MEMORANDUM

DATE: December 16, 2015

SUBJECT: Science Review of “Davis et al., 2008. Assessing Intermittent Pesticide Exposure from Flea Control Collars Containing the Organophosphorus Insecticide Tetrachlorvinphos” for HSRB Consideration

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This memorandum presents EPA’s science review of the following journal article which measured tetrachlorvinphos (herein referred to as TCVP) human exposure in adults and children from contact with dogs wearing TCVP pet collars in residences, cholinesterase inhibition in treated dogs, and also transferable residues from the fur of treated dogs. The agency is currently planning to use the journal article as a source of pet fur transferable residue data for a residential post-application exposure and risk assessment for adults and children who could be exposed from contact with dogs wearing TCVP pet collars.
INTRODUCTION

The Agency is currently planning to use the Davis et al. paper only as a source of transferable pet fur residue data in order to complete a residential post-application exposure and risk assessment for adults and children who could be exposed from contact with dogs wearing TCVP pet collars. The residue measurements would be used as a basis for calculations which are described in the Agency’s SOPs for Residential Exposure Assessment.\(^1\) The study was conducted in 2 parts both of which measured transferable residues from the fur of treated dogs. Other phases of the study were conducted which measured the impact of cholinesterase activity on treated dogs (Study 1 only), dermal exposure from children living with the treated dogs using passive dosimetry in the form of t-shirts (Study 2 only), and internal dose based on residues in urine of children and adults living with treated dogs (Study 2 only).

The transferable residue data have been determined to be acceptable for regulatory purposes given that the sample collection method was the scientific standard at the time the study was conducted and all phases of the sample collection and analysis appear adequate for those samples. Also, importantly, the risks predicted are higher than previously calculated for TCVP pet collar products which is an important consideration related to EPA’s use of the data as discussed in the Agency’s ethics review (Lydon 2015) which indicates:

> “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. §26.1706 provides an exception. Under 40 CFR §26.1706, EPA can only rely on this research if it is crucial to making a decision to impose a more stringent regulatory restriction than could be justified without the data. If EPA’s Office of Pesticide Programs (OPP) decides to rely on the TCVP glove residue data, under 40 CFR

\(^1\) [http://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/standard-operating-procedures-residential-pesticide]
§26.1706, OPP 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).”

EPA is not planning on using the other results from the research in EPA’s risk assessment based on the following: 1) the t-shirt dosimetry data likely underestimate TCVP exposures to children and result in a negligible impact on estimated risks 2) pharmacokinetic data are lacking to reliably quantify exposures based on the urinary measures of the TCVP metabolite, 2,4,5-trichloromandelic acid (TCMA) and 3) the dog plasma ChE activity measures are not human relevant.

EXECUTIVE SUMMARY:

Davis et al., 2008, investigated the exposures to TCVP that could occur in children and adults from the use of a TCVP-containing collar on pet dogs. A single product was tested, Hartz Mountain Ultimate Flea Collar, which is composed of 14.55% TCVP. Two separate studies were conducted with the test product as a part of the journal article. Both were conducted in Oktibbeha County, Mississippi, with volunteer households having pet dogs.

Study 1: The first study was about 4 months (112 days) and it evaluated the time course of TCVP residue dissipation using a transferable residue collection method published by Boone et al., 20012, which at the time of the study was the scientific standard for collecting residues that could be transferred from treated pets. In this method technicians use white cotton gloves to pet a treated dogs’ fur in a rigorous, reproducible manner. Twenty three dogs of different breeds and weights were treated with a TCVP flea collar in study 1. Dogs were rubbed by technicians continuously for a 5 minute period with use of cotton glove following a defined protocol that required contact with 1) the neck with collar, 2) the neck without collar) and 3 along the tail region for each dog. Study 1 also analyzed plasma cholinesterase (ChE) activity from blood samples taken from each dog at the same time as the rubbing samples. Pre-- and post-collar application samples were collected for the evaluation of residue transfer to gloves and the dogs blood ChE activity over the course of the 112 day sampling period.

Significant increases in transferable TCVP residues were observed compared to pretreatment concentrations as would be anticipated. In study 1, transferable residues from all three sampling locations decreased (86% decline) throughout the 112 days following a peak at day 7 post-collar application, 24,000 ± 4,000 µg/glove over the collar. Similar trends were also observed for residues characterized as around the neck without the collar in place and in the tail region where there were 94% and 71% decreases, 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.

No significant changes in dog plasma ChE activities from pretreatment levels were observed except for one increase of 10% at 84 days post-collar application.

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Study 2: The second, subsequent study was conducted on the basis that results from study 1 indicated that TCVP residues peaked and then suddenly dropped within 3 weeks of collar placement. Therefore, the second study was conducted over a 3 week (21 day) period. The research plan included additional transferable residue measures, but it also included human biomonitoring of the TCVP metabolite, 2,4,5-trichloromandelic acid (TCMA), in urine of adults and children. In addition, potential TCVP exposures were measured using dosimetry to quantify the potential exposures of children (i.e., cotton t-shirts).

In study 2, TCVP residues obtained over the collar and around the neck without the collar in place decreased (30% decline) from 5 to 12 days post-collar application, while residues obtained from the tail region remained fairly constant (81 µg/glove at 5 days and 82 µg/glove at 12 days). The peak transferable residues collected over the collar at 5 days post-collar application were of a similar magnitude to those observed in study 1. Mean residues (for all gloves analyzed) post-collar application 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.

The average amount of TCVP residues detected on children’s t-shirts on sampling days 7-11 post-collar application was 1.8 ± 0.8 µg/shirt, with no significant differences among the sampling days. These exposures were significantly greater than the mean pre-treatment residue of 0.03 ± 0.006 µg/shirt as would be expected.

Urine samples collected from children generally contained more urinary TCMA than that from the adults with significant differences between the ages occurring on only 1 of the 5 sampling days (day 11). No significant differences in urinary TCMA concentrations were observed among the adults or among the children throughout the study. The ranges of TCMA concentrations were large; 1.4 - 582 ng/ml urine for adults, and 2.1 - 1,558 ng/ml urine in children. The urinary TCMA concentrations were all adjusted for creatinine content; however, there were no differences in outcomes and, as a result, reported values were unadjusted.

No significant correlations were identified among t-shirt TCVP residues, the amount of time spent with treated dogs, and urinary TCMA concentrations.

As described in the Introduction, the Agency is currently planning to use the Davis et al paper only as a source of transferable pet fur residue data in order to complete a residential post-application exposure and risk assessment for adults and children who could be exposed from contact with dogs wearing TCVP pet collars. EPA is not planning on using the other results in risk assessment based on the following: 1) the t-shirt dosimetry data likely underestimate TCVP exposures to children and result in a negligible impact on estimated risks 2) pharmacokinetic data are lacking to reliably quantify exposures based on the urinary measures of the TCVP metabolite, TCMA, and 3) the dog plasma ChE activity measures are not human relevant.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: The test material used is the TCVP pet collar, Hartz Control Ultimate Flea Collar (EPA Reg. No.: 2596-139) which contains 14.55% active ingredient.
2. **Relevance of Test Material:** The test material used is a pet collar product that was in active usage at the time of the conduct of the study. The journal article reports that the product was purchased from a local department store.

B. **STUDY DESIGN**

The study was conducted (two separate studies/parts) to investigate the exposures to TCVP that could occur in children and adults from the use of a TCVP-containing collar on pet dogs. The evaluation of transferable residues was conducted by means of Mississippi State technicians rubbing the back of treated dogs at 3 locations: at the neck with the collar in place, at the neck with the collar removed, and near the base of the tail. Children’s potential exposure to the TCVP pet collar were estimated by passive dosimetry (t-shirts) worn for at least 4 hours at selected time points following collar application. TCVP exposures were also measured by means of the collection and analysis of adult’s and children’s urine for the TCVP metabolite, TCMA.

1. **Site Description:** Study 1 and 2 were both conducted in Oktibbeha County, Mississippi, with volunteer households having pet dogs.

2. **Animal(s) Monitored:** The dogs which were monitored included a variety short and long hair breeds and ranged from 8-85 lbs. All dogs were healthy adults and were of both genders; no pregnant females were used. All procedures were approved by the Mississippi State University Animal Care and Use Committee. The care and use of dogs were in accordance with the Guide for Care and Use of Laboratory Animals (1996). The dogs were used during both studies were monitored by the project veterinarian.

3. **Physical State of the Formulation as Applied:** Ready-to-use pet collar formulation. Hartz Control Ultimate Flea Collar - 14.55% active ingredient (TCVP); EPA Reg. No.: 2596-139.

4. **Application Rates and Regimes:** The Davis study reports that the amount of active ingredient in the TCVP pet collars used was 4,800 mg of active ingredient. The pet collar product used in the Davis study, EPA Reg. No. 2596-139 - 14.55% TCVP. No information was provided as to how the pet collar was applied to the dogs, or whether any remaining length of the collar was removed following fitting.

5. **Sampling Procedures For All Media:**

**Transferable Residues**

*Method and Equipment:* Volunteer technicians from the College of Veterinary Medicine, Mississippi State University, Mississippi, rubbed in marked areas on the dogs with use of a clean, cotton glove for a continuous 5 minute period as previously described in the Chambers et al., 2007 publication. The

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3 There is uncertainty as to whether the TCVP pet collar, which remains a currently registered product, is a dust or liquid formulation. That is, whether TCVP releases from the pet collar matrix onto the treated animal as a liquid or dust form. The Agency intends to seek additional information from the product registrant to address this uncertainty.

gloves used for rubbing were 100% cotton. Each glove was washed once with laundry detergent, and three times without detergent, pre-extracted for 8 hours with methylene chloride, dried, and stored in glass jars that were washed and pre-rinsed three times with water and acetone. All samples were collected by technicians in the participants’ homes.

Sampling Procedure: Dogs were rubbed in a marked 10 x 4 inch area with cotton gloves for a continuous 5 minute period. Three samples were taken in the following order to avoid cross contamination: 1) near the base of the tail 2) at the neck with the collar removed and 3) at the neck with the collar in place, rubbing over the collar. Rubbing was with firm pressure in both anterior and posterior directions (back-and-forth motions), but not one that would cause discomfort to the dog. The gloves were then inverted and removed and placed into a clean, labeled glass jar.

Times of Sampling: For study 1, rubbing samples were obtained prior to collar placement (day 0) and at 4 hours, and 3, 7, 14, 28, 56, 84, and 112 days post-collar application, and for study 2, prior to collar placement, and again at 5 and 12 days following collar placement.

Surface Area Sampled: An individual sample was acquired for a marked 10 x 4 inch area (40 square inches) of each of the following: 1) near the base of the tail 2) at the neck with the collar removed and 3) at the neck with the collar in place.

Sampling Time: For each of the 3 samples acquired from each dog, including the tail and two neck samples, a 5 minute rubbing phase was repeated.

Replicates per Sampling Time Period: For study 1, 23 dogs were each sampled 3 separate times (tail and two neck samples) per dog over 9 time periods (prior to collar placement and at 4 hours, and 3, 7, 14, 28, 56, 84, and 112 days post-collar application) for a total of 69 samples per time period and a grand total of 621 individual samples. For study 2, 22 dogs were each sampled 3 times per dog over 3 time periods (prior to collar placement, and again at 5 and 12 days post-collar application) for a total of 66 samples per sample time period and a grand total of 198 samples.

Plasma Cholinesterase Assay Phase (Study 1)

Method and Equipment: Blood samples were collected from each dog during each rubbing sample. These samples were centrifuged after collection to obtain plasma, which was stored at 4°C overnight, and ChE activity was determined within 24 hours after collection. No further information was provided regarding sample collection.

Sampling Procedure and Timing: Blood samples were taken from each dog at the same time as rubbing samples. Samples were collected for study 1 prior to collar placement and at 4 hours, and 3, 7, 14, 28, 56, 84, and 112 days post-collar application, and for study 2 prior to collar placement, and again at 5 and 12 days post-collar application.

T-Shirt Study Phase (Study 2)

Method and Equipment: Child participants (ages and genders were not reported) were supplied with new, laundered cotton t-shirts to wear on each pre- and post-collar application. The t-shirts were prepared in the same manner as described for the gloves, except for the methylene chloride step which was not
conducted. Each child was instructed not to alter his/her normal behavior with respect to the dog. At the end of each sampling day, a 100 cm² section from the chest area was cut out and used for extraction of the t-shirt. These sections were placed into a solvent-washed glass bottle for subsequent extraction. This region was used because it was indicated it is the most likely region of the shirt to contain residues from the child’s interaction with the dog.

**Sampling Procedure:** Children were instructed to wear the supplied t-shirts on sampling days for a minimum of 4 hours. T-shirts were collected by researchers at the end of each sampling day for analysis.

**Time of Sampling:** T-shirts were worn and collected on the day before treatment (collar application) and on each of the day 7-11 post-collar placement. Each t-shirt was worn during the afternoon and evening of the sampling day for an average 4-hour period.

**Urine Sampling Phase (Study 2)**

**Method and Equipment:** Urine samples were collected from children wearing t-shirts and from one adult in the same household and analyzed for the TCVP metabolite, TCMA. Because TCMA was unable to be prepared due to a lack of commercial starting material, the structurally similar 2,3,6-trichloromandelic acid analog was synthesized from 2,3,6-trichlorobenzaldehyde and used as an internal standard.

**Sampling Procedure and Timing:** First morning urine samples (entire void) were collected prior to the day of treatment and then again on each of days 8-12 post-collar placement.

6. **Sample Handling**

**Gloves (Studies 1 and 2) and T-Shirts (Study 2) Analysis**

Following sampling, the gloves were placed into a clean, labeled glass jar and the t-shirts were placed into a solvent-washed glass bottle for subsequent extraction. During method development, gloves and t-shirts used for petting the dogs were quantified and various concentrations were applied to different gloves to check for recovery rates and extraction parameters. After collection, the sample and spiked gloves and t-shirts were stored at 4°C until extraction.

**Plasma ChE Assay (Study 1)**

Blood samples were collected from each dog at each sample time. These samples were centrifuged after collection to obtain plasma, which was stored at 4°C overnight until ChE activity was conducted (within 24 hours).

**Urine Sampling (Study 2)**

Following collection, urine samples were brought to Mississippi State laboratories. There a subsample of 3 ml was removed from each sample and both the original and subsamples were placed in an upright freezer and held at -20°C. The subsamples were shipped frozen for analysis.
7. Analytical Methodology

Gloves (Studies 1 and 2) and T-Shirts (Study 2) Analysis

Extraction Method(s): The entire glove was analyzed for TCVP. For the t-shirts, a 100 cm² section from the front chest area of each shirt was cut out and used for extraction, as this region is the most likely region of the shirt to contain residues from the child’s interaction with the dog. After sampling, the gloves and t-shirts were extracted with acetone using an Accelerated Solvent Extractor (ASE) by Dionex. The operating conditions were heat for 5 minutes at 75°C and 1500 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, three spiked gloves were included at the time of sampling and extracted with the samples. After the gloves were extracted using an ASE, the extract was evaporated under a nitrogen stream using an N-EVAP (1–3 mL), transferred to graduated test tubes, and adjusted to 10 mL with acetone.

Detection Method(s): All samples were analyzed on an HP5890 gas chromatograph equipped with an electron capture detect (ECD). Separation of the analyte was achieved by using an RTX-5 Amine column (30m_0.53mm inside diameter/1.0 m film thickness, Restek, Bellefonte, PA, USA). The oven temperature was ramped at a rate of 3°C/min from 205°C to 225°C and held for 5 minutes, followed by a second ramp of 5°C/min to a final temperature of 290°C. The ECD injector and detector temperatures were set at 290°C and 325°C, respectively.

Method validation: The limit of detection (LOD) was 2 ppb and the limit of quantification (LOQ) was 6 ppb.

Plasma ChE Assay (Study 1)

Extraction Method(s): Cholinesterase (ChE) determinations were done using the following combinations of inhibitors and substrates to investigate plasma enzymes: butyrylcholinesterase (BChE) using butyrylthiocholine (BTCh) iodide as a substrate and tetraisopropyl pyrophosphoramide (iso-OMPA) as an inhibitor, and acetylcholinesterase (AChE) using acetylthiocholine (ATCh) iodide as a substrate and eserine sulfate as an inhibitor. All assays were a modification of the procedure described in Chambers and Chambers (1989), which is based on the spectrophotometric method of Ellman et al. (1961). Protein concentration was determined by the method of Lowry et al. (1951) for standardization.

Urinalysis (Study 2)

Urine samples were thawed at ambient room temperature, vortexed, and aliquoted (1.0 ml) to 1.8 ml glass autosampler vials, and fortified with the 2,3,6-trichloromandelic acid internal standard. Sufficient volume of concentrated (12 N) hydrochloric acid (typically 0.1 ml) was then added to each sample to achieve a final volume dilution of 1N. Each vial was then sealed with a PTFE/rubber-lined aluminum crimp cap, vortexed to mix, and placed in a water bath maintained at 80°C for a period of 1 hour, after which they were allowed to cool to ambient temperature before being analyzed.

Samples were analyzed by reverse-phase liquid chromatography mass spectroscopy using an Applied Biosystems/MDS SCIEX API 3000 triple quadrupole mass spectrometer and Analyst™ version 1.4 software. Analyses were performed in the negative ionization mode using Turbo Ionspray™ ionization. Two MS/MS transitions were monitored: 253-209 and 255-211, which are associated with a loss of CO₂ from the corresponding molecular ions [M-H] at 253 AMU (³⁵Cl) and its associated ³⁷Cl at 255 AMU. Quantitation was by integration of peak area for the single fragmentation at m/z 209.
Separation of the two analytes (TCMA and 2,3,6-trichloromandelic acid) from each other was achieved by reverse-phase C-18 liquid chromatography. The target analyte, TCMA, was identified by monitoring the two MS/MS transitions previously mentioned and observing the presence of a similar ion-intensity ratio of approximately 1:1 (for the fragment masses 209:211) as observed for the internal standard. Because of an interfering, co-eluting component detected in a large number of urine hydrolysates, further identification of TCMA at the retention time of 13.9 minutes was confirmed by its anticipated longer retention time relative to that of the 2,3,6-trichloromandelic acid internal standard, which eluted at 12.4 minutes. The longer retention of TCMA can be attributed to the differences in polarity (TCMA is less polar than the 2,3,6-trichloromandelic standard).

A typical injection sequence for sample analysis involved an initial acetonitrile solvent blank followed by a 20 ng/ml internal standard quality control (prepared in acetonitrile) and 10 experimental samples. This sequence was then repeated until all samples were analyzed.

Method Validation: The linear response of the liquid chromatography mass spectroscopy was verified by sequential injections of calibrants prepared in acetonitrile at nominal concentrations. Quantitation of the target analyte (TCMA) was done by comparing the peak areas of the internal standard (2,3,6-trichloromandelic acid) and TCMA. The LOD was 0.63 ppb and LOQ was 2 ppb. Samples that were below the LOQ were assigned the value of the LOQ for the purpose of sample analysis. Two percent of the adult samples were below the LOQ, and 10% of the children’s samples were below the LOQ. Except for pretreatment samples, none of the samples were below the LOD.

8. Quality Control

Please note that the following quality control information presented are drawn directly from the publication. No further information were provided.

Gloves (Studies 1 and 2) and T-Shirts (Study 2) Analysis

During method development, gloves and t-shirts used for petting the dogs were quantified and various concentrations (0.5–2500 mg) were applied to different gloves to check for recovery rates and extraction parameters. The limit of detection LOD was 2 ppb and the limit of quantification was 6 ppb. The percent recovery obtained during these tests ranged from 85% to 102%, with a mean of 95%.

Urinalysis (Study 2)

The accuracy of the method was evaluated by examining the relative error of triplicate concentrations for a selected analysis (family no. 110). Relative error was calculated as (experimentally determined concentration-theoretical concentration) x 100 ÷ theoretical concentration. Magnitudes of relative errors were less than 17% at the lowest concentration, less than 12% at the midlevel concentration, and less than 5% at the highest concentration. The percent recoveries were in the range of 88.5% to 116.3%, with a mean of 102.1%.

9. Statistics

Analysis of variance calculations for glove and t-shirt residues were performed with the GLM procedure of the SAS® System for Windows, Version 9.1, using the 0.05 level of significance. When significant differences between treatment groups were found, means were separated using the least significant difference test. The clinical importance of the differences was assessed using confidence intervals.
Each data set (gloves, t-shirts and adjusted urine) 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 and t-shirt 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 (SAS Institute Inc., Cary, NC, USA) at 0.05 level of significance.

Urinary TCMA data were statistically analyzed to compare the amount of TCMA between adults and children. The analyses were implemented in SAS System for Windows, Version 9.1.3 using PROC MIXED with the REPEATED statement and the autoregressive order one (AR 1) covariance structure. The AR 1 was a covariance structure with the desired property of correlations being larger for nearby time than for far-apart times, which was a better fit in this statistical model than the compound symmetric or unstructured covariance.

II. RESULTS

Rubbing Glove Study Phase (Studies 1 and 2)

Significant increases in transferable TCVP residues were observed from cotton gloves used to pet dogs compare to pretreatment concentrations. In study 1, transferable residues from all three sampling locations decreased (86% decline) throughout the 112 days following a peak at day 7 post-collar application, 24,000 ± 4,000 µg/glove over the collar. Similar trends were also observed in detectable residues around the neck without the collar in place and in the tail region where there were 94% and 71% decreases, 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.

In study 2, TCVP residues obtained over the collar and around the neck without the collar in place decreased (30% decline) from 5 to 12 days post-collar application, while residues obtained from the tail region remained fairly constant (81 µg/glove at 5 days and 82 µg/glove at 12 days). The peak transferable residues collected over the collar at 5 days post-collar application were of a similar magnitude to those observed in study 1. Mean residues (for all gloves analyzed) post-collar application 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.

Pet fur residue transfer studies conducted around the time of the Davis study typically used a repeated petting motion to a single sample collection area for a defined number of strokes (e.g., 5 to 10 strokes per animal per sample time point) and/or for a defined period of time. (e.g., 5 to 10 minutes per sampling period). Therefore, the Davis study collection methodology is consistent with other pet fur residue transfer studies conducted around the same time. Further, the Davis study sampled from the neck area, as well as the tail, and is not expected to underestimate residue transfer when compared to other residue transfer studies conducted around the same time which typically sampled only from one area of the body.

The analytical methods used for analysis of the rubbing glove phase of the study are scientifically valid. Each glove was washed once with laundry detergent, and three times without detergent, pre-extracted for 8 hours with methylene chloride, dried, and stored in glass jars that were washed and pre-rinsed three times with water and acetone. Gloves were stored after sampling at 4°C for an undisclosed period of time until analysis. Storage at 4°C until analyzed is sufficient assuming samples were not stored for more than 2 weeks. Samples were extracted using ASE, which is a technique known to be successful for extraction of pesticides, including organophosphates, from most matrices. The TCVP standard used was of acceptable purity. The study authors verified that the method extraction conditions were sufficient since
they obtained a recovery ranging from 85% to 102%, after spiking blank gloves with different amounts of TCVP. Glove samples were analyzed on an HP5890 gas chromatograph equipped with an ECD for the detection of TCVP. Detection of TCVP using GC-ECD is acceptable and achieves the required detection limits.

Study authors performed analysis of variance method for glove and tee shirt residues with the GLM procedure of the SAS System for Windows, version 9.1, using 0.05 level of significance. The glove data were also examined for the presence of statistically significant correlations using Spearman’s correlation coefficient. The calculations were performed using the CORR procedure of the SAS System for Windows, version 9.1 at 0.05 level of significance.

The study authors did not specify whether any transformation of residue data was conducted before performing analysis of variance approach. Although MIXED procedure in SAS using AR(1) covariance matrix was applied for modeling TCMA data it was not explained in the article why the same procedure with the similar correlation structure was not used for modeling glove exposure data. It should be noted that MIXED procedure in SAS offers a richer selection of variance-covariance structures for modeling longitudinal data than GLM procedure. For example autoregressive covariance structure cannot be implemented using PROC GLM but it can be implemented using PROC MIXED. EPA is proposing using only the estimate of mean glove transferable residues for all sampling times. Although the selection of variance-covariance structure will impact the estimation of standard error, test statistic, and p value, it will not significantly influence the estimation of overall mean from all sampling times.

For purpose of risk assessment, the mean transferable residue measures from study 1 and 2 are used to quantify a fraction of application rate applied (FAR) value. The $F_{AR}$ value was determined by dividing the sum of the mean residues measured from the fur over the collar and from the tail region, by the amount of active ingredient in the pet collar, 4,800 mg. The transferable residue measures from the fur of the neck with the collar removed was not summed with the other measures since the collection of residues under the collar is inconsistent with TCVP pet product label language that directs users to secure the pet collars in place. For study 1, an $F_{AR}$ value of 0.0030 (0.3%) results from the mean residues (112 days) reported from study 1 [where $(14.3 \text{ mg } + 0.013 \text{ mg})/ 4,800 \text{ mg} = 0.0030$]. For study 2, an $F_{AR}$ value of 0.0040 (0.4%) results from the mean residues (12 days) reported from study 2 [where $(19 \text{ mg } + 0.08 \text{ mg})/ 4,800 \text{ mg} = 0.0040$]. These $F_{AR}$ inputs are then used as described in EPA’s 2012 Standard Operating Procedures (SOPs) for Residential Pesticide Exposure Assessment for estimation of post-application risks from exposures to adults and children from contact with TCVP treated dogs.

**Plasma Cholinesterase Assay Phase (Study 1)**

No significant changes in dog plasma ChE activities from pretreatment levels were observed except for one increase of 10% at 84 days post-collar application; this statistical difference was not viewed as biologically significant.

The analytical methods used for determination of dog plasma ChE inhibition were conducted adequately. The ChE determinations used a combination of inhibitors, butyrylcholinesterase (BChE) and acetylcholinesterase (AChE), both of which are contained in dog plasma and are inhibited by OPs, including TCVP. The assay methods used measured the hydrolysis of enzyme-specific substrates and separated out the relative activities of the separate enzymes by using specific inhibitors of each. This method gives a true measure of inhibition of each cholinesterase.

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T-Shirt Study Phase (Study 2)

The average amount of TCVP residues detected on children’s t-shirts on sampling days 7-11 post-collar application was 1.8 ± 0.8 µg/shirt, with no significant differences among the sampling days. Transferable residues were significantly greater than the mean pre-treatment residue of 0.03 ± 0.006 µg/shirt.

The use of passive clothing dosimetry (t-shirts) is consistent with post-application exposure monitoring guidelines, for measuring dermal exposures via passive dosimetry. The guidelines recommend whole-body dosimetry for measurement of residential indoor exposures such as evaluated in the Davis study. While it would have been preferred that the entire t-shirt be analyzed for transferable residues of TCVP, the measurement of the 100 cm² section is comparable to patch dermal dosimetry which described by the guidelines as an acceptable method of residue collection. However, there are clear limitations with this approach. By sampling only one isolated section of the t-shirt, it is possible that other areas of the t-shirt that may have been more highly exposed or subject to greater residue transfer. If the full t-shirt, or more 100 cm² sections of each individual t-shirt had been analyzed, it is likely that greater residue transfer would have been measured. Therefore, with use of this approach it is likely that exposures to children wearing the t-shirts could have been underestimated.

Further, the resulting measures from the t-shirt samples, a mean of 1.8 µg/shirt, is well below the mean values reported for the residue transfer phase of the study, 14,300 µg/glove over the collar (study 1) and 19,000 µg/glove over the collar (study 2). Accordingly, the consideration of the t-shirt data results in a negligible impact on the risks from adult and child exposures to pets treated with a TCVP pet collar.

The analytical methods used for analysis of the t-shirt study phase samples are the same as those described above (Section II, Results) for the rubbing glove phase of the study which were determined to be scientifically valid. The statistical methods used for t-shirt sampling (also the same as described for the rubbing glove phase) resulted in a determination that the MIXED procedure in SAS, which was used for urinalysis, offers a richer selection of variance-covariance structures for modeling longitudinal data than the GLM procedure of the SAS System for Windows, version 9.1, used. This determination is of no consequence since EPA does not intend to rely on the t-shirt data for assessment of risks due to the limitations described above.

Urine Sampling Phase (Study 2)

Urine samples collected from children generally contained more urinary TCMA than that from the adults with significant differences between the ages occurring on only 1 of the 5 sampling days (day 11). No significant differences in urinary TCMA concentrations were observed among the adults or among the children throughout the study. The ranges of TCMA concentrations were large; 1.4 - 582 ng/ml urine for adults, and 2.1 - 1,558 ng/ml urine in children. The urinary TCMA concentrations were all adjusted for creatinine content; however, there were no differences in outcomes and, as a result, reported values were unadjusted. No attempt was made by researchers to estimate an internal dose based upon the urinary TCMA measures.

The study authors stated that the TCVP metabolite TCMA cannot be readily prepared due to lack of commercial starting material. Instead, the structurally similar 2,3,6-trichloromandelic acid analog was synthesized from 2,3,6-trichlorobenzaldehyde and used as an internal standard which was added to urine samples. TCMA was determined in urine using LC-MS/MS. Quantitation of the TCMA was done by comparing the peak areas of the internal standard (2,3,6-trichloromandelic acid) and TCMA. In this case,
TCMA (2,4,5-isomer) was quantitated against a calibration curve of another compound (2,3,6-isomer). Although both compounds are very close in structure, their responses in the mass spectrometer may be slightly different. TCMA eluted at a longer retention time (RT), 13.9 minutes, as compared to the 2,3,6-isomer internal standard which eluted at 12.4 minutes. The difference in retention time when analyzed by MS is expected, although quite little to negligible. The difference is also expected to be insignificant since the amounts of TCMA equivalent detected vary from one volunteer to another and that a small error in the calculated amount may be accepted. For more accuracy the study authors could have referred to TCMA detected as TCMA equivalent. Regardless, the detection method is acceptable for use.

Urinary TCMA data were statistically analyzed to compare the amount of TCMA between adults and children. The analyses were implemented in SAS System for Windows, version 9.1.3 using PROC MIXED with the REPEATED statement and autoregressive order one (AR 1) covariance structure. The authors stated that the AR(1) was a covariance structure with the desired property of correlations being larger for nearby time than for far apart times, which was a better fit in this statistical model than the compound symmetric or unstructured covariance.

While the urine sampling phase of the study was conducted in a manner scientifically valid, to rely on these data would require on a full understanding the pharmacokinetics of TCVP, including an adequate justification for reliance on the metabolite, TCMA, to document the time course of uptake, metabolism, and the proportion of TCVP converted to TCMA following dermal and incidental oral exposures to a controlled dose of TCVP. Without this information, EPA cannot accurately back-calculate a TCVP dose from exposures to TCVP treated pets.

III. DISCUSSION AND CONCLUSIONS

Rubbing Study Phase (Studies 1 and 2)

For purpose of risk assessment, EPA is proposing that the transferable residue data from the Davis study be used to assess the potential from residential post-application exposures to adults (non-cancer and cancer) and children (non-cancer only) from actively registered TCVP pet collars. The transferable residue data have been determined to be acceptable for regulatory purposes given that the sample collection method was the scientific standard at the time the study was conducted and all phases of the sample collection, analysis, and recoveries appear adequate for those samples. The residue transfer data resulting from the Davis study (both study 1, 112 day mean and study 2, 12 day mean) result in greater FAR values that with the best exposure data currently available.

T-Shirt Study Phase (Study 2)

For purpose of risk assessment, EPA does not intend to rely on the results of the t-shirt study phase data. While the use of passive clothing dosimetry is consistent with post-application exposure monitoring guidelines there are clear limitations with this approach. The t-shirt sampling method employed, collection and analysis of 100 cm² sections only, likely underestimates TCVP exposures to children wearing the t-shirts. Further, inclusion of the t-shirt data would result in a negligible impact on the risks from adult and child exposures to pets treated with a TCVP pet collar.

Urine Sampling Phase (Study 2)

For purpose of risk assessment, EPA does not intend to rely on the results of the urine sampling for risk quantitation. To rely on these data would require on a full understanding the pharmacokinetics of TCVP, including an adequate justification for reliance on the metabolite, TCMA, to document the time course of uptake, metabolism, and the proportion of TCVP converted to TCMA following dermal and incidental
oral exposures to a controlled dose of TCVP. Without this information, EPA cannot accurately back-calculate a TCVP dose from exposures to TCVP treated pets.

**Plasma Cholinesterase Phase (Study 1)**

While the dog plasma ChE activity analysis was conducted adequately, and the data are relevant for determining that no significant changes in dog plasma ChE activities from pretreatment levels were observed, these data are not human relevant and, therefore, are not proposed for use in assessing human health from TCVP pet collar exposures.