EPA Review of Article "Assessing Intermittent Pesticide Exposure from Flea Control Collars Containing the Organophosphorous Insecticide Tetrachlorvinphos (TCVP)"

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EPA Review of TCVP Article

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Overview of Research

- The research article (hereafter referred to as the "Davis study") measured TCVP exposures in children and adults that could occur from contact with pet dogs wearing TCVP-containing flea collars
  - Including measuring residues on treated dog's fur
- Conducted by the Center for Environmental Health Sciences, College of Veterinary Medicine, Mississippi State University (MSU)
- Research involved 2 studies, one conducted in 1998 and the other in 2002

Davis, M. et. al., *Assessing Intermittent Pesticide Exposure from Flea Control Collars Containing the Organophosphorus Insecticide Tetrachlorvinphos*. Journal of Exposure Science and Environmental Epidemiology. (2008) 18, 564-57.

Overview of Research

- Study 1 (1998)
  - TCVP residues on fur measured by rubbing/petting dogs' fur with a gloved hand (23 pet dogs)
    - Sampling was conducted by volunteer technicians from MSU veterinary school; 23 participating households
  - Dog plasma cholinesterase (ChE) measurements
- Study 2 (2002)
  - TCVP residues collected from fur by rubbing/petting dog's fur with a gloved hand (22 pet dogs)
  - Passive dosimetry (t-shirts) worn by children
  - Urinary biomonitoring of participating children and adults
    - Involved 1 child and 1 adult from the 22 participating families (22 pet dogs)

Rationale for HSRB Review

- Studies 1 and 2 meet the regulatory definition of *research*
- Through collection of urine and t-shirt samples, study 2 obtained data about individuals and meets the regulatory definition of *human subject research*
- Although the families involved in the studies already used flea collars, the researchers bought and provided specific flea collars to the participating families and asked that their dogs wear the flea collars during the studies
  - Research constitutes intentional exposure

Rationale for HSRB Review

- EPA may rely on the research to improve the protection of public health
- Specifically, only one sub-set from this research, the TCVP fur residue data collected by technicians using cotton gloves to rub the treated dogs
  - These data did not involve children
- However, the data were collected as part of broader research which involved children as study participants when they wore t-shirts and provided urine samples
- As a result, specific federal regulations come into play before EPA can potentially rely on the TCVP glove residue data

Rationale for HSRB Review

- 40 CFR Subpart Q, § 26.1703, prohibits EPA from relying 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**
- 40 CFR § 26.1706 provides an exception
- EPA can only rely on such research if it is crucial to making a decision to impose a more stringent regulatory restriction than could be justified without the data
- The use of the glove residue data could result in a more stringent regulatory restriction than with existing data

Rationale for HSRB Review

- If EPA's Office of Pesticide Programs (OPP) intends to rely on the TCVP glove residue data, under 40 CFR § 26.1706, OPP must first complete three required steps:

  1. Obtain the views of the HSRB
  2. Provide an opportunity for public comment, and
  3. Publish a full explanation of its decision to rely on the data

Science Review

Glove Residue Data: Sampling Procedure

- Glove residue samples were collected in studies 1 and 2
- MSU veterinary students collected samples by rubbing dogs in a marked 10x4 inch area with 100% cotton gloves for a continuous 5 minute period
- Before being used to collect fur residues, each glove washed with laundry detergent, 3 times without detergent, and pre-extracted

Glove Residue Data: Sampling Procedure

- Three samples collected per dog per sample day from the following sites:
  1) the back, near the base of the tail
  2) at the neck with the collar removed
  3) at the neck with the collar in place, rubbing over the collar
- One glove per dog sample site
- Rubbed with firm pressure, in back-and-forth motions

Glove Residue Data: Sampling Procedure

- Study 1
  - Sampling time periods (9): prior to collar placement, and at 4 hours, and 3, 7, 14, 28, 56, and 112 days post-collar application
  - Replicates: 23 dogs, 3 separate sites
    - 621 individual samples
- Study 2
  - Sampling time periods (3): prior to collar placement, and at 5, 12 days post-collar application
  - Replicates: 22 dogs, 3 separate sites
    - 198 individual samples

Glove Residue Data: Sample Handling

- Immediately following sampling, gloves were inverted, removed and placed into a clean, labeled glass sample jar
- During analytic method development various concentrations were applied to different gloves to check the recovery rates and extraction parameters
- The glove samples and spiked gloves were stored at 4º C in the time period between sample collection and analysis to ensure sample integrity

Glove Residue Data: Analytical Methods

- The entire glove was analyzed for TCVP
- Gloves were extracted with acetone using an accelerated solvent extractor by Dionex
  - 5 minutes at 75ºC and 1,500 psi; static for 2 minutes; flush 50% of volume; static for 2 minutes; purge with nitrogen for 150 seconds; and a final purge for 60 seconds
- For every 20 samples, 3 spiked gloves were included at the time of sampling and extracted with the samples
- After extraction, the extract was evaporated under a nitrogen stream using an N-EVAP, transferred to graduated test tubes, and adjusted to 10 mL with acetone

Glove Residue Data: Analytical Methods

- All glove samples analyzed with an HP5890 gas chromatograph equipped with an electron capture detector and RTX-5 Amine column
  - Oven temperature was ramped at a rate of 3ºC/min from 205ºC-225ºC and held for 5 minutes, followed by a second ramp of 5ºC/min to a final temperature of 290ºC
  - ECD injector and detector temperatures were set to 290ºC and 325ºC, respectively
- The limit of detection (LOD) was 2 ppb and the limit of quantification (LOQ) was 6 ppb

Glove Residue Data: Quality Control

- During method development, various concentrations (0.5–2500 mg) were applied to different gloves to check for recovery rates and extraction parameters
- The percent recovery obtained during these tests ranged from 85% to 102%, with a mean of 95%

Glove Residue Data: Statistics

- Analysis of variance calculations for glove residues were performed with the GLM procedure of the SAS® System for Windows, Version 9.1, using the 0.05 level of significance
- Each glove data set was analyzed separately for each collar or age group using one-way analysis of variance for a randomized complete block design (household is the blocking factor)
- The glove data were also examined for the presence of statistically significant correlations using Spearman's correlation coefficient
  - The calculation was performed using the CORR procedure of the SAS System for Windows, Version 9.1 at 0.05 level of significance.

Glove Residue Data: Statistics

- Statistical methods used by the study authors were scientifically appropriate, although the MIXED procedure in SAS would have been preferred since it offers a richer selection of variance-covariance structures (e.g. AR(1)) for modeling longitudinal data than the GLM procedure of SAS)
- However, EPA is proposing using only the estimate of overall mean glove residues for all post-application sampling times
  - The difference between MIXED and GLM procedures in modeling covariance structures will not significantly influence the estimation of overall mean of all sampling days

Glove Residue Data: Results

- Study 1
  - Residues decreased from all 3 sampling sites (86% decline) throughout 112 days following a peak at 7 days, 24,000 ± 4,000 µg/glove over the collar
    - Similarly, residues around the neck without the collar in place and in the tail region decreased 94% and 71%, respectively
  - Mean glove residues for all sampling times were 14,300 µg/glove over the collar, 4,300 µg/glove on the neck with the collar removed, and 130 µg/glove in the tail region

Glove Residue Data: Results

- Study 2
  - Residues obtained over the collar, and around the neck without the collar in place declined 30% from 5 to 12 days post-collar application, while residues obtained from the tail region remained fairly constant
  - Peak transferable residues collected over the collar at 5 and 12 days post-collar application were of a similar magnitude to those observed in study 1 at 7 and 14 days, respectively
  - Mean residues for all gloves analyzed post-treatment were 19,000 µg/glove over the collar, 8,000 µg/glove on the neck with the collar removed, and 80 µg/glove in the tail region.

Glove Residue Data: Discussion

- Glove residue collection methods are consistent with pet fur transferable residue collection methods conducted around the time of the Davis study:
  - Repeated petting motion to a single sample collection site (occasionally multiple) with a cotton gloved hand
  - Defined number of petting motions/strokes (5 - 10 strokes per sample collection point)
  - Defined period of time (5 - 10 minutes per sampling period)
- Analytical methods used were scientifically valid
  - Extraction recoveries ranged from 85% to 102%
- While the MIXED statistical procedure would have been preferred, the method used has no impact as the EPA intends to use the mean of all sampling times

Glove Residue Data: Conclusions

- Pending HSRB review, EPA is considering relying on the glove residue data for risk assessment
- Data have been determined to be scientifically valid and are appropriate for use in risk quantitation
- Glove residue data from the Davis study may result in a more protective risk assessment and, therefore, would be needed to support a more stringent regulatory decision

Ethics Assessment

Rationale for HSRB Review

- In order to improve the protection of public health, pending public comment, EPA intends to rely **only on the TCVP glove residue data from study 1 and study 2** in its risk assessment and to potentially impose a more stringent regulatory restriction than the Agency could do otherwise without the data

Rationale for HSRB Review 2

- Study 2 also involved the collection of data based on t-shirts worn by children and urine samples from children and adults participating in the study.
- With the exception of the dog plasma cholinesterase measurement, all of the data collected constitutes exposure assessment research
- EPA cannot reasonably separate the different types of data in these studies as different types of human research.

Rationale for HSRB Review 3

- Because study 2 encompassed more than TCVP glove residue data and involved children wearing t-shirts and providing urine samples, study 2 constitutes research involving intentional exposure of children
- For that reason, even though OPP does not wish to rely on the data involving children, OPP is submitting the research to the HSRB for review under § 26.1706

Support from EPA Grants & ORD File

- The research was funded by EPA Science to Achieve Results (STAR) grants
- ORD reviewed the grant proposal which involved human research and funding from EPA
- EPA's ethics review refers to the ORD file because it provides draft consent forms used during study 2 and recruitment information
- Primary investigator (PI) Janice Chambers confirmed that the ORD file is pertinent to the research

Role of Vet Students in Rubbing Dogs

- Technicians utilized during both studies were students enrolled at MSU's College of Veterinary Medicine
- PI Dr. Chambers confirmed that both the researchers and IRB viewed the vet students as technicians in the study, not as human subjects

Role of Vet Students in Rubbing Dogs 2

- The ORD file states that "the samplers [i.e. technicians] will be trained so that consistency in the sample collection is maintained among dogs and among samplers."
- The technicians were wearing gloves and stroking the animals in a standardized and prescribed way:
  - "in a marked 10 x 4 inch area with clean, white, cotton gloves for a continuous 5-min period"
  - The dogs were rubbed in specific locations (near the base of the tail, at the neck with collar removed, and at the neck with the collar in place).

Role of Vet Students in Rubbing Dogs 3

- EPA considers the view of the researchers and IRB on this pre-rule research to be a reasonable one
- Under § 26.1102(e), "human subject" means a living individual about whom an investigator conducting research obtains data through intervention or interaction
- Looking at the article and ORD file, the PI did not obtain data **about the technicians**, nor did she intend to do so
- The pattern of rubbing does not resemble the typical human-pet interaction and does not provide information about how a person would normally interact with a pet
- In sum, the researchers were not collecting data about the technicians in this study

Role of Vet Students in Rubbing Dogs 4

- In conclusion, there is no indication from the research article, the ORD file, or the interview with the primary investigator that the study collected data **about** the vet students who worked as technicians in the study
- Instead, the researchers were collecting data only about the residues on the gloves as an indication of how much residue was available for transfer from the pet
- Therefore, EPA agrees with the PI and IRB that the individuals collecting data on residues in pet fur by wearing gloves and rubbing the dogs were not human subjects in these studies

Ethical Considerations – Value of Research

- Value of Research to Society:
  - The objective was to assess the amount of exposure to TCVP that could occur in children and adults from the use of a TCVP-containing collar on a pet dog
  - EPA is considering some of the data from this research in its risk assessment for TCVP

Ethical Considerations - Recruitment

- The article states that, "the studies were conducted in Oktibbeha County, Mississippi (USA), with volunteer households having pet dogs" and that "participating families were volunteers who routinely used flea control products on their pet dogs."
- "One child and one adult were selected from each participating family" for study 2, which included 44 subjects

Ethical Considerations – Recruitment 2

- The ORD file states that:

- "Dogs selected for this study will be owned by professional (DVM) or graduate students enrolled in the College of Veterinary Medicine, or staff/faculty members of Mississippi State University with a child aged 4-10 years in the household who routinely plays with this dog."

- "Students or staff should be the most reliable group of owners (in contrast to the general public) in that they are accessible daily, their dogs can readily be treated and sampled when the students are in class or the staff members are at work, and as members of the academic community, the compliance and appreciation of the value of research should be high."

Ethical Considerations – Recruitment 3

- The ORD file further states that:
- "Dogs participating in this study must be enrolled in the Small Animal Community Practice Health Maintenance Program, so that their health status and vaccination history are known.."
- PI Janice Chambers confirmed that MSU's College of Veterinary Medicine is located in Oktibbeha County, Mississippi
- Therefore, the recruitment area referenced in the ORD file and the article is the same

Ethical Considerations – Independent Ethics Review

- The IRB for Research on Human Subjects at MSU reviewed and approved the sampling protocols and consent forms
- ORD, the National Center for Environmental Research and Quality Assurance (NCERQA) reviewed STAR grant proposal focusing on this research
- ORD supported the research dependent on incorporation of NCERQA comments on the consent forms

Ethical Considerations – Informed Consent

- The article states that "A copy of the protocol was distributed to each participating household, and informed consent was obtained from the adults. Children were informed verbally of the procedures and oral or written assent was obtained from them. The Institutional Review Board for Research on Human Subjects at Mississippi State University approved all sampling protocols and informed consent forms."

Ethical Considerations – Informed Consent 2

- The ORD file contains a draft consent form for adults and a minor's assent form
- The consent form states that the study involves research and identifies its purpose, the expected duration, number of urine and tee shirt samples to be provided, that research results will be coded, participants are free to withdraw, provides a contact for information and identifies compensation of $150 for each participating household

Ethical Considerations – Informed Consent 3

- The consent form also states that "no risks are anticipated to the participants"
- The implication is that since the families already used flea collars on their dogs, there was no added risk from participating in the study
- However, the researchers proposed the following hypothesis on the bottom of page 10 and top of page 11 of the research abstract submitted to ORD:

Ethical Considerations – Informed Consent 4

- "The residues of insecticides available for intermittent transfer to children from the fur of dogs treated by either a spot treatment or a collar for flea control will be appreciable and of a magnitude necessitating inclusion in cumulative risk assessments of pesticides to children; secondly, that the fur rubbing procedure developed to quantify dislodgeable residues provides a useful estimate of insecticide residues which could be transferred from the fur of dogs to children."

Ethical Considerations – Informed Consent 5

- Although the families involved already used flea collars registered by EPA, in the interest of transparency, the researchers should have shared their hypothesis with the parents of the participating children and included it in the consent form
- This information may have been stated in the protocol provided to the families but we do not know

Ethical Considerations – Informed Consent 6

- The minors' assent form states that researchers "will specifically obtain assent from the children recruited to our project...We will explain that the child's parent or guardian has given us permission to request his/her help participation in the research project. We will then explain the urine collection protocol and the tee shirt protocol to the children in language appropriate to the age of the child and obtain his/her assent to participate. We will not explain the connection to the pesticide residues on the dog so as not to alter the behavior of the child with the dog. We will obtain the children's assent orally because of the age range of the children involved."

Ethical Considerations – Respect for Subjects

- Researchers:
  - Did not reveal subjects' identities
  - Obtained informed consent
  - Provided light weight short-sleeve T shirts to kids which would not embarrass them
  - Gave written assurance that urine samples will only be used to quantify insecticide urinary metabolites
  - Provided compensation

Ethical Considerations – Compensation

- $100 equivalent of veterinary care provided by Animal Health Center of MSU College of Veterinary Medicine

- $150 to participating households in Study 2

Standards Applicable to Conduct of Research

- Study 1 was conducted in 1998 and study 2 was conducted in 2002, both before EPA's Rule for Protection of Human Subjects of Research became effective in 2006. Thus, 40 CFR Part 26, subparts B through Q, did not apply when this research was conducted.
- However, EPA's codification of the Common Rule 40 CFR Part 26 subpart A was in place and applies to the underlying research which received EPA STAR grant funding.

Standards Applicable - Conduct of Research 2

- Key elements of the Common Rule are IRB oversight and prior approval, an acceptable informed consent process and consent form, risk minimization, a favorable risk-benefit balance, equitable subject selection, and fully informed, fully voluntary participation by subjects

Standards Applicable - Conduct of Research 3

- In addition, § 12(a)(2)(P) of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) existed at the time of these studies. States that it's unlawful to use any pesticide in tests on humans unless they are fully informed of the nature and purposes of the test, of any physical and mental health consequences which are reasonably foreseeable from the tests, and that participants freely volunteer

Substantive Acceptance Standards

- 40 CFR § 26.1703
  - Except as provided in § 26.1706, prohibits reliance on data involving intentional exposure of pregnant or nursing women or of children
- 40 CFR § 26.1704
  - Except as provided in § 26.1706, prohibits reliance on data if research fundamentally unethical or deficient relative to prevailing ethical standards at time of research

FIFRA § 12(a)(2)(P)

- Makes it unlawful to use a pesticide in human tests without fully informed, fully voluntary consent

Substantive Acceptance Standards 2

- 40 CFR § 26.1706
  - EPA may rely on data that's unacceptable under the standards in § 26.1703 through 26.1705 only if EPA has:

1. Obtained the views of the HSRB

2. Provided an opportunity for public comment

3. Determined that relying on the data is crucial to a decision that would impose a more stringent regulatory restriction to protect public health than could be justified without the data; and

4. Published full explanation of decision to rely on the data

Compliance with Applicable Standards

- Regarding 40 CFR § 26.1703

- Study 2 involved tee shirt and urine samples that came from children. EPA only wants to rely on the glove residue data from Study 1 and 2 that did not involve kids. § 26.1703 does not allow reliance on research involving kids except as provided in § 26.1706

Compliance with Applicable Standards 2

- Regarding 40 CFR § 26.1704

  - There is no clear and convincing evidence that this pre-rule research was fundamentally unethical

  - The conduct of the research was not deficient relative to the ethical standards prevailing at the time the research was conducted

The research complied with FIFRA § 12(a)(2)(P)

Compliance with Applicable Standards 3

- Regarding 40 CFR § 26.1706

  - Pending public comment, OPP may wish to rely on the TCVP glove residue data generated in study 1 and 2. The data may be crucial to a potential EPA decision to improve public health protection by imposing a more stringent regulatory restriction than could be justified without the data.

Conclusion

- Under 40 CFR § 26.1706, EPA must first complete three required steps. EPA must obtain the views of the Human Studies Review Board, provide an opportunity for public comment, and publish a full explanation of its decision to rely on the data, including a thorough discussion of the ethical deficiencies of the underlying research and the full rationale for finding that EPA met the standard in 40 CFR § 26.1706 (c) (i.e., that the research is essential to a more stringent regulatory action to improve protection of public health).

Charge Questions for HSRB

- Is this research scientifically sound, providing reliable pet fur transferable residue data for use in evaluating potential exposures of adults and children from contact with pets treated with tetrachlorvinphos containing pet collars?
- Does the HSRB have any comments on EPA's determination that the samplers were not human subjects?
- Does the HSRB have any comments on the ethical conduct of the research?