UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C., 20460

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

February 22, 2023

MEMORANDUM

SUBJECT: Ethics Review of Unpublished Study of Acephate Oral Dosing Study in Humans

FROM: Michelle Arling
Human Research Ethics Reviewer (Acting)
Office of Pesticide Programs

TO: Dana Vogel, Director
Health Effects Division
Office of Pesticide Programs

REF: 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. Unpublished. (MRID 45388301)

I have reviewed available information concerning the ethical conduct of the study referenced in the documents titled "A Single Oral Dose Study with Acephate Technical in Humans; Report Amendment 2" by S. Freestone and P. McFarlane, and performed by Inveresk Research in Scotland. If the research is determined to be scientifically acceptable, I find no barrier in regulation to the U.S. Environmental Protection Agency's reliance on this study in actions under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) or §408 of the Federal Food, Drug and Cosmetic Act (FFDCA). EPA will ask the Human Studies Review Board (HSRB) to comment on this study.

Summary Characteristics of the Research

This is a pre-rule study involving intentional dosing of human subjects with acephate, conducted " to determine the highest of 4 proposed dose levels of acephate technical causing no effect or the lowest dose causing a slight inhibitory effect on blood cholinesterase activity in humans" (p. 7). The subject monitoring occurred from June 9, 1999, to August 23, 1999.

A total of 50 subjects, 40 males and 10 females, were enrolled in and completed the study. The study was conducted by physicians, nurses, and a pharmacist. Each subject received a single dose of acephate. Men received a dose of 0.35, 0.7, 1.0, or 1.25 mg/kg^{-1} or a placebo (lactose in a capsule). Women received a single dose of 1.0 mg/kg^{-1} or the placebo following the identification at the maximum no effect level identified in males. The dosing for the study occurred sequentially with increasing doses for each subgroup as set out in Table 1 of the study report (p. 26).

Table 1

Dosing Schedule

	Placebo mg.kg^{-1}	0.35 mg.kg^{-1}	0.7 mg.kg^{-1}	1.0 mg.kg^{-1}	1.25 mg.kg^{-1}	1.0 mg.kg^{-1} females
Session 1 Sub 001-002	1	1				
Session 2 Sub 003-011	2	6	1			
Session 3 Sub 012-021	3		6	1		
Session 4 Sub 022-031	3			6	1	
Session 5 Sub 032-040	3				6	
Session 6 (♀) 041-050	3					7

The study was conducted by physicians, nurses, and a pharmacist. Potential subjects visited the clinic performing the study prior to the dosing study for a pre-enrollment health evaluation and pre-dosing blood tests. For the administration of the single dose, subjects went to the clinic the day before the dose was administered and stayed in residence until two days after the dose was administered under the supervision of medical staff. On the day of dosing, subjects had breakfast and about 5 minutes after finishing, the test substance or placebo "was administered to the subjects in the sitting position with 150 ml of water… subjects remained seated or recumbent in the dosing area until 8 h after dosing (p. 36).

Prior to leaving the clinic, each subject had another physical examination. Subjects returned to the clinic at 7 and 14 days after the dose was administered for blood sampling and adverse effect reporting. Subjects were monitored during the study before dosing, at the time of dosing, and following dosing by hematology and clinical chemistry, urinalysis, blood cholinesterase assay, blood plasma, and urine.

The subjects were selected from a pool of volunteers recruited by the Inveresk Research Clinic, according to the inclusion and exclusion criteria. Prior to enrollment in the study, "each subject was informed of the nature and risks associated with the study and agreed in writing by a signature on the consent form to participate before entry into the study" (p. 28) Participants were free to discontinue participation at any time.

The protocol, information to volunteers, and informed consent form were reviewed and approved by the Inveresk Research Independent Ethics Review Committee. The protocol, amendments, ethics committee constitution and approvals, written volunteer information, and sample consent forms are included with the study report as Appendices A and B (pp. 87-264).

1. **Value of the Research to Society**: Acephate is an insecticide that was, at the time this study was conducted, used in agricultural production. The study was designed to identify doses that cause no effect or a slight inhibitory effect on blood cholinesterase. The results of the study "will be used to provide a more accurate assessment of the margin of safety associated with currently estimated human exposures" (p. 22). According to the materials provided to volunteers, "[i]t is hoped that the results of this study will confirm that the use of acephate technical does not pose an unreasonable risk to either workers or consumers" (p. 187).

   This study was not published. EPA is proposing to use the data from this study to validate a physiologically-based pharmacokinetic (PBPK) model. If validated and accepted for use, EPA will use this PBPK model in human health risk assessments, which will allow for a more refined risk assessment.

2. **Subject Selection**:

   a. ***Demographics.*** A total of 50 subjects (40 males and 10 females) were enrolled in and completed the study. Female subjects ranged in age from 20 to 46 years old, and male subjects ranged from 18 to 48 years old.

   b. ***Inclusion/Exclusion Criteria.*** The inclusion and exclusion criteria are detailed on pp. 20-22 of the study report. The study's inclusion criteria were: 18-50 years old; no clinically important abnormal physical findings at the screening examination or in the results of laboratory screening evaluation including plasma and RBC ChE; normal ECG, arterial pressure and heart rate; body weight between 50 kg – 100 kg (110 lbs – 220 lbs) and within 15% of ideal body weight; able to communicate well with the investigator and to comply with the requirements of the study; and willing to give written informed consent to participate (p. 30)

      Subjects were excluded from the study if: they took any test compound within 3 months of enrollment into the study or a new chemical entity within 4 months of enrollment into the study; needed any medication in the 5 days prior to enrollment

into the study; had a pre-existing condition that may have interfered with absorption, distribution, metabolism, or excretion of the test compound; had an allergy requiring treatment; had donated or lost more than 400 ml of blood within 12 weeks prior to enrolling in the study; had a serious adverse reaction or hypersensitivity to any drug; or "had a resting pulse of <45 b.p.m., a systolic BP of <100 mmHg or a PR interval on ECG of >210 ms," (p. 31). Agricultural workers, pest control operators, and persons "who had been exposed to an anti-ChE (including home pest control products) within one month of dosing" were excluded from the study. (p. 31) People who smoked and could not go from 2 hours before the dose was administered until 8 hours after the dose was administered without smoking were also excluded.

Females who had a positive pregnancy test at the screening exam or on the day before the test substance was administered study (p. 45), or who were of childbearing potential and were not taking adequate contraceptive precautions were excluded from the study.

Potential subjects were excluded if they had "an inability to communicate or co-operate with the investigator because of a language problem, poor mental development or impaired cerebral function" (p. 31).

Potential subjects' general practitioners were consulted about their patients' participation in the study. If a general practitioner objected to his or her patient's participation in the study, the person was excluded from participation (p. 31).

Up to 21 days prior to administration of the dose, volunteers were screened according to the inclusion and exclusion criteria and underwent a screening examination performed by a physician consisting of medical history; complete physical examination; ECG; hematology, clinical chemistry, plasma and RBC cholinesterase analysis, and urinalysis; screening for Hepatitis B and C, and HIV; and urine-based drug screening. Female subjects also were tested for pregnancy. The physician performing the screening also informed volunteers orally "and in writing about the objectives, procedures and risks involved in participating in the study" and "that the test compound was a pesticide." (p. 23) Assignment of volunteers who satisfied the inclusion and exclusion criteria to the test period and to receive the test substance or the placebo was randomized.

c. ***Pregnancy and Nursing Status.*** Per the exclusion criteria, no pregnant females were included in the study. Pregnancy status was confirmed by urinary test at the screening visit and as part of the testing performed the day before the dosing occurred. There is no discussion of females' nursing status in the study report or protocol.

d. ***Recruitment.*** Subjects were selected from a pool of volunteers recruited by

Inveresk Research. The study report notes that "healthy volunteers were recruited from the surrounding area through a generic advertisement for volunteer participation" (p. 22). A copy of the advertisement is not included in the study report.

During the recruitment phase, 63 males were screened for the study; of these 12 were rejected during the screening visit and 11 did not participate because they did not meet the eligibility criteria, or they failed to attend the clinic for admission (pp. 57-58). Twelve female volunteers were screened; of these, one was rejected at the screening visit and one was not needed as a test subject (p. 58).

3. **Risks and Benefits**:

a. ***Risks.*** The written information sheet provided to subjects included a section on physical risks involved with participation in the study and anticipated inconveniences. This section described the side effects including headache, nausea, chest tightness, coughing, and "vomiting, diarrhea, abdominal pain, blurred vision weakness, sweating, constricted pupils, excess saliva production, slow pulse, and involuntary muscle twitching" (p. 143), and goes on to note that it is unlikely that any of the side effects would occur based on the doses that would be used in the study. Subjects were also at risk of soreness and bruising from the blood tests, whether by needle and syringe, or cannula. Subjects were informed that they were free to withdraw from the study at any time for any reason; they were also informed that leaving the clinical unit within 24 hours of dosing could involve risks to their health. The protocol called for subjects withdrawing for non-medical reasons to receive additional information about the potential risks to their health (p. 155); no subjects withdrew during this period.

Risks were minimized by selecting a dose that was not expected to cause any adverse effects beyond cholinesterase inhibition, and staging the doses to increase until the maximum dose that does not significantly inhibit cholinesterase was identified. The screening process ensured that only apparently healthy adults were eligible to participate as subjects. Testing during the study period that showed abnormal values "were repeated as often as deemed necessary by the clinical investigator until the test values returned to accepted limits or until an explanation other than the compound effect was given" (p. 38). The study was conducted by physicians, registered nurses, and a pharmacist, and the subjects stayed on site at the clinic from one day before dosing until two days after dosing. Further, subjects returned to the clinic at 1 week and 2 weeks after their dosing and were asked about adverse events. The final protocol included "criteria for dose escalation to be set so that dose escalation would not occur if there were any significant inhibition of ChE activity." (p. 19) Subjects were monitored at the clinic for 2 days post-dosing and returned twice after being released from the residential portion of the study for follow up and assessment by the clinicians. Clinical staff including physicians and nurses were on site at all times to ensure the subjects' safety.

There were a total of 9 adverse events in 6 of the 50 subjects (pp. 62-65); none of the adverse events was serious and non were deemed to be related to study participation. There were 5 complaints of headache (some treated with paracetamol). In addition, one subject complained of dyspepsia, one subject reported leg pains, one subject reported a cough and sore throat, and one subject complained of dizziness. 20 of the 48 subjects; 40 adverse effects occurred after the dosing involving 18 subjects.

b. ***Benefits.*** There were no benefits to the subjects. The study evaluated the effects of a single dose of acephate on cholinesterase levels, as well as how acephate is metabolized. The potential benefits of the research according to the study report are described in Section 1, Value of Research to Society. Further, EPA is proposing to use the data generated by this research to validate a PBPK model. If validated and accepted for use, EPA will use this PBPK model in human risk assessments, which will allow for a more refined risk assessment.

c. ***Risk-Benefit Balance.*** Overall, the potential societal benefits of understanding the excretion of acephate outweigh the risks to subjects associated with the study.

4. **Independent Ethics Review**: The protocol, information provided to volunteers, and consent form were reviewed by the Independent Ethics Review Committee of Inveresk Research. The ethics committee met on November 5, 1988 and provided conditional approval of the protocol. The final protocol and materials were dated April 21, 1999, and approved on April 24, 1999 (p. 238). The standards under which the protocol was conducted included the Declaration of Helsinki (up to the 1996 amendments) and the principles of the United States Common Rule (p. 27). The ethics committee followed standard operation procedures that included "a statement of principles, a description of the membership, written procedures which the IRB follow [sic], written procedures for ensuring prompt reporting to the IRB by ICR, and authority of the IRB to approve, modify or disapprove of research activities (p. 28)". The criteria to be met for the ethics committee to approve research include minimizing risks to subjects, making the risks to the subjects reasonable in relation to anticipated benefits to subjects, ensuring equitable selection of subject, requiring informed consent from subjects, and collecting adequate monitoring data to ensure the safety of the subjects (p. 28).

   There were 3 amendments to the protocol. On July 9, 1999, the ethics committee approved the Amendment 1 (p. 239), which added to the schedule PK blood sampling at days 7 and 14, to update the consent form to include information that the subjects' urine would be monitored, and to amend the parameters for the normal range of red cell cholinesterase (p. 167). Amendment 2 to the protocol was made on August 17, 1999 and was sent to the IRB for informational purposes only (p. 240), as it set the dose chosen for females based on the protocol criteria and amended the consent form (pp. 257-264).

There were several reported deviations to the protocol (p. 59). Some occurred before the test substance was administered (screened outside the 21-day target window, drank alcohol within 48 hours of the test substance administration, took paracetamol within 5 days of dosing, screening heart rate outside the acceptable range but reviewed by the Study Director) (p. 59). Some deviations involved missing or mistimed sample collection (Subject 48 did not provide a cholinesterase sample 12 days before test substance administration, Subject 50 dosed earlier than planned so did not have full 30 minutes of ECG monitoring, all subjects had cholinesterase samples collected with other pre-dose blood collection, which was outside the protocol-specified 30 minutes) (p. 59). From the EPA's perspective, the reported deviations did not negatively affect the health, safety, and/or rights of the subjects.

5. **Informed Consent**: According to the study report, "the scope and intent of the study was clearly explained to the volunteers by the screening physician. All participants were informed verbally and in writing about the objectives, procedures, and risks involved in participating in the study and that the test compound was a pesticide" (p. 23). Subjects were given the opportunity to ask questions of a doctor associated with the study prior to signing the consent form. They received a copy of a document titled "Volunteer Information," which identifies acephate as the test subject and a pesticide; describes the risks and benefits of the research; explains the study procedure and purpose, and intended use of the study results; lists the inclusion and exclusion criteria; outlines the circumstances for stopping a subject's participation; notes that all subjects' information would be treated in a confidential manner; and provides contact information for the supervising physicians (p. 187-190).

   The consent form references the information in the "Volunteer Information" document and acknowledges receipt of the document and the same information in an oral manner, describes the study, restates that participants are free to withdraw from the study at any time, and outlines the compensation for participation. The consent form also includes authorization for Dr. Freestone, a principal investigator on the study, to contact the subject's general practitioner and for the general practitioner to report relevant details from the subject's medical history to the Dr. Freestone (p. 183-186).

   Neither the informed consent form nor the "Volunteer Information" document provide participants with specific information about who to contact in the event of a study-related question or who to contact after the study's completion with questions or concerns.

6. **Respect for Potential and Enrolled Subjects**: At the screening session, a physician provided volunteers with written and oral information about the study, and a copy of the informed consent form. Volunteers had time to review the materials and ask questions prior to signing the informed consent form the day prior to enrolling in the study. According to the informed consent form, subjects received £450 (~$550 at February 2023 conversion rate) for completing participation in the study (p. 184).

Subjects were free to withdraw from the study at any time for any reason. Subjects who withdrew from the study prior to completion for medical reasons were eligible for compensation in proportion to the time they were enrolled in the study. Subjects who withdrew for non-medical reasons were eligible to receive compensation at the discretion of the supervising doctor. No subjects withdrew from the study or were removed from participation by the Study Director.

The study sponsor agreed to compensate participants for any injuries caused directly by participation in the study; however, the compensation could be reduced if the subject bore some responsibility for the injuries. Participant confidentiality was maintained by providing each participant a random, individual identification number. Subjects' privacy was not compromised in the report.

Standards Applicable to the Conduct of the Research

The study reported in documents titled "A Single Oral Dose Study with Acephate Technical in Humans; Report Amendment 2" (MRID 45388301) was completed and submitted to EPA before the effective date of EPA's amended Rule for the Protection of Human Subjects of Research (40 CFR part 26) on April 7, 2006.

The portions of EPA's regulations regarding the conduct of research with human subjects, 40 CFR part 26 subpart A - L, do not apply since the research was neither conducted nor supported by EPA, nor was it initiated on or after to the effective date of the amended Rule for the Protection of Human Subjects.

The study report notes that the research was conducted in accordance with the Declaration of Helsinki. Some of the key principles from the 1996 Declaration of Helsinki are:

1. Research must be scientifically sound and conducted by qualified personnel.
2. There must be a clear purpose and protocol, reviewed and approved by an independent ethics committee.
3. The importance of the study's objective must outweigh the inherent risks to subjects, and measures to minimize risks must be implemented. The interests of science and society should never take precedence over considerations related to the well-being of the subject.
4. Respect the privacy of subjects and confidentiality of their personal information.
5. Participants should give prior, informed, voluntary consent and have the freedom to withdraw from the study. Steps should be taken to avoid situations where subjects feel pressure to provide consent for any reason.

The study report also notes that the research followed the principles of the Common Rule, which required appropriate review and approval of research by IRBs, that the benefits of the research outweigh the risks, that subjects are treated with respect, and that researchers get

informed consent from those who participate in research. This includes giving them information they would need to make an informed decision about participation in language they would understand.

Finally, the report includes the criteria considered by the IRB when reviewing and approving a study, which includes whether risks to subjects are minimized and reasonable in relation to anticipated benefits to the subjects, the selection of subjects is equitable, whether informed consent is required, and whether there is adequate monitoring of the data collected to ensure safety of the subjects.

Standards Applicable to the Documentation of the Research

The study reports were submitted to EPA on April 24, 2001, prior to the effective date of EPA's amended Rule for the Protection of Human Subjects of Research (40 CFR part 26) on April 7, 2006. Consequently, the requirements for the submission of information concerning the ethical conduct of completed human research contained in EPA regulations at 40 CFR part 26, subpart M do not apply.

Standards Applicable to EPA's Reliance on the Research

The Agency's rule (40 CFR part 26 subpart Q) defines standards for EPA to apply in deciding whether to rely on research—like this study—involving intentional exposure of human subjects. This study was initiated prior to the effective date of the rule; therefore, the applicable acceptance standards from 40 CFR part 26 subpart Q are these:

> **§26.1703.** Except as provided in §26.1706, EPA must not rely on data from any research subject to this subpart involving intentional exposure of any human subject who is a pregnant woman (and therefore her fetus), a nursing woman, or a child.
>
> **§26.1704(b).** EPA must not rely on data from any research subject to this section if there is clear and convincing evidence that: (1) The conduct of the research was fundamentally unethical (e.g., the research was intended to seriously harm participants or failed to obtain informed consent); or (2) The conduct of the research was deficient relative to the ethical standards prevailing at the time the research was conducted in a way that placed participants at increased risk of harm (based on knowledge available at the time the study was conducted) or impaired their informed consent.
>
> In addition, FIFRA §12(a)(2)(P) applies. This passage reads:
>
> In general, [i]t shall be unlawful for any person . . . to use any pesticide in tests on human beings unless such human beings (i) are fully informed of the nature and purposes of the test and of any physical and mental health consequences which are reasonably foreseeable therefrom, and (ii) freely volunteer to participate in the test.

EPA has submitted this study for review by the HSRB in conformance with 40 CFR §26.1604.

Compliance with Standards

The subjects were all adults. Pregnant women were excluded from participation in the study. Although the study report has no information on whether nursing women participated, there is no evidence to indicate that any nursing woman participated in the study. Therefore, EPA's reliance on this research is not prohibited by 40 CFR §26.1703.

40 CFR §26.1704 forbids EPA to rely on data from pre-rule research—such as this study—if there is "clear and convincing evidence that the conduct of the research was fundamentally unethical…or was significantly deficient relative to the ethical standards prevailing at the time the research was conducted in a way that placed participants at increased risk of harm (based on knowledge available at the time the study was conducted) or impaired their informed consent."

The research was reviewed and approved by an IRB prior to implementation of the study. The IRB was an independent ethics board that evaluated the research under several ethical standards and found that the protocol met the necessary criteria. Subjects received information about the study (including risks and benefits) in a language they understood, had the opportunity to ask questions, and provided written informed consent prior to their enrollment in the study. Subjects' safety was carefully monitored throughout their participation by appropriately qualified medical personnel. Subjects were compensated for their participation.

I find no evidence that this research was fundamentally unethical, or that its conduct was significantly deficient relative to the ethical standards prevailing at the time the research was conducted.

The study report includes the protocol, informed consent form, and informational materials for participants that were approved by the independent ethics committee. My review of this documentation supports a conclusion that the research was not deficient relative to the ethical standards prevailing at the time the research was conducted. Therefore, 40 CFR §26.1704 does not prohibit EPA reliance on this research.

FIFRA §12(a)(2)(P) requires that human subjects of research with pesticides be "fully informed of the nature and purposes of the test and of any physical and mental health consequences which are reasonably foreseeable" from their participation and freely volunteer to participate. The reported descriptions of the consent process appear to meet the substantive requirements of FIFRA §12(a)(2)(P).

Conclusion

Based on the ethics review, I find no barrier in law or regulation to EPA reliance on this research (MRID 45388301) in actions taken under FIFRA or §408 of FFDCA. I defer to others for a review of the scientific validity of the study; if it were determined not to have scientific validity, it would also not be ethically acceptable.