Session 3: Reconsideration of two concerns previously raised by the HSRB in its June 2009 review of an intentional human dosing study with chlorpyrifos (Kisicki et al., 1999); additional pertinent information was made available by the sponsor related to these two concerns.

Background and context:

At its June 2009 meeting, the Human Studies Review Board considered several completed studies involving intentional human exposure to the organophosphate insecticide chlorpyrifos. In its review, the Board raised a number of scientific concerns about one of these studies: 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. Dated April 15-19, 1999. Unpublished study prepared by MDS Harris Laboratories under Project No. 21438 and Dow AgroSciences Study No. DR K-0044793-284. 578 p. MRID 44811002.

Two of the concerns about the Kisicki et al. (1999) study were considered to be major, and raised questions about whether the data arising from the study were scientifically valid. One concern was whether the urine samples were subjected to acid hydrolysis before processing and quantifying the chlorpyrifos metabolite trichloropyridinol. The second concern was why the absorption of chlorpyrifos was so much different compared to an earlier study of chlorpyrifos by Nolan et al. (1982) that was also reviewed at the June 2009 meeting. The relevant section of the Board's final report is appended below, with these two concerns highlighted yellow.

Following the June 2009 meeting, the study sponsor, Dow Agrosciences was asked by EPA to provide a written response to Agency regarding HSRB concerns. This response was not provided to the HSRB.

At the recent EPA Scientific Advisory Panel (SAP) meeting held in February 2011, data on chlorpyrifos from many sources were discussed, including the human intentional dosing data. The final report from the June 2009 HSRB was also provided to the SAP as part of the background materials. When the HSRB's concerns were discussed, Dow Agrosciences provided the SAP with its earlier response; this response was taken into consideration in the subsequent SAP discussion.

The HSRB may have come to a different conclusion regarding these two issues - the analytical procedures used to analyze the urine samplesfor trichloropyridinol in Kisicki et al. (1999) and the difference in observed absorption between Kisicki et al. (1999) and Nolan et al. (1982) - had this information been provided to the Board prior to completion of the June 2009 final report. If this is the case, the HSRB should request that its conclusions and recommendations about the Kisicki et al. (1999) study be corrected in the public record.

The original HSRB report and Dow Agrosciences' response to the Agency are thus being provided to the HSRB to determine whether a correction to the public record is warranted.

Charge questions:

1. Do the recommendations provided by the HSRB at its June 2009 meeting and in its subsequent report regarding the reliability of data on the urinary metabolite trichloropyridinol in the Kisicki et al. (1999) study change in light of the new information provided?

2. Do the recommendations provided by the HSRB at its June 2009 meeting and in its subsequent report regarding the level of absorption of chlorpyrifos in the Kisicki et al. (1999) study change in light of the new information provided?

Extract from the Final Report of the June 2009 HSRB Meeting re: Kisicki et al. (1999)

Assessment of Completed Research Study MRID 44811002: Kisicki *et al.* (1999) A Rising Dose Toxicology Study to Determine the No-Observable Effect-Levels (NOEL) For Erythrocytes Acetylcholinesterase (AChE) Inhibition and Cholinergic Signs and Symptoms of Chlorpyrifos at Three Dose Levels.

Overview of the Study

This study was a double blind, randomized and placebo-controlled rising dose study designed to determine the no-observable effect level (NOEL) for inhibition of erythrocyte acetylcholinesterase in human volunteers. The dosages used in the study were 0.5, 1 and 2 mg/kg, with the starting dose of 0.5 mg/kg based on the results of Nolan *et al*. (1982) discussed previously. In addition to assessing cholinesterase activity, blood and urine were collected and analyzed for chlorpyrifos and TCP to help define the pharmacokinetic properties in humans. Finally, paraoxonase status of each subject was determined (but will not be discussed here).

Chlorpyrifos was administered orally in a gelatin capsule with 0 (lactose), 0.5 and 1 mg chlorpyrifos/kg administered in Phase I of the study followed by a second phase that include 0 (control) and 2 mg/kg dosages. There were six male and six female participants for each dose level. Subjects were confined to the testing facility overnight prior to treatment through the first 48 hr after dosing. Additional samples were collected thereafter at 24 hr intervals through 168 hr (one week) post dosing. Blood, collected pre-dose (-10 and 0 hr) and at 2, 4, 8, 24, 36, 48, 72, 96, 120, 144 and 168 hr post treatment, was used for determining erythrocyte acetylcholinesterase activity and chlorpyrifos and TCP levels. Voided urine was collected at 12 hr intervals starting 48 hr prior to dosing and through the 168 hr dosing period (with the exception that immediately after dosing urine was collected at 0-6 and 6-12 hr).

Science

Charge to the Board

Are the blood and urine measurements of chlorpyrifos and/or TCP from the Kisicki *et al.* (1999) study reliable and appropriate for use in characterizing the results of epidemiological studies with chlorpyrifos?

Board Response to the Charge

HSRB Recommendation

Because of concerns about the analytic procedures' potential inability to control for the glucuronide-conjugated TCP, and apparent discrepancies in the absorption data when compared

with the data from Nolan *et al*. (1982), the Board questioned the reliability and utility of the and urine measurements of chlorpyrifos and/or TCP from Kisicki *et al.*(1999) risk assessment purposes.

HSRB Detailed Recommendations and Rationale

With respect to the general elements considered in the evaluation of the scientific reliability of the chlorpyrifos exposure data, this study provided clear documentation of the administered dose. Chlorpyrifos was added to a gelatin capsule normalized with lactose powder and the weight of the filled capsule was determined. There was one noted discrepancy that one participants in the 2 mg/kg dose group may have received a lower dose (1.63 mg/kg) based on conflicting data on body weight and administered dose data, but overall the documentation of the administered dose was satisfactory.

There was also generally good reliability with respect to sample collections. Participants remained in the testing facility for the first 48 hrs after dosing and 48 of the 60 subjects provided all periodic urine samples. Urine collections were judged to be complete based on creatinine excretion for 19 of the 60 subjects. One participant in the 2-mg/kg-dose group failed to return for sample collection and clinical monitoring after the initial 48 hr post-dosing period.

Chlorpyrifos and TCP were measured in blood and urine with GC/MS detection, and TCP was derivatized with N-methyl-(N-tertbutyldimethylsilyl)-trifloroacetamide. In general, these methods were similar to those described by Honeycutt and DeGeare with the exception that stable labels of chlorpyrifos and TCP (13C and/or 15N-labeled) were used as internal standards in the present analyses.

In reviewing this study, the HSRB identified several limitations or issues that may impact the overall reliability of the data. These included:

1. The amount of chlorpyrifos absorbed after oral administration in the present study was markedly lower than that reported by Nolan *et al*. (1982). A dose of 0.5 mg/kg was common to both studies, with Nolan *et al.*reporting approximately 70%, and Kisicki *et al.*reporting approximately 35% absorbed. The rationale provided for this large difference was that chlorpyrifos was administered using lactose tablets and gelatin capsules in the Nolan *et al.*and Kisicki *et al.*studies, respectively. Kisicki *et al.*suggested that oral absorption is slowed by the dissolution of the gelatin capsule with a concurrent reduction in the total amount absorbed. Although the Board agreed that absorption of chlorpyrifos from the gelatin capsule might be slower than that from a lactose tablet, there was some skepticism that the difference in dosage form would yield the large discrepancy in total percent absorbed in the two studies.

2. In the methodological details provided for the analysis of urinary levels of TCP, there was no indication that urine samples were subjected to acid hydrolysis required to

liberate the glucuronide conjugate of TCP, which is the major urinary metabolite of chlorpyrifos. This is an important distinction, as both Nolan *et al.*(1982) and Honeycutt and DeGeare (1993) indicated that urine samples were treated with concentrated sulfuric or hydrochloric acid and heated in order to hydrolyze the glucuronide conjugate and liberate TCP which is then derivatized (in the methods used by Honeycutt and DeGeare and Kisicki *et al.*). In the analytical portion of the Kisicki *et al.* (Brzak 2000), there is a clear designation that blood samples were treated with concentrated hydrochloric acid prior to extraction, but no similar details are provided for the analysis of the urine samples. Although it seems unlikely that this important (and necessary) step was omitted, the lack of explicit indication of this critical analytical step raises some doubt that it was done. If urine samples were not properly hydrolyzed prior to analysis, then the validity and reliability of these data are uncertain. Furthermore, since urinary TCP is used to estimate oral absorption of chlorpyrifos, it is possible that differences in sample handling may explain or at least contribute to the lower percent absorption reported by Kisicki *et al*.

3. The total mass balance (total recovery of the administered chlorpyrifos) was not determined. Inclusion of total recovery could increase the confidence in the estimated percent of absorption in the study and possibly clarify whether there was an issue with the TCP analysis conducted by Kisicki *et al*.

4. There were several questions regarding the designation and use of alternate participants in the study. The basis for participant replacement and verification of body weight and dose administered to the alternate participants were not adequately provided.

Because of concerns about the analytic procedures' potential inability to control for the glucuronide-conjugated TCP, and apparent discrepancies in the absorption data when compared with the data from Nolan *et al*. (1982), the Board thus questioned the reliability and utility of the and urine measurements of chlorpyrifos and/or TCP from Kisicki *et al.*(1999) risk assessment purposes.

Charge to the Board

Are the measurements of cholinesterase activity/inhibition from the Kisicki *et al.* (1999) study reliable?

Board Response to the Charge

HSRB Recommendation

The Board concluded that these measurements likely represent a reliable set of data, but cautioned the Agency about relying on data from the incomplete profile for the one responder at

the highest dose level. The Board also cautioned against relying on the statistical analyses as presented in the report.

HSRB Detailed Recommendations and Rationale

In this study, erythrocyte acetylcholinesterase activities were determined pre-dose (2 samples) and 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144 and 168 after dosing (Ellman method). Plasma cholinesterase activity was not measured. The results showed a relatively high degree of variability, and samples collected at 96 hr (Phase I; 0, 0.5 and 1 mg/kg) were judged to be low relative to all other treatment times. One participant in the 1-mg/kg dose group showed a 24.5% decrease in activity at the 96 hr time point. However, given the decreased activity observed in the entire group of samples collected at 96 hr along with the fact that peak inhibition would be expected to occur before 96 hr, this result was not considered to be a real finding. Only one participant in the 2-mg/kg-dose group showed a reduction in erythrocyte cholinesterase activity in the study, with a nadir (72% of her baseline level) observed 12 hr after treatment. However, this volunteer did not return for blood collections after the initial 48 hr period, resulting in an incomplete profile. This participant did not appear to show adverse clinical signs associated with cholinesterase inhibition. Based on the reduction in erythrocyte cholinesterase activity in this subject, the NOEL for enzyme inhibition was considered to be 1 mg/kg.

In reviewing this study, the Board identified several limitations or issues that may impact the overall reliability of the data. These included:

1. There was generally poor documentation provided for the grouping or batch processing of the blood samples. Given the recognized inter-day and inter-assay variability associated with the Ellman method, this is a critical weakness. The study records were noted to be insufficient to confirm that samples were batched correctly to allow the proper utilization of the unexposed control data to correct for day-to-day or batch-to-batch variations in the mean lab results. This limitation is particularly important to detect or to quantify accurately small changes within an exposed population.

2. The interpretation of the results is centered on the single individual who showed more than a 25% inhibition of erythrocyte cholinesterase activity at the 2-mg/kg-dose level. The conclusions regarding both the duration and magnitude of inhibition depend upon the inclusion of this participant, and the lack of follow-up in this participant beyond 48 hr thereby limits the interpretation of the results.

3. The reported statistical analyses of these data are highly problematic, and the results in Appendix 3 were judged to be clearly wrong. Specifically, it was noted that a univariate repeated measures ANOVA was performed (Kisicki *et al*. 1999, 25), and Appendix 3 summarizes the results of such analyses (Kisicki *et al*. 1999, 151-158, 184-188, 206-211). Although it is not possible to replicate these analyses, the report

suggests that the variable being analyzed is the Normalized Percent of Baseline (Kisicki *et al*. 1999, 24). The report indicates that the Group Mean Square (Kisicki *et al*. 1999, 157) is 19.1207 while the Error Mean Square is 52,835,395,914.644. It is virtually impossible that these two values can be this different for real data. Furthermore, the corresponding Individual Hour Error Mean Squares from the corresponding mixed model analysis range from about 14.39 to 48.26. These are much more reasonable values. The report (Kisicki *et al*. 1999, 25) also indicates that mixed effects models were used where gender and treatment were considered to be fixed factors, and that time was considered to be a random continuous covariate. According to the mixed model analyses reported in Appendix 3, time is not being considered a random continuous covariate. Time is treated as a continuous covariate, but not as a random continuous covariate. Overall, the Board concluded that the statistical analyses are likely incorrect, and there should be some effort to perform correct analyses before relying on these data.

4. Plasma cholinesterase activity was not determined in the study. It is recognized that the erythrocyte cholinesterase activity is more biologically relevant for assessing potential adverse effect of chlorpyrifos, and as such, the lack of data on plasma cholinesterase is not a serious limitation. However, given the issues raised concerning sample handling, batch processing and statistical evaluations, inclusion of plasma cholinesterase may have helped to assess the overall reliability of the data.

The Board thus concluded that the measurements of erythrocyte acetylcholinesterase activities were generally reliable. However, Board members cautioned that the incomplete profile obtained from the only subject who showed inhibition at the 2 m/kg dose level limited the utility of the data. Moreover, the Board recommended that, although the data might be reliable, the statistical analyses in general and particularly for the data in Appendix 3 should be replicated prior to applying these results to any risk assessment or model development.

Response to EPA (John Doherty) re: TCP hydrolysis and analysis methods and differences in oral absorption of chlorpyrifos between human volunteer studies

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INTRODUCTION

The Health Effects Division (HED) of EPA's Office of Pesticide Programs recently brought several human volunteer studies involving chlorpyrifos to the EPA Human Studies Review Board (HSRB) for scientific and ethical review. Based on discussion at the HSRB meeting, EPA on July 13, 2009 requested additional information regarding conduct of chemical analyses and oral absorption results in two of the studies. Dow AgroSciences (DAS) is providing information in response to this request.

ANALYTICAL PROCEDURES FOR TCP

John Doherty of the HED, in an email inquiry to Dow Chemical of September 16, 2009, posed the following question: *"The issue is that the HSRB raised concerns that the Nolan (~70%) and Kisicki (~30%) studies have such a large recovery difference for TCP indicating more was absorbed in the Nolan study. One of the HSRB panel members challenged the methodology used to detect TCP in blood and urine. Previously, Ken Racke addressed the issue in blood indicating that no hydrolysis was necessary since based on previous work with rat blood, nearly all TCP in blood is unconjugated. The assumption that TCP in human blood was also unconjugated has been made as a defense for not attempting to hydrolyze the blood to free conjugated TCP. This is still an assumption that at least one person here is challenging. The HSRB appears to consider the Nolan study more reliable than the Kisicki study with respect to TCP analysis in the urine. For the Kisicki study the Board has "concerns about the analytical procedures' potential inability to control for the*

glucuronide-conjugated TCP, and apparent discrepancies in the absorption data when compared with the data from Nolan et. al (1982), the Board questioned the reliability and utility of the blood and urine measurements of chlorpyrifos and/or TCP from Kisicki et al (1999) for risk assessment purposes." The Board also implied that the difference in absorption is related to differences in the methodology used to detect TCP.

I went over both the Brzak original supplement to the Kisicki study (page 16) and the response the Ken Racke sent previously (July 24, 2009, you are the second author) but cannot find where it specifically states that the sulfuric acid hydrolysis step was included in the preparation of the urine for TCP analysis. If the acid hydrolysis step was the same for both Nolan and Kisicki and it is the established method to free conjugated TCP, then we can eliminate that the difference in apparent absorption between Nolan and Kisicki is due to differences in sample preparation. Therefore if you can provide written documentation that there was the appropriate acid step in the preparation of the urine for TCP analysis and any other information that will resolve the reliability issue for TCP analysis in the Kisicki study, then the HSRB should reconsider its concerns. The documentation should include the amount of acid, temperature and time allowed for hydrolysis."

To clearly address this question, a summary of the analytical methods used for analysis of total 3,5,6-trichloropyridinol (TCP), has been prepared (Table 1). This table summarizes the study, species, hydrolysis, extraction, derivatization and separation/detection methods for Nolan 1982 and Kisicki 1999 human volunteer studies. For comparision, the analogous TCP methods employed on two rat metabolism or pharmacokinetic studies are included.

TCP in Urine: As shown by Nolan et al. in the Dow Chemical 1987 rat metabolism study, ^{14}C-chlorpyrifos-derived metabolites are excreted primarily in the urine of rats, accounting for 84-92% of the administered dose. The major metabolites found were free TCP and the glucuronide and sulfate conjugates of TCP. The authors showed that a 1 hr hydrolysis of the urine, at 80 degrees C, with a 1:10 acidification with 12M HCl was sufficient to completely hydrolyze the two conjugates of TCP back to the free TCP metabolite. The TCP

analytical methods employed for analysis of urine samples from both the Nolan 1982 and Kisicki 1999 human volunteer studies utilized hydrolysis methods of at least this acid strength and hydrolysis temperature and duration. In the Nolan 1982 study, urine samples were hydrolyzed with 2:10 (acid:urine) ratio of 18M H_2SO_4, at 90 degrees C for 1 hr. In the Kisicki 1999 study, the urine samples were analyzed for total TCP by Dr. Steven Summer of Battelle Laboratory (Columbus, Ohio). The acid hydrolysis method employed in this study was the same as in the original Nolan 1987 rat metabolism study (1:10, 12M HCl:urine @ 80 degC x 1 hr). Summer also verified that these conditions were sufficient for complete hydrolysis of the conjugated TCP in the Kisicki study urine samples by evaluating hydrolysis of numerous samples at 3 hr and 1hr. The extended hydrolysis time afforded no increase in total TCP found (<2% change).

TCP in Blood: As shown by Mendrala et al. in the Dow Chemical 1997 rat pharmacokinetic study, free TCP accounted for virtually all of the radioactivity in blood of rats administered either 5 or 100 mg/kg of ^{14}C-chlorpyrifos. Based on these results, the analysis of total TCP in the Nolan 1982 and Kisicki 1999 human volunteer studies did not require any acid hydrolysis step, as the TCP in human blood samples is assumed to be non-conjugated (see more complete discussion of this in Dow AgroSciences comments of July 24, 2009 (Racke 2009)). Some acid was added to the blood samples in both human volunteer studies, to protonate the analyte prior to SPE cleanup and/or subsequent extraction.

Overall Extraction, Derivatization, Analysis Conditions: All of the quantitative analytical methods for TCP in blood or urine from the rat or human pharmacokinetic studies were fairly comparable. Following acidification (with subsequent hydrolysis for urine), the samples were extracted into an organic solvent. Note that the samples from the Nolan 1982 human volunteer study employed an intial solid-phase extraction (SPE) column cleanup step, as the GC detection method of Electron-Capture (ECD) is not as specific as the detection method employed in the later studies (GC/MS). However, once in an extraction solvent, the TCP in the sample extracts was derivatized with a silylating reagent to enhance volatility of this analyte. The derivatized TCP was then separated on a GC column and quantitated via either ECD or mass spectral detection. The reference for the

methods employed in any of the human or rat studies is listed in the Comments column of Table 1. Note that the recovery of TCP from any of the listed methods was quite high, ranging from 86 to 114%. More specifically, the methods used for analysis of urinary TCP from the two human volunteer studies was quite high, at 97 and 99%. Note that the method for urine analysis in the Kisicki study was a Dow AgroSciences Report by E. Olberding (1998), which is included as an Appendix in the TCP analysis report of Summer 1999.

ORAL ABSORPTION OF CHLORPYRIFOS

John Doherty of the HED, in an email inquiry to Dow Chemical of September 16, 2009, also posed a second question: *"If the acid step was included and total TCP was detected in the urine in the Kisicki study, then the difference in apparent absorption needs to be explained. In both cases either the pill or the capsule have to be dissolved in the stomach before the chlorpyrifos can pass the pyloric valve to enter the g-i tract to be absorbed. I discussed this with another toxicologist, particularly involved with metabolism and PK, and he agrees that there may be some differences in absorption but not such a large difference as seen with the Nolan and Kisicki studies. Therefore, HED is requesting that DOW provide additional support for the assumption that the differences in apparent absorption is related to differences in dosing method (pill vs. capsule). It is also suggested that any other explanations that can explain the large difference in apparent absorption between the Nolan and Kisicki studies be provided especially to remove concerns that differences in analytical procedure were responsible."*

As discussed above, the analysis of TCP in blood and urine from the Nolan 1982 and Kisicki 1999 studies used comparable methods for analysis of the total TCP present in either matrix. An adequate acid-hydrolysis step was also employed in the analysis of urine samples from the Kisicki 1999 study samples. As a result, the difference in oral absorption between the two studies is most probably a result of test material formulation differences.

In the study of Nolan et al. (1982), the chlorpyrifos test material was dissolved in methylene chloride and the resulting solution applied to a lactose tablet. Following evaporation of methylene chloride carrier, the tablet was taken orally with water by the study volunteers. In contrast, the chlorpyfos test material used in the Kisicki 1999 study was formulated by weighing in crystalline test material into an empty capsule and filling the remainder of the capsule with lactose powder.

It would be expected that the chlorpyrifos added as a solution to lactose would afford dried test material of a substantially smaller particle size and/or larger surface area than the corresponding crystalline product. Numerous authors have shown that altering either property of drugs that are poorly water soluble in water can substantially enhance oral absorption in animals or humans. Garner et al. (2002) showed that micronizing diosmin from a mean particle size of 36.5 µm to 1.79 µm resulting in an increase in oral absorption in human volunteers from 33% to 58%. Evans et al. (2006) have also shown a 4-7 fold increase in systemic bioavailability of ketoconazole in dogs upon nanostructuring to increase drug surface area via rapid freezing. Elder et al (2006) have shown subtantial increases in systemic bioavailability of danazol following reducing drug particle size via a controlled precipitation technique. Finally, Liu (2008) has also discussed the fact that oral absorption generally increases with reduced particle size, since for most poorly soluble compounds, dissolution into the GI fluids is the rate-limiting step in oral uptake. In fact, this author describes that the oral absorption of the antifungal agent griseofulvin was found to increase 2.5 fold when the surface area was increased about six-fold. This increase in absorption is quite consistent with the 2.2-fold increase in absorption seen for chlorpyrifos, when adminstered as a solution applied to a lactose tablet (then dried) vs. the crystalline solid (70% from Nolan 1982 vs. 32% from Kisicki 1999).

Based on these examples, and the known differences in test material formulation techniques between the Nolan and Kisicki human volunteer studies, it is expected that the lower oral bioavailability measured in the 1999 Kisicki study was due to a larger mean chlorpyrifos particle size (affording a smaller net surface area/mg test material) than that of the formulation employed by Nolan et al (1982).

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Table 1. Summary of TCP analytical methods employed in human volunteer studies.

Study	Species	Matrix	Hydrolysis Conditions	Extraction Solvent	Derivatization Solvent	Derivatization Reagent	Derivatization Conditions	Analysis Conditions	Comments
Nolan, 1987 *Dow report* *HET-K-044793-(76)*	**Rat**	Urine	1:10 (12M HCl:urine) 80 degC x 1 hr	None	None	None	None	HPLC- 14C detection	84-92% of dose recovered in urine, as free TCP, glucuronide and sulfate conjugates. hydrolysis for 80 degC x 1hr completely cleaved both glucuronide and sulfate conjugates
Mendrala, 1997 *Dow report* *DECO HET T2.02-191-000-003A*	**Rat**	Blood	None (HCl added to protonate TCP prior to extraction)	Ethyl acetate	Toluene	MTBSTFA silylating reagent	60 degC x 1hr	NCI-GC/MS	Free TCP accounted for virtually all of test material-derived radioactivity found in blood (at 5 and 100 mg/kg) Original TCP method: Brzak et al (1998). J. Anal. Tox. 22: 203-210. Method recovery of TCP from Blood was 96-114%.
Nolan, 1982 *Dow report* *HET K-044793-061*	**Human volunteer**	Blood	None (HCl added to protonate TCP prior to extraction)	C_{18}-SPE cleanup, then benzene extraction	Benzene	BSA silylating reagent	None listed-derivatization assumed to be instantaneous	GC/ECD	Original TCP method: McKellar (1979) Dow report ACR 79.9 B 600-216-79 Method recovery of TCP from Blood and Urine was 86% and 97%, respectively
Brzak, 2000 *Dow report DECO HET K-044763-284B*	**Human volunteer** (samples from Kisicki 1999 study)	Blood	None (HCl added to protonate TCP prior to extraction)	Ethyl acetate	Toluene	MTBSTFA silylating reagent	60 degC x 1hr	NCI-GC/MS	Original TCP method: Brzak et al (1998). J. Anal. Tox. 22: 203-210. Method recovery of TCP from Blood was 96-114%.
Nolan, 1982 *Dow report* *HET K-044793-061*	**Human volunteer**	Urine	2:10 (18M H_2SO_4:urine) 90 degC x 1 hr	C_{18}-SPE cleanup, then benzene extraction	Benzene	BSA silylating reagent	None listed-derivatization assumed to be instantaneous	GC/ECD	Original TCP method: McKellar (1979) Dow report ACR 79.9 B 600-216-79 Method recovery of TCP from Blood and Urine was 86% and 97%, respectively.
Summer, 1999 *Battelle report* *N101121A*	**Human volunteer** (samples from Kisicki 1999 study)	Urine	1:10 (12M HCl:urine) 80 degC x 1 hr Authors also investigated 3hr hydrolysis time	1-chlorobutane	1-chlorobutane	MTBSTFA silylating reagent	60 degC x 1hr	EI-GC/MS	Original TCP method: Olberding (1999). Dow AgroSciences report GRM-97.04.R1 (appended to Summer 1999 Battelle report) Method recovery of TCP from Urine was 99%. Study results show no increase in urine TCP levels with 3hr hydrolysis time vs. 1 hr hyd