, and fair participant selection.

2. Voluntary and informed consent of all participants.

   The Board concluded that it did not have adequate information to determine that participant selection in the Gulson *et al*. (2010) study was conducted in substantial compliance with procedures at least as protective as those in subparts A - L of 40 CFR Part 26.

   Board members raised a concerns about the fairness and voluntariness of participant selection, including: 1) the investigator holding recruitment discussions with potential participants in his home; 2) selecting participants who were "personal contacts" of the investigator; 3) inclusion of participants from the same family, suggesting that the study was conducted in a context where subjects may have experienced undue pressure to participate; and 4) a lack of information about the type and level of compensation that participants were offered. In addition, although the published paper states that "venous blood and urine samples were collected 8 days before exposure" (Gulson *et al*. 2010, 140) an email from the study investigator to the Agency (Parsons 2011) stated that "the subjects attended a meeting at my house 1 week before the trial started and I described the trial and any risks." This apparent inconsistency in the timing of the informed consent process and the collection of study-related samples raises some concerns.

Although the Board recognizes that this research was reviewed and approved by the Macquarie University Human Research Ethics Committee, which is registered with the Australian National Health and Medical Research Council, it concluded that ethics committee approval alone does not provide adequate information to determine that the Gulson *et al*. (2010) study was conducted in substantial compliance with procedures at least as protective as those in subparts A - L of EPA's regulation at 40 CFR Part 26. The Board thus recommended that EPA seek additional information from Macquarie University or the study investigator, including a copy of the research protocol and unsigned informed consent form, and that this information be provided to the HSRB for re-consideration.

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