HSRB Human Studies Review Board

June 9, 2023

H. Christopher Frey, Ph.D.
Assistant Administrator, Office of Research and Development
United States Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Subject: April 18, 2023, EPA Human Studies Review Board Meeting Report

Dear Dr. Frey:

The United States Environmental Protection Agency (EPA) requested that the Human Studies Review Board (HSRB) provide scientific and ethics review of a non-guideline study involving human participants.

On April 18, 2023, the HSRB considered a study conducted by S. Freestone and P. McFarlane (2001): "A Single Oral Dose Study with Acephate Technical in Humans; Report Amendment 2." Briefly, this was a non-guideline, double-blind, placebo controlled, pharmacokinetic and cholinesterase (ChE) inhibition study. EPA proposes to use information from the study to improve predictability of future physiologically based pharmacokinetic (PBPK) modeling.

The HSRB's responses to the charge questions for the non-guideline pharmacokinetic and cholinesterase inhibition study presented at the meeting on April 18, 2023, along with detailed rationale and recommendations for their conclusions, are provided in the enclosed final meeting report.

Sincerely,

Lisa Corey

Julia A. Sharp

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

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

Report of the U.S. Environmental Protection Agency Human Subjects Review Board

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Eun Um, Ed.D.
HSRB Statistical Reviewer
AMSTAT Consulting

David Williams, Ph.D.
HSRB Science Reviewer
Oregon State University

EPA Contact

Tom Tracy, Designated Federal Officer

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Contents

List of Acronyms – Freestone and McFarlane (2001)

AUC	Area under the curve
BIC	Bayesian Information Criterion
CEB	Health Effect's Division Chemistry and Exposure Branch
CFR	Code of Federal Regulations
ChE	Cholinesterase
CL/F	Clearance
Cmax	Maximum concentration
ECG	Electrocardiogram
EPA	Environmental Protection Agency
HSRB	Human Studies Review Board
NOAEL	No-observed-adverse-effect level

PBPK	Physiologically based pharmacokinetic
SD	Standard Deviation
Tlag	First-order absorption lag time
Tmax	Time to maximum concentration
T1/2(el)	Half-life elimination

HSRB Meeting Report – Acephate Technical Study

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.

Introduction

On April 18, 2023, the Human Studies Review Board (HSRB) considered a study conducted by S. Freestone and P. McFarlane (2001): "A Single Oral Dose Study with Acephate Technical in Humans; Report Amendment 2." Briefly, this was a non-guideline, double-blind, placebo controlled, pharmacokinetic and cholinesterase (ChE) inhibition study. EPA proposes to use information from the study to improve predictability of future physiologically based pharmacokinetic (PBPK) modeling.

Review Process

The Board conducted a public meeting on April 18, 2023. Advance notice of the meeting was published in the *Federal Register* as "Human Studies Review Board; Notification of a Public Meeting" (EPA, FRL-9328-01-ORD). This Final Report of the meeting describes the HSRB's discussion, recommendations, rationale, and consensus in response to the charge questions on ethical and scientific aspects of the research.

For each agenda item, the Agency staff presented their review of the scientific and ethical aspects of the research. Each presentation was followed by clarifying questions from the Board. The HSRB solicited public comments and then proceeded to address the charge questions under consideration. The Board discussed the science and ethics charge questions and developed a consensus response to each question. For each of the charge questions, the Chair called for the Board to vote to confirm concurrence on a summary statement reflecting the Board's response.

In their evaluation and discussion, the Board considered materials presented at the meeting, research articles, and related materials, the Agency's science and ethics reviews of the research studies, the Agency's statistical analysis of the research data and oral comments from Agency staff during the HSRB meeting discussions. A comprehensive list of background documents is available at https://www.epa.gov/osa/april-18-20-2023-hsrb-meeting.

Charge Questions and Context

Charge to the Board – Science

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)," considered scientifically sound for the purposes of validating future PBPK models?

HSRB 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 partial information to support evaluation of future PBPK model performance, given the limitations and recommendations provided by the HSRB are taken into account.

Study Summary

This study was originally designed to "*determine the highest of four proposed dose levels of acephate technical causing no effect, or the lowest dose causing a light inhibitory effect, on blood cholinesterases in humans.*"

Subjects

This non-guideline, double-blind, placebo-controlled study enrolled 50 healthy adult volunteers (40 male and 10 female). Appropriate inclusion/exclusion criteria were designed for the study. Subjects were allocated to the experimental or placebo condition based on computer randomization.

Design

Male subjects were randomized into a placebo group or one of four single-dose oral treatments: (1) 0.35 mg/kg, (2) 0.70 mg/kg, (3) 1.00 mg/kg, or (4) 1.25 mg/kg at a ratio of 7 subjects to treatment and 3 to placebo. Female subjects were limited to the 1.00 mg/kg dosage, with 7 subjects in the exposure group and 3 in the placebo group. Dosages were selected based on previous animal studies. The experiment was conducted as a staggered lead-dose design with a single subject initially tested at each of the next higher doses before the remaining subjects were exposed.

Measures

Blood samples were collected pre-test (-10, -3, -2, -1 days and 30 minutes prior to drug administration) and then post-test at hours 1, 2, 4, 8, 12, 24, 48 and 72 as well as days 7 and 14 post dose and analyzed for plasma concentrations of acephate and its metabolite methamidophos. Repeated blood samples were collected for some individuals (male at 1.25 mg/kg at 72 hours; male placebo at 48 hours; male placebo at 72 hours; and female at 1.00 mg/kg at 8 hours) presumably owing to >20% variability from baseline in some cases. Urine samples were collected at hours 0-12, 12-24 and 24-48 post dose and analyzed for acephate and methamidophos. Several other clinical measures of all participants (e.g., systolic blood pressure, diastolic blood pressure, electrocardiogram (ECG) readings, hematology etc.) were monitored during the experiment.

Pharmacokinetic parameters (i.e., C_{max}, T_{max}, T_{lag}, $T_{1/2}$, CL/F and AUC) in blood plasma and dose recovery in urine samples were also calculated for each subject. Pre and post dose blood samples were also collected from each subject to determine pre and post dose ChE inhibition.

Statistical Analysis

A sample-size determination indicated a total of 50 subjects (n = 10 per dose level) would be adequate under the following assumptions: a subject:placebo ratio of 7:3 (2.33), effect size = 20% change from baseline, standard deviation (SD) = 8.7, and level of significance (α) = 0.05.

Descriptive statistics (mean and SD concentration for blood plasma ChE) were reported for each time point. Percent change in ChE inhibition from baseline was used in a repeated-measures analysis of variance (RM-ANOVA) for main effect (dose level), time, and interaction (dose X time) with subjects entered as the random effect. Pairwise comparisons against baseline were carried out using a Bonferroni adjustment if linear trend was not significant.

Pharmacokinetic models were developed to estimate mean parameter estimates based on ChE profiles of individual participants (male subjects only) for acephate and methamidophos. Models predicted area under the curve (AUC) as the outcome variable to estimate several traditional parameters: maximum concentration (Cmax), time to Cmax (Tmax), first-order absorption lag time (Tlag), half-life elimination (T1/2(el)), and clearance (CL/F). A data imputation methodology was outlined in the document to allow modeling.

Results

The study found all but one of the subjects showed acephate Cmax in plasma within 1-4 hours post dose. Acephate levels in plasma decreased to undetectable (<0.01 ppm) by 48 hr post dose. T1/2 values for acephate in plasma were 3.47-6.61 hr. Tmax for methamidophos in plasma was similar (1-4 hrs. except one subject) to that for acephate, and the methamidophos levels decreased to undetectable (<0.01ppm) by 24 hr post dose. T1/2 values for methamidophos in plasma were 3.54-11.57 hr. The dose recoveries, determined by measuring urinary acephate and methamidophos levels within 48 hr post dose, were at 25.8-61.8 % in males (four dose levels) and 12.4-52.6 % in females (only one dose level). The non-recovered portion of the administered doses remained unknown and were assumed to "be related to incomplete gastrointestinal absorption or formation of additional metabolites". This study did not observe a dose-related response of ChE inhibition or any other adverse effects that were attributable to treatment. The authors concluded the top doses in the male and female groups (1.25 and 1.0 mg/kg, respectively), were the no-observed-adverse-effect level (NOAEL) values.

Science and Statistical Review

The HSRB has the following comments regarding the science and statistics review:

- We agree that the data from the study could be used as part of a PBPK modeling effort for acephate. However, this study should not be the primary source of information used for modeling because the study does have serious limitations (as discussed below). This study may be considered as a supplemental piece of information to support other pharmacokinetic studies that are used to develop a PBPK model for acephate.
- This study recruited 40 male and only 10 female subjects, and all of them were healthy adults. The comparison was conducted at one dose rate (1.0 mg/kg) for females, and at this dose rate

the comparison results showed relatively lower recoveries of acephate and methamidophos in urine samples from females than those from males. Without thoroughly characterizing the differences among different doses and between genders, the estimated pharmacokinetic parameter values will have limited use and it is also difficult to evaluate potential variations of these values.

- A key concern relates to the apparent low percentage of acephate recovered in this study. The recovery of acephate and methamidophos in urine ranged from 25-62% in males and 12%-51% in females. This is a very poor mass balance. Some suggestions are made about the possibility of other (presumably minor) metabolites but that seems insufficient to account for the low recovery. The portion and fate of the non-absorbed acephate remains unknown. The apparent low oral recovery would need to be accounted for in any use of the data for pharmacokinetic modeling. Are there rodent data indicating bioavailability via the oral route? Is it known to be very low in other studies? Is it known whether significant metabolites (methamidophos) are detected in blood and urine and excreted in the feces? Such questions should be investigated when considering the use of this study.
- In EPA's Science Review, the Agency's contractor states that there was no evidence of a significant lag phase. This was true for most subjects, but for one subject (no. 12) the Cmax occurred at 8 hours (Study report, PDF page 70).
- The study sample was not representative of the general population (e.g., fairly strong restrictions such as no caffeine or smoking during the entire study period; all subjects were healthy). There is also an important lack of racial diversity in the subject group (all but 1 of the 50 subjects was apparently Caucasian). It is unknown if this would have any demonstrable impact on the experimental outcomes, particularly since the Asian participant was included in the placebo group.
- The administered acephate doses in this study ranged ~4-time difference (0.35-1.25 mg/kg). According to EPA's 2018 draft human health risk assessment for acephate, acephate exposures can vary significantly depending on the exposure scenario and the amount of acephate used as described on the product labels. For instance, according to Table 6.2.1, for residential post-application exposure scenarios, the calculated dermal exposure for adults varied from 0.09 to 6.4 mg/kg/d, and the calculated oral exposure for children varied from 0.001 to 0.05 mg/kg/d. Even greater variations of exposures (up to four orders of magnitude difference) were seen for occupational handler scenarios, as shown in Appendix C. This study observed that acephate exposure "was not considered to exhibit dose proportionality". EPA should keep in mind the range of doses tested in this study versus a much greater range of potential acephate exposures under different use conditions.
- It is noted in EPA's Science Review that some blood and urine results were outside the calibration range (i.e., 10-1,000 ng/mL). Additional discussion and quantification on this point would be helpful. A review of Table 8 in the Science Review suggests urinary results were far above the calibration range. For example, in males at 12 hours the mean plus standard deviation concentration in urine was 34,957 ± 21,973 ng/mL. For plasma values, the degree of out-of-calibration was less severe. For example, in Table 6 of the Science Review, the maximum acephate range reported is 1,598.6 ± 240.9 ng/mL, which is outside the 10-1,000 ng/mL calibration range but to a lesser degree. Does this suggest the plasma data are reliable, but the urinary data are not? Moreover, we also note that on page 43 of the study report, the report states "samples are diluted by the addition of acetone containing polyethylene glycol and

aliquots of the diluted samples were analyzed ...". This seems to imply that urine samples, when needed, were diluted before analysis to accommodate the 10-1000 ng/ml range of the analytical method. It is unclear whether this is what EPA refers to in the Science Review and whether the potential pre-analysis dilution has addressed EPA's concern.

- Acephate exposures could occur through routes other than oral ingestion. For instance, EPA assessed acephate exposures through inhalation, dermal contact, incidental oral ingestion and dietary intake (US EPA, 2006). In addition, in certain scenarios (e.g., occupational handlers), repeated acephate exposures may occur. Pharmacokinetic parameter values are needed and should be validated for different exposure routes and for repeated exposures. EPA should keep in mind the dosing method of this study (single-dose oral treatment) when using the data for evaluation of future PBPK models.
- EPA does not plan to use the health effects data (i.e., ChE inhibition in plasma and red blood cell for risk assessment. The HSRB agrees with this decision given that the results are highly variable among individuals and there is no apparent dose response pattern.
- Minor Comment. Note that if EPA's goal is to use the pharmacokinetic data for model evaluation, a review by chemists rather than toxicologists might be appropriate.
- The sample size determination of the study was unclear. Page 27 of 1312 states: "No formal sample size calculation was performed." Page 126 of 1312 states: "No formal sample size calculation is being performed." However, page 47 of 1312 provides an entire section on sample size determination ("6.9.1 Determination of Sample Size"). Sample size for repeated measures requires an assumption concerning correlation among the repeated measures, and this was not stated in the sample size section. Additionally, if 50 subjects were determined sufficient under the applied assumptions, it is unclear why 40/50 were male and 10/50 were female unless an assumption is 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 for female subjects. Even though there were no sex-related differences at 1.0 mg/kg dosing, this is not sufficient to conclude that 1.0 mg/kg is the NOAEL for females compared to 1.25 mg/kg for males.
- Several resulting concentrations were noted in the EPA Science Review to have fallen outside of the method evaluation range at several doses and time periods. It is unclear how these data points were handled in the analyses conducted in the original study.
- As noted in the EPA Science Review (p. 26, App. I), 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 EPA noted, the original use of Bonferroni corrections is likely overly conservative; hence, the suggested use of the many-to-one (i.e., several treatments vs. a single control) test by Dunnett is supported. Further, the sensitivity analyses conducted by the Health Effect's Division Chemistry and Exposure Branch (CEB) statisticians to include additional RBC ChE measurements from 4 subjects that were presumably ignored in the original analysis was appropriate.

Recommendations

Based on the review of the documents provided, including the EPA Science Review, the HSRB recommends that EPA:

- Change the wording of 'theatrical plates' in Table 2 of the EPA review to 'theoretical plates'.
- Clearly include as part of any modeling application a discussion on limitations of the study including the variability of the results (e.g., by using error bars), potential extreme observations (e.g., Tmax for subject 12 was at 8 hours which is much larger than the other subjects), and lack of representativeness of the general US population should be.
- Keep in mind the dose range of this study when using the data for evaluation of future PBPK models and consider the variations of acephate exposure potentials of different use scenarios in the US.
- Note that the more robust analyses performed by CEB statisticians utilizing mixed models with variance-covariance matrix chosen to minimize Bayesian Information Criterion (BIC) values is justified and the appropriate analytical strategy for these data.
- Note the lack of a clear sample size determination description in the US EPA Science Review. EPA should clarify in their review the impacts of the 80:20% gender allocation on the PBPK model evaluation given the sample size calculation is unclear in the original study.
- Note 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. Though the profiles are statistically similar to male subjects at this dose, this does not provide evidence at other doses. EPA should recognize the limitations of the data from the females (representing one dose rate) when conducting their evaluation exercise.

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 Code of Federal Regulations (CFR) part 26?

HSRB 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.

Ethics Review

Participant Recruitment and Selection

Volunteers were recruited through an advertisement.

Participants were then selected from this pool who were between 18 and 50 years of age, with no clinically important abnormal physical findings at the screening examination, no clinically relevant abnormalities in the results of laboratory screening evaluation, normal ECG, normal arterial pressure and heart rate, body weight between 50 and 100 kg and within +/- 15% of ideal body weight, able to

communicate well with the investigator and to comply with the requirements of the entire study, and who had provided written informed consent to participate in the study.

Subjects being considered for participation in this study were excluded for meeting any of the following criteria: administration of any investigational drug 0 to 3 months before entry to the study, a need for any medication from 0 to 5 days before entry to the study, any surgical or medical condition which might interfere with the absorption, distribution, metabolism or excretion of the test compound, presence or history of allergy requiring treatment, donation or loss of more than 400 ml of blood 0 to 12 weeks before entry to the study, serious adverse reaction or hypersensitivity to any drug, inability to communicate or cooperate with the investigator, objection by the subject's general practitioner to their patient's participation in the study, females of childbearing potential who were not taking adequate contraceptive precautions, females with a positive urine pregnancy test, smokers who could not abstain from smoking from 2 hours before the dose was administered to 8 hours post dose, any subject with a resting pulse of <45 bpm, a systolic BP of <100 mmHg or a PR interval on ECG of >210 ms, any subject who had exposure to anti-ChE's within one month of dosing, and all agriculture workers or pest control applicators were excluded from the study.

Informed Consent Process

Review of the information provided to potential subjects, including the consent form, indicates that general requirements for informed consent were met, and various elements of informed consent were accounted for, including: explanation of the scope and intent of the study, verbal and written communication of objectives, procedures, and risks involved with participation, societal benefits of the research, the subjects' ability to withdraw from the study at any time, the maintenance of participant confidentiality, and the provision of contact information for the supervising physicians.

Risks and Benefits

Risks of this study were minimized through (1) the selection and staging of dose sizes, (2) implementing a screening process ensuring only participation of apparently healthy adults, (3) having clinical staff on site at all times, and (4) tracking and responding appropriately to any participant reports of adverse effects.

Potential benefits of this study include the ability to "provide a more accurate assessment of the margin of safety associated with currently estimated human exposures" to acephate (p. 22). In addition, the EPA is proposing to use data from this study (pending validation and acceptance for use) to evaluate a PBPK model which would improve human health risk assessments.

While there were no benefits to the subjects, HSRB agrees with EPA's ethics review that the potential societal benefits outweigh the risks to subjects associated with the study. Nine adverse events were reported by 6 of the 50 subjects but were not considered serious or related to the subjects' participation in the study.

Independent Ethics Review

The protocol, information provided to volunteers, and consent form were reviewed by an independent ethics committee, which approved 3 amendments to the protocol. Some amendments result in updates to the consent form (e.g., information regarding the collection/monitoring of subjects' urine). HSRB agrees with EPA's perspective that reported deviations to the protocol during the study did not negatively affect the health, safety, and/or rights of the subjects.

Review Summary

In compliance with subpart K, risks to subjects were minimized, any risks to subjects were reasonable in relation to anticipated benefits (though there were no benefits to the subjects themselves), and selection of subjects appear to have been equitable based on the available information. Further, informed consent was sought from each prospective subject and informed consent appears to have been appropriately documented. The safety of subjects was ensured, and adequate provisions were provided to protect the privacy of subjects and to maintain the confidentiality of data.

In compliance with subpart L, the study required that potential subjects be at least 18 years of age (which excludes the participation of children). Pregnant females were also excluded from the study. As mentioned in the EPA's ethics review, the study report does not include discussion of females' nursing status.