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

OFFICE OF
PREVENTION, PESTICIDES AND TOXIC
SUBSTANCES

May 29, 2009

MEMORANDUM

SUBJECT: Ethics Review of Chlorpyrifos Human Toxicity Study

FROM: John M. Carley
Human Research Ethics Review Officer
Office of Pesticide Programs

TO: Anna Lowit, Ph.D.
Health Effects Division

RE: Kisicki, J.; Seip, C.; Combs, M. (1999) A Rising Dose Toxicological Study to Determine the No-Observable-Effect-Levels (NOEL) For Erythrocyte Acetylcholinesterase (AChE) Inhibition and Cholinergic Signs and Symptoms of Chlorpyrifos at Three Dose Levels. Unpublished study prepared by MDS Harris Laboratories under Project No. 21438 and Dow AgroSciences Study No. DR K-0044793-284. 578 p. (MRID 44811002)

Juberg, D.; Mattsson, J. (2008) Dow AgroSciences Response to EPA Query Regarding Two Toxicology Reports. Unpublished document prepared by Dow AgroSciences LLC under Study ID DRJ05142008. 20 p. (MRID 47429401)

Juberg, D.; Mattsson, J. (2008) Updated Dow AgroSciences Response to EPA Query Regarding Two Toxicology Reports. Unpublished document prepared by Dow AgroSciences LLC under Study ID DRJ05282008. 27 p. (MRID 47436401)

I have reviewed the cited documents with care, and have concluded that EPA is forbidden by 40 CFR §26.1704 to rely on the Kisicki *et al.* study, MRID 44811002, in actions taken under FIFRA or §408 of FFDCA. It is possible that the circumstances and purposes for which you propose to consider it may be such that the provisions of 40 CFR §26.1706 for an exception to the prohibition in 40 CFR §26.1704 may be satisfied.

A. Summary Assessment of Ethical Conduct of the Research

In this study 60 healthy adult male and female volunteers were administered one of three oral doses of chlorpyrifos or placebo, confined for the first 48 h post-dosing, and further monitored at intervals up to 7 days post-treatment. Subjects provided urine from 48 hours pre-dosing through the study period. Blood and urine were analyzed for chlorpyrifos and TCP, its principal metabolite, and for red blood cell acetylcholinesterase activity. As part of initial screening of subjects, blood samples were collected several days before dosing for paraoxonase analysis and for clinical laboratory tests.

No signs of toxicity were observed. No treatment-related differences in clinical laboratory tests were observed. One subject in the high dose group experienced statistically significant inhibition of RBC cholinesterase, but withdrew when released from confinement 48 hours post-treatment and was lost to follow-up. Investigators reported a NOEL for toxic signs and symptoms of 2.0 mg/kg, and a NOEL for RBC AChE inhibition of 1.0 mg/kg.

The study was sponsored by Dow AgroSciences and conducted by MDS-Harris Clinical Laboratory in Lincoln, Nebraska in September and October of 1998. The final report was completed and submitted to EPA in April 1999, and assigned MRID 44811002. In April 2008 EPA asked the sponsor to address a series of questions, focusing on tracking of individual subjects. An initial response was submitted by DAS on May 14, 2008 (MRID 47429401); expanded responses to the same questions were submitted by DAS on May 28, 2008 (MRID 47436401).

Value of the Research to Society: The objective of this study was “to determine the no-observable-effect-level (NOEL) for erythrocyte (RBC) acetylcholinesterase (AChE) inhibition following a single oral dose of 0.0 (placebo), 0.5, 1.0, or 2.0 mg chlorpyrifos/kg body weight. The RBC AChE data will better define the relationship between exposure to chlorpyrifos and inhibition of this frequently used marker of organophosphate (OP) exposure.” The study was funded by Dow AgroSciences, the principal registrant of chlorpyrifos, and was undertaken and submitted to EPA voluntarily. EPA now proposes to use RBC cholinesterase data and blood and urine analyses from this study to support the interpretation of animal testing and epidemiological data concerning developmental effects of chlorpyrifos.

Subject Selection: Although the figure of 60 subjects is reported at many points¹, there were actually many more than 60 candidates and volunteers involved in this research. Investigators report screening 140 candidates who responded to a “standard advertisement” approved by the in-house IRB. Of these 140 candidates, 82 (58.6%) were enrolled as primary or alternate subjects, as shown in Table 1 below. Demographic and other information is reported only for the “final subjects” in the study, after all substitutions of alternates had been made.

¹ “A total of 30 male and 30 female subjects between the ages of 18 and 55 participated in this study.” (p. 15)

Table 1: Summary Disposition of Enrolled Subjects in Kisicki Study
Based on Appendices II and III, MRID 47346401

		Phase I	Phase II	Total
Enrollees	Primary subjects enrolled	36	24	60
	Alternate subjects enrolled	11	11	22
	Total subjects enrolled	47	35	82
Disposition	Pre-trial cancellations	1	0	1
	No-Shows	3	6	9
	Dropped for cause	3	2	5
	Alternates not used	4	3	7
	Subjects treated	36	24	60

The protocol reports the “source of subjects” as “non-institutionalized subjects consisting of college students and members of the community at large.” The age of participants ranged from 19 to 54 with a mean of 31. Inclusion criteria required that females be using an acceptable means of birth control and that all subjects be of normal body weight and in general good health.

Exclusions were extensive, and repeated in the consent form as “instructions” to the subjects. Candidates were excluded if they had any clinically significant disease or condition, or a history of responding to AChE inhibitors. In addition, it was disqualifying for a candidate to have participated in a previous clinical trial within 30 days, to have donated blood within 30 days, to have donated plasma within 7 days, to have an abnormal diet or to have changed diet within 30 days, to be treated with known enzyme-altering agents within 30 days, to have used any prescription medication within 14 days, to have used any OTC medication within 7 days, to have had known exposure to a cholinesterase inhibitor within the previous month, or to have a positive pregnancy test. Volunteers were further restricted during the testing period from consuming alcohol and from strenuous activity.

The eventual 60 subjects included 57 Caucasians and one American Indian, one Asian, and one Black. The representativeness of the sample was not discussed.

The consent form states that “at the conclusion of the study, MDS Harris will pay me up to \$460 for my participation in this study.” No explanation is provided concerning what factors might lead to payments of less than \$460, whether subjects who withdrew would forfeit payment, whether alternates not used would be paid or in what amount, or how payment would be made. This is not clarified in the study report.

Dose Selection: “The 0.5 mg/kg oral dose level was included in this study so there would be a common dose level which would link this study with the previous study . . . (Nolan et al., 1984). In the previous study, the 0.5 mg/kg dose level depressed plasma cholinesterase activity by 80% but had no effect on RBC AChE activity and did not cause any signs or symptoms of toxicity. Because no effects on RBC AChE activity or signs and symptoms were expected following the 0.5 mg/kg dose level, a decision was made to test the 0.5 and 1.0 mg/kg dose levels at the same time. The 1.0 mg/kg dose level had not been tested previously in humans and was selected as a dose level that might cause a small depression in RBC AChE activity. However, the animal data indicated it was unlikely to produce any signs or symptoms. The last dose level, 2.0 mg/kg, was to be tested only if the RBC AChE activity and signs and symptoms data obtained through 72 hour post-treatment confirmed that the 1.0 mg/kg dose level did not affect these parameters. The 2.0 mg/kg dose level had not been tested previously in humans and was selected as a dose level that might cause some depression in RBC AChE activity but was unlikely to produce any signs or symptoms.” (p. 19)

The additional “unlikely possibility” that effects would be observed at the 0.5 mg/kg dose level was provided for as well—had this occurred, phase II would have used a dose level lower than 0.5 mg/kg, in an effort to identify a NOEL.

Risks to Subjects: Risks to subjects are not discussed in the protocol. The consent form describes “potential side effects” of cholinesterase inhibitors ranging from “improved performance on numerous tests of mental function” to adverse effects including headache, dizziness, nausea, diarrhea, sweating, blurred vision, and tightness in the chest. These side effects of ChE inhibitors are characterized as “nonspecific and . . . not unique to organophosphate poisoning.”

Risks specific to chlorpyrifos are characterized in the consent form (p. 128) as follows:

No adverse effects are anticipated from the quantity of chlorpyrifos you will be given but since there is always the possibility that some unexpected adverse reaction may develop in some persons who take this or any other compound, trained medical personnel are available at MDS Harris for immediate medical attention. Although animal studies indicate little or no risk in humans, the possible side effects to a fetus or embryo are unknown. There are specific and effective antidotes available to treat overexposure to chlorpyrifos. . . . In all but exceptional cases, persons seriously poisoned with chlorpyrifos recover rapidly leaving no long term effects. Persons showing mild symptoms of poisoning show a rapid and complete recovery even if antidote therapy is not used.

This reassuring language is reinforced later in the consent form (p. 131):

It has been indicated that this procedure may be associated with undesirable effects, some of which are not predictable. However, I understand that, in the opinion of MDS Harris’ medical consultants, those risks are not great enough to keep me from participating in this study.

Between these two passages the volunteer is told (p. 130):

I understand that while involved in this study, I will have direct access to medical attention. I further understand that it may be very unsafe for me to leave the clinic and medical attention before being examined by MDS Harris medical personnel.

In addition to these incomplete and potentially confusing passages concerning the risks of ingesting chlorpyrifos, the consent form also mentions in the context of a discussion of the numerous blood draws involved in the study that “risks involved in drawing blood include pain and discomfort, bleeding and/or bruising at the puncture site, and fainting.”

All female subjects were required to take a serum pregnancy test; no risks were identified either with this test or with disclosure of results, and methods for handling the results were not described in either the protocol or the consent form.

Risk Minimization: Although the protocol contains a background discussion of antidotes (from which the consent form passage quoted above was extracted), it does not call for having antidotes to chlorpyrifos on hand in the clinic where testing will take place.

The study design included a rule restricting escalation to phase II based on the results of phase I. This rule was not explained to the subjects, nor was the consent form used in phase II amended to reflect the results of phase I.

Each time investigators took vital signs from subjects, they asked open-ended questions about how the subjects felt.

Subjects were told in the consent form (p. 130) that MDS-Harris would provide medical treatment necessary to assist their recovery from injury or illness “as a direct result of participation in this research study.” The consent form goes on:

This agreement to provide free medical treatment does not include treatment for any illness or injury I might experience during the course of the study if the illness or injury is not the result of the research study. . . . I further understand that provision of medical treatment shall not be admission by MDS Harris that my injury or illness is study related.

Benefits: Subjects were told in the consent form “I understand that I will receive no direct medical benefit from this study; however, this study will be part of an effort to develop additional information about this test compound that may provide potential benefit to others.” Anticipated benefits of the research are not discussed in the protocol.

Relation of Risks and Benefits: No discussion of the relationship between risks to subjects and benefits to others is documented either in the protocol or in the IRB records provided.

Independent Ethics Review: The protocol, MSDS, consent form, “volunteer stipend, and standard advertisements” for this study were reviewed by the MDS-Harris internal Institutional Review Board, which held and holds a Federal-Wide Assurance from

OHRP. At the initial review on September 15, 1998, the IRB approved the proposal with two comments: (1) The IRB asked the investigators to “add the word ‘are’ in the eleventh sentence of the first paragraph” in the INTRODUCTION/PURPOSE section of the consent form—a paragraph which contains only seven sentences in the consent form included in the study report, and (2) The IRB noted that “additional changes to the protocol and informed consent form were submitted by the study sponsor. Copies of these changes were submitted to the IRB at the meeting and approved. . .” (p. 134)

On September 18 the Chairman of the MDS-Harris IRB approved Amendment One to the protocol (which modified the blood sampling schedule) and associated changes to the consent form. At the same time an “administrative revision” to the consent form correcting an error in stating the mid-dose levels was also accepted. The consent form provided in the primary study report (pp. 126-132) is reflective of the changes approved on September 18. On September 29 the full IRB acknowledged receipt of the administrative correction to the consent form.

All three letters from the IRB include a statement that “the Board is fully aware of the regulations governing institutional review boards (Part 56 of Title 21 of the Code of Federal Regulations) and believes that its operations are in compliance with those regulations.” The regulatory criteria for IRB approval of proposed research at 21 CFR §56.111 are not mentioned.

Informed Consent procedures: The study protocol in §6.5 (p. 106) states:

All prospective subjects will have the study explained by a member of the research team or a member of their staff. The nature of the drug substance to be evaluated will be explained together with potential hazards involving drug allergies and possible adverse reactions.

Prior to study initiation, acknowledgment of the receipt of this information and the subject’s freely-tendered offer to participate will be obtained in writing from each subject in the study.

These appear to be standard, ‘boiler-plate’ paragraphs used at the laboratory; the reference here to a “drug substance to be evaluated” is of no consequence to the integrity of the consent process, since this language was not provided to subjects.

Elsewhere in the protocol (p. 108, §8.2) the check-in procedures for the night before dosing are defined to include signing of the informed consent form.

As noted earlier in this review, by the time of check-in—October 1, for phase I—subjects had given blood and urine samples, provided medical and surgical history, and been subjected to a physical examination at screening, had visited the clinic 48 h before check-in to obtain urine collection containers, and had been collecting their urine for two days. Notwithstanding that the QA Unit reported study initiation on October 1, 1998 (p. 4), in my judgment the subjects should all have provided written informed consent at or before

the time of screening, which for phase I subjects was reported in Appendix 4.6.1 (p. 253-258) as September 23 or 30.

Finally, the evening of check-in appears to have been hectic at MDS Harris, and may have been an inappropriate time to seek or obtain informed consent. Subjects may not have had “sufficient opportunity to consider whether or not to participate” under these circumstances, as required by the applicable regulations.

Substance of Informed Consent: The consent form includes extensive technical language taken directly from the protocol which was unlikely to be understood by subjects. The Flesch-Kincaid Grade Level score for the first full paragraph of the consent form is 17.7, equivalent to an advanced graduate student. The form is poorly organized and difficult to follow, shifting suddenly from the first to second persons and back again, and including both dire warnings of “very serious consequences for [subject’s] well-being” and reassurances that unidentified MDS Harris medical consultants think the risks involved should not deter the subjects from participating. An analysis of the content of the consent form in terms of the requirements of 21 CFR §50.20 and §50.25 is attached to this review.

Respect for Potential and Enrolled Subjects: The consent form informed subjects that they were free to decline to participate or to withdraw from the research at any time without penalty or loss of benefits to which they were otherwise entitled. (p. 131) Privacy of the subjects was not compromised in the study report or supplemental materials.

The recruiting and screening processes were overly intrusive. Since a subject’s surgical history was irrelevant to the inclusion and exclusion factors and not used in any way, it is unclear why this information was collected and reported (Appendix 4.5, pp. 250-252). Females were required both to certify that they were surgically sterile or post-menopausal or using an acceptable method of birth control and to take a serum pregnancy test.

Protocol Deviations: The protocol asserts that:

Adverse events, whether serious or non-serious, will be followed to resolution regardless of whether the subject(s) is(are) still participating in the study.

In fact, the only test subject for whom a statistically significant reduction in RBC AChE was reported

. . . withdrew from the study following collection of the 48 hour blood specimen and it was not possible to determine how long it took for RBC AChE activity to return to baseline.

This is not acknowledged in the study report as a deviation from the protocol. There is no indication that it was reported to the IRB.

B. Applicable Standards

This research was initiated in the fall of 1998, several years before EPA's amended Rule for the Protection of Human Subjects of Research became effective on April 7, 2006. It was conducted in the United States by a Contract Research Organization (CRO) laboratory, and overseen by the laboratory's in-house Institutional Review Board. It is reported to have been "conducted in accordance with all applicable U.S. guidelines as specified in Title 21 of the Code of Federal Regulations parts 50, 56, and 321 . . . and the International Guidelines for Human Testing as promulgated in the Declaration of Helsinki (1964; amended 1996)." This review accepts the FDA rules at 21 CFR parts 50 and 56 as the standards governing the ethical conduct of this research; these are substantially identical to the requirements of the Common Rule.

The report of this research was submitted to EPA in April 1999, before the effective date of EPA's amended Rule for the Protection of Human Subjects of Research, and thus it was not subject to the requirement of 40 CFR §26.1303 for submitters to document the ethical conduct of the research.

This research meets the definition of "research involving intentional exposure of a human subject" in the amended rule at 40 CFR §26.1102(i). The Agency's amended rule defines standards for EPA to apply in deciding whether to rely on research involving intentional exposure of human subjects. (See 40 CFR §26 subpart Q.) The acceptance standards applicable to this research are these:

§26.1703. Prohibition of reliance on research involving intentional exposure of human subjects who are pregnant women (and therefore their fetuses), nursing women, or children. Except as provided in §26.1706, in actions within the scope of §26.1701 EPA shall not rely on data from any research involving intentional exposure of any human subject who is a pregnant woman (and therefore her fetus), a nursing woman, or a child.

§26.1704. Prohibition of reliance on unethical human research with nonpregnant adults conducted before April 7, 2006. Except as provided in §26.1706, in actions within the scope of §26.1701, EPA shall not rely on data from any research initiated before April 7, 2006, if there is clear and convincing evidence that 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 was significantly deficient relative to the ethical standards prevailing at the time the research was conducted. This prohibition is in addition to the prohibition in §26.1703.

FIFRA §12(a)(2)(P) also applied to this research. This provision 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.

C. Compliance with Applicable Standards

All subjects were at least 19 years old, and the 30 female subjects monitored in the study were all incapable of pregnancy or using birth control, and all were negative in a serum pregnancy test. EPA reliance on this study is thus not prohibited by 40 CFR §26.1703.

The applicable FDA rules, like the Common Rule, require (among other things) IRB oversight and prior approval, risk minimization, a favorable risk:benefit balance, an acceptable informed consent process and consent form, equitable subject selection, and fully voluntary participation by subjects.

An approved IRB oversaw this research, but in its first review failed to note the discrepancy between dose levels in the protocol and consent form, corrected by Amendment 1. The IRB also failed to notice other inconsistencies between the protocol and the consent form, deferral of consent until several days after collection of tissue samples and medical information had begun, and clear deficiencies in the consent process and form relative to the requirements of the FDA rule. The IRB's letters of approval notably did not assert that the standards for IRB approval in 21 CFR §56.111 had been satisfied, or that risks had been minimized and were reasonable in relation to . . . the importance of the knowledge that may be expected to result from the research. The IRB does not appear to have assessed the adequacy of the consent form and process in terms of the requirements of 21 CFR 50.20 and 50.25; instead they merely asserted that the IRB was "fully aware" of the FDA regulations in 21 CFR Part 56, and believed themselves to be in compliance with them.

40 CFR §26.1704 forbids EPA to rely on data from pre-rule research 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."

Although there are significant gaps in the documentation of the ethical conduct of this research, such gaps do not in themselves constitute "clear and convincing evidence." The conduct of this study is, in fact, better documented than some other research from this period. I found no evidence that this research was fundamentally unethical. But based on my review of the consent form against the standard of 21 CFR §50.20 and §50.25 and the other concerns noted in this review—primary among them the collection of tissue samples and personal information well before subjects had provided informed consent—I conclude that its conduct was significantly deficient relative to the FDA regulations cited as governing its ethical conduct. I found the evidence supporting this conclusion to be clear and convincing.

Because of the significant and substantive deficiencies in the informed consent process, I conclude that this study was also non-compliant with the substantive requirements of FIFRA §12(a)(2)(P) for fully informed, fully voluntary participation.

D. Conclusion

I conclude that EPA is forbidden by 40 CFR §26.1704 to rely on the Kisicki *et al.* study, MRID 44811002, in actions taken under FIFRA or §408 of FFDCA. It is possible that the circumstances and purposes for which you propose to consider it may be such that the provisions of 40 CFR §26.1706 for an exception to the prohibition in 40 CFR §26.1704 may be satisfied.

I defer to others for a full review of the scientific validity of this study. If it were determined not to have scientific validity, it would also not, of course, be ethically acceptable.

Attachment: Analysis of FDA 21 CFR Requirements for and Elements of Informed Consent

**Analysis of FDA 21 CFR Requirements for and Elements of Informed Consent
MRID 44811002: Kisicki et al. (1999)**

Criterion		OK	Comments/Page Reference	
§ 50.20: General requirements for informed consent	No investigator may involve a human being as a subject in research covered by these regulations unless the investigator has obtained the legally effective informed consent of the subject or the subject's legally authorized representative	N	Initial blood and urine samples and history were collected at screening. Consent forms were signed at check-in.	
	An investigator shall seek such consent only under circumstances that provide the prospective subject or the representative sufficient opportunity to consider whether or not to participate and that minimize the possibility of coercion or undue influence	?	Circumstances and procedures poorly described; consent sought at time of check-in, when circumstances were hectic and staff were harried	
	The information that is given to the subject or the representative shall be in language understandable to the subject or the representative	N	Reading level of CF is extremely high. Poorly organized; hard to follow.	
	No informed consent, whether oral or written, may include any exculpatory language through which the subject or the representative is made to waive or appear to waive any of the subject's legal rights, or releases or appears to release the investigator, the sponsor, the institution or its agents from liability for negligence	Y	CF contains no exculpatory language	
§ 50.25: Elements of informed consent	(a) In seeking informed consent the following information shall be provided to each subject	(1) A statement that the study involves research, an explanation of the purposes of the research and the expected duration of the subject's participation, a description of the procedures to be followed, and identification of any procedures which are experimental	Y	Research & purpose p. 126 Duration p. 127 Procedures pp. 127-129
		(2) A description of any reasonably foreseeable risks or discomforts to the subject	Y	Side effects of Cpfs p. 128 Risks of venipuncture p. 129
		(3) A description of any benefits to the subject or to others which may reasonably be expected from the research	Y	p. 130
		(4) A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the subject	NA	
		(5) A statement describing the extent, if any, to which confidentiality of records identifying the subject will be maintained and that notes the possibility that the Food and Drug Administration may inspect the records.	Y	p. 130
		(6) For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be obtained	Y	Compensation p. 130 Treatment p. 130
		(7) An explanation of whom to contact for answers to pertinent questions about the research and research subjects' rights, and whom to contact in the event of a research-related injury to the subject	Y	Rights p. 131 Injury p. 130
		(8) A statement that participation is voluntary, refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled	Y	p. 131
	(b) When appropriate, one or more of the following elements of information shall also be provided to each subject	(1) A statement that the particular treatment or procedure may involve risks to the subject (or to the embryo or fetus, if the subject may become pregnant) which are currently unforeseeable	Y	p. 128
		(2) Anticipated circumstances under which the subject's participation may be terminated by the investigator without regard to the subject's consent	Y	p. 131
		(3) Any additional costs to the subject that may result from participation in the research	N	
		(4) The consequences of a subject's decision to withdraw from the research and procedures for orderly termination of participation by the subject	Y	p. 130
		(5) A statement that significant new findings developed during the course of the research which may relate to the subject's willingness to continue participation will be provided to the subject	Y	p. 131
		(6) The approximate number of subjects involved in the study	Y	p. 127