EPA Human Studies Review Board (HSRB)
April 18–19, 2023 Meeting Minutes

Committee Members: (See EPA HSRB Members List – Attachment A.)
Date and Time: Tuesday, April 18, 2023, and Wednesday, April 19, 2023, 1:00 to 5:00 p.m. EDT.
Location: Via Zoom
Purpose: The HSRB provides advice, information, and recommendations on issues related to scientific and ethical aspects of human subjects research.
HSRB Website: https://www.epa.gov/osa/human-studies-review-board

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Tuesday, April 18, 2023:

A. Meeting Topic and Charge Questions

Topic: S. Freestone and P. McFarlane (2001); A Single Oral Dose Study with Acephate Technical in Humans; Report Amendment 2. Inveresk Research, Elphinstone Research Centre, Tranent, Scotland. March 23, 2001.

Charge to the Board – Science: Are the plasma and urine concentration data for acephate and methamidophos, as described in the study "A single oral dose study with acephate technical in humans (Freestone, S. and McFarlane, P., 2001)," considered scientifically sound for the purposes of validating a physiologically based pharmacokinetic (PBPK) model?

Charge to the Board – Ethics: Does available information support a determination that the study was conducted in substantial compliance with subparts K and L of 40 CFR part 26?

B. Convene Meeting and Introduction of Members

Tom Tracy, Designated Federal Official (DFO), EPA Human Studies Review Board (HSRB), Office of the Science Advisor, Policy and Engagement (OSAPE)

Mr. Tom Tracy, DFO for HSRB, called the meeting to order at 1:00 p.m., EDT. He introduced the meeting, outlined the Federal Advisory Committee Act procedures, and performed a roll call of meeting participants. The following members and observers were present:

HSRB members	Lisa Corey, Ph.D., Co-Chair (Intertox, Inc.) Julia Sharp, Ph.D., Co-Chair (National Institute of Standards and Technology) Albert J. Allen, M.D., Ph.D. (Consulting Specialist) Chad Cross, Ph.D. (University of Nevada – Las Vegas) Philip Day, Ph.D. (University of Massachusetts, Chan Medical School) Nicole Deming, J.D., M.A. (Case Western Reserve University, School of Medicine) Weiying Jiang, Ph.D. (California Environmental Protection Agency) Thomas Lewandowski, Ph.D. (Gradient) Srikumaran Melethil, Ph.D., J.D. (University of Missouri – Kansas City) George Milliken, Ph.D. (Milliken Consultants) Sinziana Seicean-Boose, M.D., Ph.D., M.P.H. (Case Western Reserve University) Joseph Tuminello, Ph.D. (McNeese State University) Eun Um, Ed.D. (AMSTAT Consulting) David Williams, Ph.D. (Oregon State University)
EPA staff members	Michelle Arling (EPA, Office of Pesticide Programs (OPP)) Tom Tracy (EPA, OSAPE) Monique Tadeo (EPA, Program in Human Research Ethics and Oversight (PHREO))

	Rosanna Louie-Juzwiak (EPA, OPP) Frank Antwi (EPA, OPP) Cecilia Tan (EPA, OPP) Monique Perron (EPA, OPP) Ruthanne Louden, (EPA, OPP) Tamica Cain (EPA, OPP) James Nguyen (EPA, OPP) Emily Sokol (EPA, Oak Ridge Associated Universities (ORAU)) Madison Clark (EPA, OSAPE)
Members of the public, representatives of research sponsor, and research team:	Ann Jonynas (American Vanguard Corporation (Amvac)) Niamh McMahon (Amvac) Afroditi Katsigiannakis (ICF, Contractor Support) Katie Lenae (ICF, Contractor Support) Angelina Guiducci (ICF, Contractor Support) Lucas Rocha Melogno (ICF, Contractor Support)

C. Meeting Administrative Procedures

Tom Tracy, DFO, HSRB, OSAPE

Mr. Tom Tracy reviewed the Zoom platform tools and features and stated the purpose of the meeting was to review the paper by Inveresk Research, "A Single Oral Dose Study with Acephate Technical in Humans." He noted minutes of the meeting and a report will be prepared, certified, and posted on the website within 90 days of April 19, 2023.

D. Welcome and Updates

Monique Tadeo, M.S., CIP, HSRB Official and Director

Ms. Monique Tadeo had no updates to report.

E. Opening Remarks

Lisa Corey, Ph.D., HSRB Co-Chair
Julia Sharp, Ph.D., HSRB Co-Chair

Dr. Julia Sharp welcomed everyone to the EPA HSRB meeting, briefly introduced the study under discussion, and outlined the meeting process. She then asked for updates from OPP.

F. Updates from OPP

Michelle Arling, J.D., OPP

Ms. Michelle Arling provided updates from the last few meetings. In October, the HSRB reviewed two studies involving exposure to formaldehyde. EPA is reviewing two additional studies, along with the weight-of-evidence document, that will be presented to the Board in May 2023. In October and December, two insect repellent studies were discussed. EPA is finalizing its feedback for the registrants.

EPA was notified that the registrant who provided the protocol the HSRB reviewed for the next meeting has withdrawn its submission. Consequently, EPA and HSRB cannot work or comment on this protocol. Ms. Arling acknowledged the time HSRB members spent reviewing the protocol and apologized for the cancelation. She also noted this was an unusual occurrence and that the meeting on April 19 was thereby canceled. Lastly, she asked whether Board members had any questions.

- **Julia Sharp**: Thank you for letting us know. I have started drafting a report and will send that along to Michelle. Who should inform our consultant?
- **Tom Tracy**: I will inform the consultant now.
- **Thomas Lewandowski**: Will something be resubmitted so the work we have done will be useful in the future?
    - **Michelle Arling**: That is possible. If it is not a resubmission from the same company, we still receive an assortment of protocols for testing skin repellents; thus, regardless of the source of the future submission, the feedback from HSRB is always valuable.

Dr. Sharp then asked whether Board members had started their reviews and requested they send them to her so she can share them with EPA.

G. EPA Science Review Highlights

Ruthanne Louden, M.P.H., OPP Health Effects Division

Ms. Ruthanne Louden introduced herself while sharing the first slide of the EPA Science Review Highlights presentation. Slide 2 provided an outline for the presentation, which included an introduction, PBPK model overview, science review, and the charge question. Slide 3 provided the introduction for the study, including biological background information on acephate. Acephate is an organophosphate pesticide. The initiating event in the adverse outcome pathway involves inhibition of the enzyme acetylcholinesterase (AChE) via phosphorylation of the same residue at the active site of the enzyme. Additionally, methamidophos (acephate degradant) is a more potent inhibitor of AChE.

Slide 4 introduced the study, "Acephate Acute Oral Study in Humans." This was a single oral dose study with acephate technical product in humans to determine the pharmacokinetic and acetylcholinesterase inhibition activity. The study included the determination of concentrations of acephate and methamidophos in plasma and urine over time, as well as red blood cell count and plasma and AChE activity following a single dose of acephate in human subjects.

Slide 5 listed potential uses of the data alongside the author's objectives, noting these uses differed. Slide 6 listed questions and topics related to physiologically based pharmacokinetics (PBPK) models. Slide 7 highlighted traditional risk assessments with a graph that described a no-observed-adverse-effect level (NOAEL) and a benchmark dose, lower concentration limit (BMDL).

Slide 8 highlighted how PBPK modeling can be used as a scientific tool to revise defaults. The

underlying assumption is that an equivalent biological response occurs at an equal internal dose within the test species. Slide 9 continued highlighting PBPK models, discussing how a chemical moves through the body. PBPK models are mathematical representations of a pharmacokinetic (PK) process. A PBPK model predicts plasma/tissue concentrations, given an external dose. Slide 10 showed how animal and human PBPK models can be related in terms of point of departure and target tissue dose or a surrogate of target tissue dose. Slide 11 presented a flow chart illustrating the process of PBPK model development. Models begin with problem formulation and end with a comparison to human data. Slide 12 showed an example of comparing model predictions with observed data. Slide 13 discussed the utility of acephate/methamidophos human data for PBPK modeling. The registrant will use acephate/methamidophos concentrations in plasma and urine to validate/calibrate a future PBPK model, which will be evaluated by EPA for its appropriateness for risk assessment. The registrant is not proposing to use the red blood cell count or plasma AChE data.

Slide 14 provided an overview of OPP model review criteria. The model purpose should address specific risk assessment purposes; the model structure should contain the target organ or surrogates; the mathematical representation should be based on known PK mechanisms and ensure mass balance; the parameter estimation needs to be reasonable and justified; the mathematical equations should be accurately coded in programming software with appropriate integration algorithms; and model-simulated PK profiles should be evaluated to ensure they are consistent with the experimental data.

Slide 15 introduced the science review portion of the presentation. Slide 16 included information about the study design. The study was a double-blind, placebo-controlled, single ascending oral dose study with acephate and human volunteers. Males and females were sorted into separate dose groups. Following exposure, concentration profiles of acephate and methamidophos in plasma (up to 14 days post-dose) and urine (up to 48 hours post-dose) were determined. Additionally, plasma and red blood cell AChE activity was measured prior to exposure; at 1, 2, 4, 8, 12, 24, 48, and 72 hours; and 7 and 14 days post-dose.

Slide 17 provided additional information on the study design, specifically for sample collection. Vital signs (blood pressure and heart rate), oral temperature, electrocardiogram (ECG), hematology, clinical chemistry, blood sample collections, and urine samples were all collected/recorded. Slide 18 provided additional information on the study design, specifically for analytical methods. Red blood cell count and plasma AChE activity were analyzed using a colorimetric assay by Ellman (1961).[1] Plasma and urine acephate and methamidophos concentrations were determined by gas chromatography (GC) with flame photometric detection. All methods were validated. The method-validated range for acephate and methamidophos in plasma and urine was 10–1,000 ng/mL.

Slide 19 highlighted the results of the study. Treatment-related effects on vital signs, ECGs, hematology, clinical chemistry, urinalysis, and physical examinations were not observed over the course of the study. Adverse clinical signs were not considered related to treatment. Dose levels

[1] Ellman, G.L. (1961) Biochem. Pharmacol. 7:78.

of acephate were considered safe and well tolerated throughout the study period. Adverse effects or clinically relevant reductions in plasma or red blood cell AChE activity were not observed.

Slide 20 provided graphic representation of the results. The two concentration time plot graphs differed by sex. Slide 21 provided the concentration time plots for methamidophos in both sexes. Slide 22 provided results of the urinalysis. Total recovery of acephate and methamidophos (as acephate equivalents) in urine through 48 hours post-dose ranged from 25% to 62% in males and from 12% to 51% in females. Most recovered during the first 12 hours; less than 1% of the acephate-recovered dose was methamidophos. Lower recoveries in females versus males were likely attributed to decreases in urine collection volumes and fractions of unchanged parent observed in females compared to males.

Slide 23 highlighted the method validation and storage/stability for the study. Analytical methods and storage/stability for the determination of acephate and methamidophos in human blood and urine were described and validated. The method-validated concentration range (for both plasma and urine) of approximately 10–1,000 ng/mL and the storage/stability results were considered acceptable. Data fell outside of the method-validated ranges for both human blood/plasma and urine in several instances. However, PK data from this study are still considered useful for the purpose of improving predictability of future PBPK modeling.

Slide 24 provided study conclusions. This study was conducted in compliance with the Principles of Good Laboratory Practice (GLP). The AChE inhibition data will not be used due to the variability and lack of a clear dose-response relationship. Although some of the urinary and plasma concentration data fell outside of the method-validated range, they may still be used to calibrate/validate future PBPK modeling. Slide 25 presented the charge question, "Are the plasma and urine concentration data for acephate and methamidophos, as described in the study 'A single dose study with acephate technical in humans (Freestone, S. and McFarlane, P., 2001)' considered scientifically sound for the purposes of validating a PBPK model?"

H. Board Questions of Clarification

- **Thomas Lewandowski**: Can we use this modeling if we do not know whether a model will be developed? Does EPA expect a model to be developed, and would the sponsors develop it when they use this study, or would EPA develop it?
    - **Ruthanne Louden**: A model is in development, but we have not received it yet.
- **Srikumaran Melethil**: How would this product appear on the market in relation to the doses used in this study?
    - **Michelle Arling**: Looking at this study, we are investigating whether we can validate a PK model. Then we will look at the risks and hazards and complete an assessment to determine the appropriate application levels.
- **Srikumaran Melethil**: Does the year in which it was developed, 1961, raise any red flags?
    - **Ruthanne Louden**: Yes, maybe someone else can comment.
    - **Monique Perron**: We have a long history including those basic measurements, and the approaches have remained similar throughout the years. I would not be

surprised to see more variability in data in an older study. If they were able to detect it back then, we could probably detect a much lower value now and have more confidence in those lower values. We will follow up with our chemists and get more information on this topic.

- **Weiying Jiang**: The last Reregistration Eligibility Decision (RED) document was issued in 2006. Is EPA currently developing another RED document, and are there any data calling for a RED document at this time?
    - **Ruthanne Louden**: EPA has a risk assessment from 2017, but we have a new risk assessment that will be published soon.
    - **Rosanna Louie-Juzwiak**: EPA expects to release an updated risk assessment. It is currently undergoing registration review.
- **David Williams**: I know these data are not released as a PK model, but I see they selected a non-compartmental model. I wonder whether an alternative model would be better.
    - **Cecilia Tan**: The non-compartmental model is used to calculate PK parameters. That describes the animal data. However, this type of model cannot be used to make predictions. For our purpose, the PK model will be used as a data analysis tool to replace the default uncertainty factor for animal-to-human extrapolation and it will be used for predicting human departure .
    - **Thomas Lewandowski**: This is a standard procedure for estimating PK parameters. No one would use those estimates for a specific application of the chemical. The non-compartmental model is a quick analysis tool.
    - **Michelle Arling**: We expect a contract company will develop the model and submit it to EPA for review and analysis. EPA will not be building models from the ground up.
- **Srikumaran Melethil**: What is an internal dose?
    - **Cecilia Tan**: An internal dose is the concentration inside the body.
    - **Srikumaran Melethil**: Would an internal dose be the same thing as a target concentration?
    - **Cecilia Tan**: In most cases, yes.
    - **Srikumaran Melethil**: To confirm, internal and target dose is the same thing as concentration?
    - **Cecilia Tan**: Not necessarily. Blood concentration may not be the target. For example, for cholinesterase inhibition it is in in the brain. That would be the target tissue concentration. In this study they measured plasma concentrations. So, it is considered the internal dose but not necessarily the target dose.

I. EPA Ethics Review Highlights

Michelle Arling, J.D., OPP

Ms. Michelle Arling presented the slides for the Acephate Oral Dosing Study Ethics Review. She shared some background regarding the study, noting it was conducted before the promulgation of

the 2006 Human Studies Rule, it was considered an intentional exposure human toxicity study since humans were dosed with acephate and the effects on red blood cells and cholinesterase were measured, and EPA requires an HSRB review for pre-rule intentional exposure toxicity studies (when the Agency intends to rely on those studies). A total of 50 subjects (10 females and 40 males) were enrolled in the study. They were recruited from the area surrounding the test facility through a generic advertisement for volunteer participation. All subjects were randomly assigned to the dose and placebo groups.

Slide 5 detailed the eligibility criteria. Participants had to be aged 18–50 years, with normal physical findings at prescreening examinations, and able to communicate with investigators and comply with instructions. Applicants were excluded if they had used any test compounds 3–4 months prior to study start, had used medications 5 days prior to study start, were women who were pregnant or not taking contraceptives, had allergies, had pre-existing conditions, donated blood within 12 weeks of the study, conducted agricultural work (including pest control), were exposed to anti-ChE compounds within 1 month of dosing, or could not refrain from smoking 8 hours after dosing.

Slide 6 detailed the consent process, which included screening prior to enrollment, physician notification, and receipt of a consent form that explained study procedures, risk, and discomforts. All participants were told they could withdraw at any point during the study period. All participants provided written informed consent at the pre-dose screening.

Slide 7 detailed the risks of participation and risk-minimization efforts. Risks included headache, nausea, chest tightness, coughing, sweating, abdominal distress, and discomfort from blood draws. Risk-minimization efforts included enrolling only healthy subjects, physician notification, increasing doses only after evaluation at lower doses, and close monitoring by medical staff during and after dosing.

Slide 8 detailed the adverse side effects observed in the study. In total, nine adverse side effects were reported among six subjects. The side effects included headache, dyspepsia, leg pain, dizziness, and cough/sore throat. It was concluded that no adverse effects were serious, and none were likely related to study participation.

Slide 9 detailed respect for participants. All participants were free to withdraw at any time and were paid £450 at the completion of the study. Participant privacy was protected throughout the study.

Slide 10 detailed the independent ethics review that was completed on the study. The research was reviewed and approved by the Inveresk Research Independent Ethics Review Committee. Correspondence from the Institutional Review Board (IRB) includes specific comments, approval of protocol and some amendments, and the members present at the meeting. The study report includes the protocol, correspondence from the IRB, consent materials, and other relevant information (revisions based on amendments).

Slide 11 detailed three amendments to the study protocol. One was in response to IRB comments: update volunteer information, include product information, and add criteria for

stopping the study. Protocol Amendment 1 included additional sampling, consent for urine monitoring, and updated ChE parameters. Protocol Amendment 2 included setting the female dose level lower than the male dose level.

Slide 12 detailed the substantive acceptance standards of the Board. These standards include 40 CFR §26.1703, which prohibits reliance on data involving intentional exposure of pregnant or nursing women or of children; 40 CFR §26.1704, which prohibits EPA relying on data if it has clear and convincing evidence that the research was conducted unethically or was deficient relative to the ethical standards prevailing at the time it was conducted in a way that placed participants at increased risk of harm or impaired their informed consent; and FIFRA §12(a)(2)(P), which makes it unlawful to use a pesticide in human tests without fully informed, fully voluntary consent.

Slide 13 detailed the findings of the Board. All subjects were at least 18, there was no evidence nursing women were included, and pregnant women were excluded. . The amendments and deviations did not compromise safety, consent, or the rights of subjects. All subjects were fully informed, and their consent was fully voluntary, without coercion. Slide 14 stated the conclusion: No legal, regulatory, or ethical barriers prevent EPA from relying on this research.

Dr. Julia Sharp asked whether Board members had any questions regarding the ethics review. No questions were raised.

J. Public Comment

Mr. Tom Tracy stated the Board had received no public comments and asked whether anyone on the call wanted to provide a comment. No comments were raised.

K. Board Discussion: Charge to the Board – Science

Weiying Jiang, Ph.D., and Thomas Lewandowski, Ph.D., Science Review
Chad Cross, Ph.D., Statistics Review

Dr. Julia Sharp introduced the Board Discussion and the speakers for the science session. Dr. Sharp shared her screen with the notes from the speakers and invited Dr. Thomas Lewandowski to present his observations.

- **Thomas Lewandowski**: We reviewed and summarized the study similarly to how EPA did. We generally agree that the data from the study can support PBPK modeling. We agree with the proposal from EPA to use the data to validate the model, but limitations should be acknowledged. These limitations include the following: Only 50 subjects were included in the study, 40 males and 10 females. Males had multiple doses, whereas females had a single dose. The enrollment was very selective (e.g., all participants were healthy adults, with a small and highly specific study population, and they could not drink coffee or smoke during the study). How well would that group represent the general population? The study population was not diverse either, and that should be considered by EPA. The study also had an issue regarding dose proportionality: At higher doses in males, higher concentrations of acetate were detected in plasma, but the relationship was

not proportional. The data for females were limited; as such, we cannot infer conclusions about the effects of increasing doses in females. The recoveries were low (e.g., only 12% of dosage was recovered in females). Other metabolites may have been present that were not detected, but they do not seem to account for such a large difference in observed recoveries. This recovery issue should be considered in PBPK dose modeling, as dosage cannot be assumed in absorbed dose. The authors found no evidence of a significant lag phase of absorption. Nonetheless, that statement in the manuscript seems conclusive even though one subject had maximum concentrations after hours, suggesting a delayed absorption. Therefore, we suggest EPA review this finding again. The doses were narrow, but we know of the potential for more variable doses in an occupational setting. The range of exposure was limited, therefore resulting in limitations of exposure generalizations. The study had an issue with the calibration range (e.g., in urine, the mean acephate concentration was around 35,000 ng/mL with a standard deviation of around 22,000 ng/mL). These values are beyond the calibration range of 10–1,000 ng/mL. Are the urine data unreliable? The authors mentioned some dilution was performed prior to the samples being analyzed. Did that dilution account for the correction of the total concentration after back-calculation? Dermal contact and other exposure pathways should also be explored in future studies. The authors mentioned the limitations in the time course data for methamidophos on page 70 of the PDF. We understand EPA plans to use the data itself, but if pharmacokinetic parameters will be used, that limitation should be considered. We understand EPA does not plan to use the health effects data, and we agree. No clear dose-response pattern is found in the data. The PK data may need a review from chemists instead of toxicologists, and this approach should be considered by EPA. Finally, we noticed a typo in the EPA review ("theoretical plates," not "theatrical plates").

    - **Weiying Jiang**: I have no additional comments.

- **Julia Sharp**: Should any of the previous points be addressed during the meeting?
    - **Thomas Lewandowski**: Can EPA talk more about the calibration observations?
    - **Ruthanne Louden**: To validate the PBPK model, we would use individual data, maybe data within a validated range. I am not exactly sure. The plasma data are not as concerning as the urine data. However, I agree with Dr. Lewandowski that the urine data were outside the upper range.
    - **Cecilia Tan**: If the study has data that are questionable, those data should not be used. It is unclear currently, so we would use the data to see how the model performs.
    - **Thomas Lewandowski**: When analyzing a sample, we dilute to prevent overwhelming the instrument, and then we do a back-calculation. For the urine samples being above the 10,000 ng/mL concentration range: Does it mean that what went in the instrument had those concentrations, or was it the result of a back-calculation? I suggest confirming what occurred.
    - **Cecilia Tan**: I agree.

- **Julia Sharp**: Are there any other questions or observations?
    - **Srikumaran Melethil**: The study talked about non-dose proportionality. Dose non-linearity is an important topic to consider further for pharmacokinetics.
    - **Thomas Lewandowski**: I also have some concern given the variability in the data. The study has a conclusive statement about proportionality and non-linearity, but we see a lot of variability from one person to another. I would not provide such a conclusive statement.
    - **Julia Sharp**: Dr. Melethil, do you have a specific recommendation?
    - **Srikumaran Melethil**: I agree with Dr. Lewandowski. The authors mentioned significant differences were observed and that we do not know what future doses would be. I would say the data suggest non-linearity. That would be helpful for someone doing future studies.
    - **Thomas Lewandowski**: I agree.
    - **Weiying Jiang**: We should not give quantitative conclusions considering the data quality and data variability. About the proportionality and linearity, we do not know (e.g., females had only one dose, and males had data variability). Second, it seems that the EPA PBPK model will use animal data first, and then use the human data to validate the model. So, my general conclusion is that we should not give quantitative suggestions.
    - **Thomas Lewandowski**: Is it reasonable to say the data "suggest" non-linearity?
    - **Weiying Jiang**: The study says there was a proportional increase, but we cannot say whether it is linear or not.
- **David Williams**: In a 1987 paper, the authors saw a significant amount of methylation, which would not show up in GC. Other metabolites could explain the mass balance issues. I also did not see whether the authors corrected for creatinine data. Would that be a standard procedure?
    - **Thomas Lewandowski**: The study focused on a metabolite, but it is not the only one. I do not recall seeing a correction for creatinine, which is very variable between individuals, but it is certainly something we should note.
    - **Srikumaran Melethil**: It seems this paper has a quantitative problem due to variability, which suggests the paper is not useful if we cannot do anything quantitative, which is the focus of PBPK modeling.
    - **Thomas Lewandowski**: I think the data are useful, but the conclusions drawn from the study need to acknowledge the limitations clearly.
    - **Weiying Jiang**: I agree; it is useful. A sentence in the study said, "systematic exposure to acephate appears to increase with dose but it was not considered to exhibit dose proportionality. There was, however, evidence of a broadly linear relationship between a systematic exposure and dose across the dose range used in the study." My understanding is that it shows a vague linear increase of exposure based on administered dose, but it may not be conclusive. In addition, we do not know how this proportionality or linearity could apply to other doses in scenarios

with higher exposures.

- **Julia Sharp**: I propose we continue this discussion after the statistical review. That review may have information that would help contribute to our discussion about linearity.

- **Chad Cross**: I wish I had an answer to the previous question, but I do not, so it will remain for discussion. The review by others has already covered some of my observations. I am stopping at the statistical analysis section before providing my comments.

Dr. Cross summarized the quantitative aspects of the study design, described in detail in his notes on page 2. He mentioned that the third paragraph of the statistical analysis had already been discussed.

- **Chad Cross:** Remarks and comments arising from the review are the following. The sample size determination needs clarification. It is confusing to read that sample size was not determined in some sections; however, an entire paragraph discusses sample size determination. We need to clarify whether autocorrelative terms were included in the sample size determination. Again, regarding sample size, if 50 subjects were sufficient, it is unclear why 40/50 were male and 10/50 were female unless an assumption was made that ChE inhibition is the same for males and females. Additionally, the inclusion of females at only one concentration (1.0 mg/kg) provides limited useful data for pharmacokinetic modeling of female subjects. Even though no sex-related differences were observed at that dose, this is not sufficient to conclude that 1.0 mg/kg is the NOEL for females compared to 1.25 mg/kg for males, as stated in the document. All participants were noted as white except for one participant, who was noted as Asian. It is unknown whether this would have had any demonstrable impact on the experimental outcomes since the Asian participant was included in the placebo group. However, this should be noted or addressed in some way. Several resulting concentrations, as we discussed before, have fallen outside of the method validation range at several doses and time periods. It is unclear how these data points were handled in the subsequent analyses. As noted in the EPA science review document on page 26, the use of RM-ANOVA cannot be verified as appropriate as used in the initial analysis owing to insufficient information concerning sphericity assumptions underlying the choice of variance/covariance structure. As noted, the original use of Bonferroni corrections is likely overly conservative; hence, the suggested use of the many-to-one (i.e., several treatments versus a single control) test by Dunnett is supported, and that was reported in the EPA scientific review document. Furthermore, the sensitivity analyses conducted to include additional RBC ChE measurements from the four subjects that were presumably ignored in the original analysis was appropriate. As for recommendations, first, the more robust analyses performed by EPA Chemical Engineering Branch (CEB) statisticians using mixed models is justified and should be noted as the appropriate analytical strategy for these data. Second, sample size determination should be verified with explanation as to the 80%:20% allocation to males:females, respectively, and the potential effect on power

under this allocation should be clarified. Lastly, it should be noted that parameter estimates for pharmacokinetic models for acephate and methamidophos may be of limited value for females given their exclusion from all concentrations except 1.0 mg/kg. Although the profiles are statistically similar to male subjects at this dose, this does not provide evidence at other doses.

- **Julia Sharp:** Thank you, Dr. Cross. I will add one more recommendation to the first point you mentioned. The CEB provided a variance/covariance matrix, so they did some sensitivity analysis to choose the most appropriate analysis for the data. The spatial power variance/covariance matrix allows for unequal dosing, so I agree with you that the mixed model approach is best, as well as the variance/covariance matrix that was selected.
- **Chad Cross:** Thank you. I will add that clarification.
- **Julia Sharp:** I have one other question. When you say, "should be noted in the document," are you making a recommendation to EPA to note those topics in its recommendations?
- **Chad Cross:** Yes, that would be the recommendation moving forward. The data insufficiencies should be documented in EPA's review.
- **Julia Sharp:** Are there any other points for discussion or clarification for the statistics review?

No questions were asked.

- **Julia Sharp:** We did not resolve the question about linearity. I propose Dr. Lewandowski provide his opinion about including data limitations in the EPA review. We could include a statement from the science review to say that data may suggest non-linearity and should be investigated further.
  - **Thomas Lewandowski:** We agree, and we will take that statement and repeat it in our review and say that it should be considered by EPA.
- **Julia Sharp**: Do we need any information from EPA about the qualitative use of the data?
  - **Michelle Arling**: We want to confirm that before we wrap up the discussion on science and statistics, you have all the information you need from EPA.
  - **Thomas Lewandowski:** I think we have everything we need.
  - **Weiying Jiang**: I agree.
- **Julia Sharp**: Thank you, science and statistics reviewers. Here is the charge question: "Are the plasma and urine concentration data for acephate and methamidophos, as described in the study 'A single dose study with acephate technical in humans (Freestone, S. and McFarlane, P., 2001)' considered scientifically sound for the purposes of validating a PBPK model?"
  - **Julia Sharp:** A proposed response to that charge question is: "The plasma and urine concentration data for acephate and methamidophos, as described in the study 'A single oral dose study with acephate technical in humans (Freestone, S.

and McFarlane, P., 2001)' are considered scientifically sound for the purposes of providing information to validate future PBPK models."

- **Julia Sharp:** I am taking suggestions for that response to the charge question, knowing it does not reflect everything that has been discussed during this meeting. I have a proposal to change the end of that response: "The plasma and urine concentration data for acephate and methamidophos, as described in the study 'A single oral dose study with acephate technical in humans (Freestone, S. and McFarlane, P., 2001)' are considered scientifically sound for the purposes of providing information to validate future PBPK models, *given the limitations and recommendations provided by the HSRB are considered*." I think there is room for improvement. I think our discussion and the review documents show that the data could be used as partial evidence. Should we add the word "partial" in the statement? Are there any other recommendations? **Thomas Lewandowski:** I would suggest adding "support validation" rather than "validate."
- **Julia Sharp:** The updated statement, with the last part updated per Dr. Lewandowski's suggestion, now reads: "The plasma and urine concentration data for acephate and methamidophos, as described in the study 'A single oral dose study with acephate technical in humans (Freestone, S. and McFarlane, P., 2001)' are considered scientifically sound for the purposes of providing information to support validation of future PBPK models, given the limitations and recommendations provided by the HSRB are considered."
- **Nicole Deming:** Do we need to delete "are considered"? It is said twice in one sentence.
- **Thomas Lewandowski:** The last one could say "are taken into account." There is also a missing preposition.
- **Julia Sharp:** Are there any other suggestions or responses to the charge question?
- **Srikumaran Melethil:** I would like to suggest the word "providing *preliminary* information."
- **Julia Sharp:** We have "preliminary" and "partial" information.
- **Srikumaran Melethil:** Yes, "partial" would work.
- **Julia Sharp:** Are there any other suggestions?
- **Michelle Arling:** Where would "preliminary" or "partial" fit, and what is it intended to convey?
- **Julia Sharp:** The discussion suggested maybe these data could be considered to validate future PBPK models, but perhaps there are other data that could be used in addition to the data being discussed today. Is that correct, Dr. Lewandowski?

Dr. Lewandowski agreed via the meeting platform reactions before commenting.

- **Thomas Lewandowski:** These data are limited, so we suggest that EPA not rely on this single study.
- **Michelle Arling:** That is very helpful. Thank you.

  - **Weiying Jiang:** I agree with Dr. Lewandowski. This study should not be used as a single piece of evidence or the primary source of evidence.
  - **Michelle Arling:** We would use it only when evaluating the study.
  - **Thomas Lewandowski:** We have limited knowledge since we have only this study. Therefore, we are looking at this one limited study but there might be other data we do not know about.
  - **Julia Sharp:** Dr. Cecilia Tan had a suggestion about using the word "evaluating," instead of "validation," in the statement we discussed earlier.
  - **Thomas Lewandowski:** That is fine with me. Do we need to say, "evaluate their performance"?
  - **Cecilia Tan:** Maybe we can say, "to support the evaluation of future PBPK model performance."
  - **George Milliken:** It seems we want to use these data to develop a process for modeling future data sets. Is that correct? This will help establish a process for PBPK modeling.
  - **Thomas Lewandowski:** I think we were asked to evaluate only this paper. It sounds like the question is for EPA.
  - **Cecilia Tan:** It is not necessarily using the data to establish a process. We already have that process in place for, e.g., animals, but we do not have anything for humans. Using this human data increases the confidence of the model performance.
- **AJ Allen:** You will have a sponsor bring in an application using these compounds, and we expect them to do a PBPK model. What we expect is that EPA could use this model to evaluate how well it predicts outcomes.
  - **Cecilia Tan:** We will not build our own model. The registrant will submit a model and show EPA how it built that model. The registrant will include how it used animal and human data to demonstrate model performance.
  - **AJ Allen:** So, EPA would not validate the model, but would evaluate how accurate the model is.
- **Srikumaran Melethil:** I want to note that the current study would be called a simplistic PBPK model because it includes no tissue or organ data, based on an online publication from EPA. A complex model would involve sampling of multiple tissues. The person who developed the question may have considered simplistic PBPK models only.
- **Julia Sharp:** Thank you for that observation. A new proposed response to the charge question is as follows: "The plasma and urine concentration data for acephate and methamidophos, as described in the study 'A single oral dose study with acephate technical in humans (Freestone, S. and McFarlane, P., 2001)' are considered scientifically sound for the purposes of providing partial information to support evaluation of future PBPK model performance, given the limitations and recommendations provided by the HSRB are taken into account." Are there any other suggestions?

No additional suggestions were raised. Julia Sharp asked the Board to vote on the new charge question. It was unanimously approved.

L. Board Discussion: Charge to the Board – Ethics

Joseph Tuminello, Ph.D., Ethics Review

Dr. Joseph Tuminello stated the charge question to the Board: "Does available information support a determination that the study was conducted in substantial compliance with subparts K and L of 40 CFR part 26?"

Dr. Tuminello then stated that his response to the draft charge was: "Yes, the available information supports a determination that the study was conducted in substantial compliance with subparts K and L of 40 CFR part 26."

He stated that while there were no benefits to the subjects, he agreed with EPA's ethics review, which indicated the potential societal benefits outweighed the risks to the subjects associated with the study. He also agreed with EPA's determination that the study subjects encountered no health or safety risks.

Dr. Tuminello stated that he did question whether the study *not* indicating that nursing and pregnant women were involved provided sufficient basis for the Board to assume they were, in fact, not involved. He then agreed with the argument the Board presented earlier: because there was no evidence that nursing and pregnant women were included in the study, it is safe to assume they were not included. Dr. Tuminello then stated that the study was conducted in compliance with subparts K and L of 40 CFR part 26.

- **Julia Sharp**: Do we need to discuss the exclusion of nursing women further?
    - **Joseph Tuminello**: No, I agree with the presentation given by the Board.
    - **David Williams**: Should we make a notation of the potential limitation of the limited demographics included in the study? **Julia Sharp**: This is noted in the science review. We could also address it in the ethics portion.
    - **AJ Allen**: This study was conducted in the U.K. One of the challenges is we are not sure where it was conducted or what the local demographics would be. The question is whether the study represents the local population. If there is not a statement that the authors were trying to exclude some groups, then EPA thinks it was conducted ethically.
    - **Phillip Day**: The way we think about these issues would belong more in the science component than the ethical component.
- **George Milliken**: No studies have been designed to test the difference between men and women. If we were to do that, we would need 50 in each group. If we wanted to do that for ethnic groups, we would also need 50 people in each group.. We need enough power to test the differences.
    - **Julia Sharp**: The science review does talk about generalizing to a representative population. That is a limitation of the study to be addressed in the science review.
- **Sinziana Seicean-Boose**: There is a science and an ethical component. Sex and race were

already addressed in the science charge question. In the ethical portion, we can say something about the heterogenicity versus the homogeneity of the study participants in regard to sex and race. When the study population is selected, we like to see that it includes an equal number between sexes and an equitable distribution of races. However, because this is a U.K. study, the ethical criteria are probably different from how they are in the U.S.

- **Nicole Deming**: I wanted to discuss something similar about the ethics and distinguishing between something that has a statistical or results-based impact and something that is an opportunity to participate in research and to have a just distribution of not only access but of benefits and risks. It is a consideration for the ethics review, and international guidelines in the U.K. are aligned with this. Yes, they are outside our purview to establish those, but they are very similar.

Dr. Julia Sharp repeated the current charge question: "Does available information support a determination that the study was conducted in substantial compliance with subparts K and L of 40 CFR part 26?" The proposed response: "The available information described in 'A single oral dose study with acephate technical in humans (Freestone, S. and McFarlane, P., 2001)' supports a determination that the study was conducted in substantial compliance with subparts K and L of 40 CFR part 26."

Dr. Sharp asked whether anyone had any suggestions or comments regarding the proposed response. There were none. A vote was taken to see whether the Board members agreed with the proposed response. The members unanimously agreed.

M. Adjournment

Mr. Tom Tracy thanked the Board. The meeting adjourned at 3:45 p.m., EDT.

Attachment A: HSRB Current Committee Membership

Name	Title	Affiliation
Lisa Corey, Ph.D.	Senior Toxicologist	Intertox, Inc. Seattle, WA
Julia Sharp, Ph.D.	Mathematical Statistician	National Institute of Standards and Technology Fort Collins, CO
Albert J. Allen, M.D., Ph.D.	Consulting Specialist	Self-employed
Chad Cross, Ph.D.	Associate Professor In-Residence	University of Nevada Las Vegas, NV
Philip Day, Ph.D.	Assistant Professor	University of Massachusetts, Chan Medical School Worcester, MA
Nicole Deming, J.D., M.A.	Assistant Dean, Faculty Affairs and Human Resources	Case Western Reserve University, School of Medicine Cleveland, OH
Weiying Jiang, Ph.D.	Staff Toxicologist	California Environmental Protection Agency, Department of Pesticide Regulation Sacramento, CA
Thomas Lewandowski, Ph.D.	Principal	Gradient Seattle, WA
Srikumaran Melethil, Ph.D., J.D.	Professor Emeritus	University of Missouri-Kansas City Kansas City, MO
George Milliken, Ph.D.	President	Milliken Consultants Manhattan, KS
Sinziana Seicean-Boose, M.D., Ph.D., M.P.H.	Assistant Professor	Case Western Reserve University Cleveland, OH
Joseph Tuminello, Ph.D.	Assistant Professor	McNeese State University Lake Charles, LA
Eun Um, Ed.D.	President and CEO	AMSTAT Consulting San Jose, CA
David Williams, Ph.D.	Distinguished Professor	Oregon State University Corvallis, OR

Attachment B: Federal Register Notice Announcing Meetings

ENVIRONMENTAL PROTECTION AGENCY

[FRL-10408-01-ORD]

Human Studies Review Board; Notification of Public Meetings

AGENCY: Environmental Protection Agency (EPA).

ACTION: Notice of public meeting.

SUMMARY: The Environmental Protection Agency (EPA), Office of Research and Development (ORD), gives notice of 2023 public meetings of the Human Studies Review Board (HSRB). The HSRB provides advice, information, and recommendations on issues related to scientific and ethical aspects of third-party human subjects' research that are submitted to the Office of Pesticide Programs (OPP) to be used for regulatory purposes.

DATES: Four three-day virtual public meetings will be held on:

1. February 15–17, 2023; and
2. April 18–20, 2023; and
3. July 25–27, 2023; and
4. October 11–13, 2023.

Meetings will be held each day from 1 p.m. to 4 p.m. Eastern Time. For each meeting, separate subsequent follow-up meetings are planned for the HSRB to finalize reports from the three-day meetings. These meetings will be held from 1 p.m. to 4 p.m. Eastern Time on the following dates: March 23, 2023; May 18, 2023; August 23, 2023; and November 16, 2023.

ADDRESSES: These meetings are open to the public and will be conducted entirely virtually and by telephone. For detailed access information and meeting materials please visit the HSRB website: *https://www.epa.gov/osa/human-studies-review-board*.

FOR FURTHER INFORMATION CONTACT: Any member of the public who wishes to receive further information should contact the HSRB Designated Federal Official (DFO), Tom Tracy, via phone/voicemail at: 919-541-4334; or via email at: *tracy.tom@epa.gov*.

SUPPLEMENTARY INFORMATION:

Background

The HSRB is a Federal advisory committee operating in accordance with the Federal Advisory Committee Act 5 U.S.C. App.2 section 9. The HSRB provides advice, information, and recommendations on issues related to scientific and ethical aspects of third-party human subjects research that are submitted to OPP to be used for regulatory purposes.

Meeting access: These meetings will be open to the public. The full agenda with access information and meeting materials will be available seven calendar days prior to the start of each meeting at the HSRB website: *https://www.epa.gov/osa/human-studies-review-board*. For questions on document availability,

or if you do not have access to the Internet, consult with the DFO, Tom Tracy, listed under **FOR FURTHER INFORMATION CONTACT**.

Special Accommodations. For information on access or services for individuals with disabilities, or to request accommodation of a disability, please contact the DFO listed under **FOR FURTHER INFORMATION CONTACT** at least 10 days prior to each meeting to give EPA as much time as possible to process your request.

How May I Participate in this Meeting?

The HSRB encourages the public's input. You may participate in these meetings by following the instructions in this section.

1. *Oral comments.* To preregister to make oral comments, please contact the DFO, Tom Tracy, listed under **FOR FURTHER INFORMATION CONTACT**. Requests to present oral comments during the meetings will be accepted up to Noon Eastern Time, seven calendar days prior to each meeting date. To the extent that time permits, interested persons who have not preregistered may be permitted by the HSRB Chair to present oral comments during the meetings at the designated time on the agenda. Oral comments before the HSRB are generally limited to five minutes per individual or organization. If additional time is available, further public comments may be possible.

2. *Written comments.* For the Board to have the best opportunity to review and consider your comments as it deliberates, you should submit your comments prior to the meetings via email by Noon Eastern Time, seven calendar days prior to each meeting date. If you submit comments after these dates, those comments will be provided to the HSRB members, but you should recognize that the HSRB members may not have adequate time to consider your comments prior to their discussion. You should submit your comments to the DFO, Tom Tracy listed under **FOR FURTHER INFORMATION CONTACT.** There is no limit on the length of written comments for consideration by the HSRB.

Topics for discussion. The agenda and meeting materials will be available seven calendar days in advance of each meeting at *https://www.epa.gov/osa/human-studies-review-board*.

Meeting minutes and final reports. Minutes of these meetings, summarizing the topics discussed and recommendations made by the HSRB, will be released within 90 calendar days of each meeting. These minutes will be available at *https://www.epa.gov/osa/human-studies-review-board*. In addition, information regarding the HSRB's Final Reports, will be found at *https://www.epa.gov/osa/human-studies-review-board* or can be requested from Tom Tracy listed under **FOR FURTHER INFORMATION CONTACT**.

Dated:

Mary Ross, Director, Office of Science Advisor, Policy and Engagement.