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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

December 19, 2016

MEMORANDUM

SUBJECT: Ethics Review of Unpublished Study of Malathion Oral Dosing Study in Humans

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

TO: Yung Yang, PhD
Health Effects Division
Office of Pesticide Programs

REF: Gilles, D., Dickson, J. (2000) A Randomised Double Blind Ascending Single Oral Dose Study with Malathion to Determine the No Effect Level on Plasma and RBC Cholinesterase Activity. Inveresk Research, Elphinstone Research Centre, Scotland. ICR 013177. March 20, 2000. Unpublished. (MRID 45125602)

Aston, L.S. (2000). Determination of residues of malathion dicarboxylic acid (DCA), malathion monocarboxylic acid (MCA), dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), and dimethyl dithiophosphate (DMDTP) in human urine. Pacific Toxicology Laboratories, 6160 Variel Avenue, Woodland Hills, CA 91367. PTL119801. October 11, 2000. Unpublished. (MRID 45244601)

I have reviewed available information concerning the ethical conduct of the study referenced in the documents titled "A Randomised Double Blind Ascending Single Oral Dose Study with Malathion to Determine the No Effect Level on Plasma and RBC Cholinesterase Activity" authored by D. Giles and J. Dickson, performed by Inveresk Research in Scotland, and sponsored by Cheminova Agro A/S of Denmark and "Determination of residues of malathion dicarboxylic acid (DCA), malathion monocarboxylic acid (MCA), dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), and dimethyl dithiophosphate (DMDTP) in human urine" authored by L.S. Aston, performed by Pacific Toxicology Laboratories, and sponsored by Cheminova Agro A/S of Denmark. 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.

The document titled "Determination of residues of malathion dicarboxylic acid (DCA), malathion monocarboxylic acid (MCA), dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), and dimethyl dithiophosphate (DMDTP) in human urine" is a report of the analysis conducted by Pacific Toxicology Laboratories of the urine samples collected under the study protocol referenced in the report "A Randomised Double Blind Ascending Single Oral Dose Study with Malathion to Determine the No Effect Level on Plasma and RBC Cholinesterase Activity." Amendment 2 to the approved protocol for this study noted that Pacific Toxicology Laboratories would conduct the analysis of urine samples collected, rather than Inveresk Research (p. 19, 208-222), and amendment 5 to the protocol noted that the urine analysis would be reported separately from the study report. (pp. 19-20, 239-244, 302) All references to page numbers in this document refer to the study report "A Randomised Double Blind Ascending Single Oral Dose Study with Malathion to Determine the No Effect Level on Plasma and RBC Cholinesterase Activity".

Summary Characteristics of the Research

This is a pre-rule study involving a pesticide, malathion, conducted from October 15, 1998 to March 22, 1999. According to the study report, "[t]he primary objective of this study was to determine the highest dose of malathion causing no effect or the lowest dose causing a slight inhibitory effect on blood cholinesterase activity in humans. … The aim was to detect the effects of single oral doses of malathion on humans and identify a dose level with no effect on plasma and red blood cell (RBC) cholinesterase (ChE) activity." (p. 14) The study also measured urinary excretion of metabolites of malathion.

A total of 48 subjects, 38 males and 10 females, were enrolled in and completed the study. A total of 51 subjects were recruited for the study (3 alternates replaced test subjects prior to the initiation of the study). The study was conducted by physicians, nurses, and a pharmacist. Each subject received either a single dose of malathion or a placebo. The doses of malathion used in the study were 0.5, 1.5, 5, 10, and 15 mg/kg^{-1}, which were administered to subjects in 7 treatment blocks. Subjects were assigned to treatment blocks and to receive malathion or the placebo randomly. The dose was increased progressively with each subsequent treatment block, and the increase "was permitted only after full review of all safety data indicated that it was safe to do so." (p. 18) Subjects received the single dose of malathion or the placebo in a capsule, while in a sitting position, approximately 5 minutes after breakfast, and remained in a sitting position for 8 hours after the dose was administered. According to the study report, the dosing occurred as follows:

> In the first block, one subject received placebo and 3 subjects received the lowest dose of malathion (0.5 $mg.kg^{-1}$). The second treatment block also consisted of 4 subjects. One subject received placebo and 3 subjects received 1.5 $mg.kg^{-1}$ of malathion. Session 3 consisted of 10 subjects. Seven subjects

> received 5.0 mg.kg^{-1} of malathion and 3 subjects received placebo. Session 4 consisted of 4 subjects. One subject received placebo and 3 subjects received 10.0 mg.kg^{-1} malathion. Session 4 consisted of 9 subjects. Since no effect was seen at 10.0 mg.kg^{-1} of malathion in Session 4, a further 4 subjects received 10.0 mg.kg^{-1}, 3 subjects received 15.0 mg.kg^{-1} and two received placebo. Session 6 consisted of 7 subjects. Since no effect was seen at 15.0 mg.kg^{-1} in Session 5, a further 4 subjects received 15.0 mg.kg^{-1} of malathion and 3 subjects received placebo. Session 7 consisted of 10 female subjects, 7 of whom received 15.0 mg.kg^{-1} of malathion and 3 placebo. Since the NOEL in males was greater than 15.0 mg.kg^{-1}, the highest level tested, the female group was dosed at 15.0 mg.kg^{-1}. (pp. 17-18)

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 morning before the dose was administered and spent three nights in residence at the clinic under the supervision of medical staff. Prior to leaving the clinic, each subject had another physical examination. Subjects returned to the clinic at 4, 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 of the study and given a copy of the volunteer consent form and information to review." (p. 20) All subjects gave written informed consent prior to participation in the dosing study. 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. 67-326) The study report notes that the study was conducted in accordance with the Declaration of Helsinki. (p. 20)

1. **Value of the Research to Society**: Malathion is an insecticide that was, at the time this study was conducted, used in agricultural production, to treat scabies and lice, and for public health insect control. According to the materials provided to volunteers:

> This study is being conducted to reduce the uncertainties of species differences in determining a level of human exposure that causes measured reduction of blood cholinesterase levels. Prior testing has shown that a relatively large reduction of blood cholinesterase is required before

any resulting clinical effects are observed. The results of this study are expected to be useful in showing that the use of malathion on crops does not pose health risks to food consumers, and also may be useful in evaluating whether workers who use malathion are thereby at risk. (p. 187)

This study was not published. EPA is proposing to use the residues of urinary metabolites reported 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. EPA anticipates that the PBPK model for malathion will be reviewed by the FIFRA Science Advisory Panel in summer 2017.

2. **Subject Selection**:

   a. ***Demographics.*** A total of 51 subjects were recruited for participation in the study. 48 subjects, 38 males (18 to 48 years old) and 10 females (18-41 years old), were enrolled in and completed the study. Three test subjects were selected to participate but did not enroll in the dosing study. Two subjects withdrew because they could not swallow the capsules with the test compound, and one subject did not participate in the dosing study because subject's plasma and RBC ChE levels were abnormal at screening and pre-dose testing. These three subjects were replaced with alternates who met the inclusion/exclusion criteria and who participated in the dosing study.

   b. ***Inclusion/Exclusion Criteria.*** The inclusion and exclusion criteria are detailed on pp. 20-22 of the study report. Volunteers were screened up to 21 days prior to dosing according to the established criteria. 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 giving written informed consent to participate. (pp. 20-21)

      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. 22). 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. 22) 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 dosing study, 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. 21)

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.

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. 15) 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. 14) A copy of the advertisement is not included in the study report.

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. It states "[i]n view of the low doses of malathion to be used in this study and the results of earlier studies, it is not anticipated that any of the subjects of the study will experience any adverse effects other than a reduced level of blood cholinesterase." (p. 188). The document also explains human studies with other organophosphates (known cholinesterase inhibitors) suggest that at higher doses, symptoms could include: "headache, nausea, chest tightness, coughing, vomiting, diarrhea, abdominal pain, blurred vision, weakness, sweating, constricted pupils, excessive saliva preparation, slow pulse, and involuntary muscle twitching or spasm. A few reported cases of coma or death from ingestion of large quantities of malathion have been reported." (p. 188) 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 by explaining the study, requirements, and potential risks to participants. The screening process ensured that only apparently healthy adults were eligible to participate as subjects. 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) With respect to potential risks associated with a single dose of malathion, subjects were monitored at the clinic for 2 days post-dosing, and periodically until the 14^{th} day after the dose was administered. Clinical staff including physicians and nurses were on site at all times to ensure the subjects' safety.

There were a total of 44 adverse events in 20 of the 48 subjects; 40 adverse effects occurred after the dosing involving 18 subjects. Evaluation of relatedness was performed by the investigator, while the study was blinded, and again later, taking into consideration a variety of factors including whether the subject received a placebo or test compound and clinical effects. There was no evidence of inhibition of plasma or RBC ChE with any adverse event; the ultimate conclusion was that no adverse events were likely related to participation in the study. One serious adverse effect occurred; it was related to a pre-existing condition of the subject and was not related to participation in the study. (pp. 54-62)

b. ***Benefits.*** There were no benefits to the subjects. The study evaluated the effects of a single dose of malathion on cholinesterase levels, as well as how malathion 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 reported residues of urinary metabolites from this study 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. EPA anticipates that the PBPK model for malathion will be reviewed by the FIFRA Science Advisory Panel in summer 2017.

c. ***Risk-Benefit Balance.*** The potential societal benefits of understanding the excretion of metabolites of malathion 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 Inveresk Research Independent Ethics Review Committee. The final protocol and materials were approved on October 28, 1999, pending incorporation of the changes requested in the committee's approval letter. There were 5 amendments to the protocol. Amendment 1 (pp. 138-206) incorporated the recommended changes included in the committee's letter and made changes based on requests from the study director. Amendment 2 (pp. 208-222) added the dose level for females to the protocol and volunteer information, amended the quality assurance section, added Pacific Toxicology Laboratories as the location where urine pharmacokinetic samples would be analyzed, and added a person as clinical research associate. Amendment 3 (pp. 223-234) amended the timeframe for the study, moved the Good Laboratory Practices (GLP) statement within the protocol, detailed the analysis of blood and urine samples under GLP, and incorporated quality assurance and other similar information into the collection and analysis of samples section of the protocol. Amendment 4 (pp. 235-238) replaced the description of the method for urine collection, storage, transportation, and analysis with a note that urine analysis by Pacific Toxicology Laboratories would be conducted under a separate protocol. Amendment 4 also included a note that plasma analysis would begin with samples from the subjects dosed at 15 mg/kg^{-1} and subjects receiving the placebo in the same test session; if analysis of these plasma samples showed no malathion or metabolites, further analysis of samples would not be required. Amendment 5 (pp. 239-243) documented that the urine analysis performed by Pacific Toxicology Laboratories would be reported separately from the results of the dosing study. Note: Amendment 5 was mistakenly titled Amendment 4. (p. 244)

   The protocol stated that each amendment would be documented, signed, and dated by the study director, and sent to the sponsor and ethics committee for approval. (p. 169) The study director documented each amendment and notified the study sponsor, but did not send all amendments to the ethics committee for approval. The ethics committee reviewed and approved Amendments 1 and 2. Amendment 3 was not sent to the ethics committee for review. (p. 300). Amendment 4 was issued after the clinical phase of the study was complete and therefore did not affect the safety of the subjects. It was not sent to the ethics committee. (p. 301) Amendment 5 was an administrative amendment and not sent to the ethics committee. (p. 302)

There were several deviations to the protocol reported on pp. 41-43. Three subjects in the study failed to meet a criterion; two subjects were underweight and one took medication during the 5-day period before the dosing. The study director maintained enrollment of these subjects in the study after concluding that the deviations would not have an effect on the study results. Other deviations occurred related to the timing of collection of samples or measuring vital signs; in general, these deviations were minor. 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:

> At screening, each subject was informed of the nature and risks of the study and given a copy of the volunteer consent form and information to review. On admission to the clinic on the evening prior to dosing, written informed consent was obtained from each volunteer. Each subject's general practitioner was asked if they had any objections to their patient's participation before the start of the study. (p. 20)

Subjects received information orally and in writing. According to the consent form, they 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 malathion 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. 219-222)

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, notes that the participant had the opportunity to ask questions of a doctor associated with 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. 215-218)

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 ($640 at Nov. 30, 2000 conversion rate) for completing participation in the study. 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. 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 Randomised Double Blind Ascending Single Oral Dose Study with Malathion to Determine the No Effect Level on Plasma and RBC Cholinesterase Activity" (MRID 45125602) and "Determination of residues of malathion dicarboxylic acid (DCA), malathion monocarboxylic acid (MCA), dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), and dimethyl dithiophosphate (DMDTP) in human urine" were 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. The key ethical principles in the Declaration of Helsinki are respect for persons, beneficence and justice.

Standards Applicable to the Documentation of the Research

The study reports were submitted to EPA on October 25, 2000, 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." 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. Although the requirements of 40 CFR §26.1303 to document the ethical conduct of the research do not apply, because the research was submitted prior the effective date of EPA's amended Rule for the

Protection of Human Subjects, the documentation supports a conclusion that the research was not deficient relative to the ethical standards prevailing at the time the research was conducted. Although several amendments to the protocol were not submitted to and approved by the ethics committee, from the EPA's perspective, there is no indication that the failure to submit these amendments to the ethics committee negatively affected the health, safety and/or rights of the subjects. 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 (MRIDs 45125602, 45244601) 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.

cc: Rick Keigwin
Jeff Dawson
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