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Understanding Variation in Atrazine Metabolism in a Nonhuman Primate Model

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Abstract

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By Christina R. Brosius

Atrazine is among the most heavily applied pesticides in the United States. Atrazine and a few of its dealkylated and mercapturic acid metabolites have been used as biomarkers of atrazine exposure. This study assessed variation in a biomarker of atrazine exposure, diaminochlorotriazine (DACT), in a nonhuman primate model in serum and urine. The aims of this study were to assess variation in a single individual subject over time and among subjects in one dosing group, to assess variation in the metabolic profile by exposure vehicle, and to perform a comparison of biomonitoring matrices with a look at the elimination of atrazine and its metabolites from plasma and their appearance in urine. A group of six Cynomolgus monkeys were dosed with atrazine via five exposure pathways and doses across five experimental phases. Serum and urine samples were collected at up to 21 time points over seven days. Atrazine, DACT and other atrazine degradates were quantified in serum and urine using solid-phase extraction (SPE) sample preparation and high performance liquid chromatography- tandem mass spectrometry (HPLC-MS/MS) analysis with isotope dilution quantification. Experimentally determined DACT elimination rate constants (K_e) were used to compare atrazine elimination across subjects and exposure pathways and between biological matrices. Curves describing DACT elimination from urine and serum were developed, and DACT half-lives were calculated. Serum DACT half-lives were approximately 11 hours; urine half-lives were approximately 13 hours. Inter-subject and intra-subject variation in K_e were low in both matrices. Elimination rate constants were significantly different (urine $p=0.02$, serum $p=0.006$) when identical doses were administered intravenously versus orally, and oral doses administered at varying concentrations produced significantly different estimates of K_e. Elimination of DACT from serum and urine was different. Serum DACT was less abundant, peaked earlier, and dropped below limits of detection earlier. These results can inform planning for future studies that involve biomonitoring of atrazine and its most abundant biomarker.

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I. INTRODUCTION

Atrazine is a chlorotriazine herbicide that is widely used for the control of grasses and broadleaf weeds in agricultural applications (Laws and Hayes 1991). It was developed in the 1950s, and has been applied to millions of hectares of land in the years since (LeBaron, McFarland, and Burnside 2010). Atrazine is the most widely applied agricultural herbicide with approximately 80 million pounds applied yearly in the United States (USGS). More than 65% of U.S. cropland is treated with atrazine (ATSDR). It is primarily applied on corn, sugarcane, and wheat, although it is also used in turf grass applications, on sorghum, pineapples, nuts, and evergreen tree farms.

Atrazine's presence and that of its degradates has been frequently reported in surface and ground waters (USGS). The parent compound and some of its primary degradation products retain their biological activity in the environment (Donaldson, Kiely, and Grube 2002). Animal toxicological studies have identified toxic effects of atrazine and its chlorine-retaining degradation products on several organ systems, though most observed harmful affects have been endocrine-mediated (Jowa, Lubow, and Howd 2011). Atrazine has low acute toxicity in mammals. The primary adverse health effects of concern are generally accepted to be from chronic exposure. Of most concern are reproductive/developmental effects. Animal studies have shown estrus cyclicity disruption and altered plasma hormone levels resulting from atrazine exposure. Atrazine's carcinogenicity has been extensively studied, but data are inconclusive. The International Agency for Research on Cancer classifies atrazine as "not classifiable as to its carcinogenicity to humans" (ATSDR).

According to the Agency for Toxic Substances and Disease Registry (ATSDR), "data regarding the health effects of atrazine in humans are limited to ecological, case-control, and cohort mortality cancer studies and reproductive/developmental toxicity studies" (ATSDR). Most of the data come from oral exposure studies in animals (Barr et al. 2007). Epidemiological studies have found associations between farm workers and communities exposed to atrazine in their drinking water and developmental endpoints. ATSDR has set minimum risk levels (MRLs) for acute-duration and intermediate-duration exposures to atrazine but deems chronic-exposure data inadequate for an MRL derivation (ATSDR).

Biomonitoring is a valuable tool for assessing exposure to environmental chemicals (Needham and Sexton, 2000; Barr et al. 2005; Barr et al. 2006). It is the analysis of biological matrices like blood, urine, adipose tissue, saliva, and others for environmental chemicals or compounds that are specifically linked to those chemicals to identify or quantify the amount of a chemical that has made its way into the body. A compound that we measure to assess an exposure is a biomarker. This is different from measuring the amount of a chemical in a person's environment because it in most cases biological measurements measure aggregate and cumulative exposures from all environmental matrices (e.g. air, water, food, dust).

The state of the science of biomonitoring exposure to atrazine has evolved rapidly within the last few decades. Many studies have relied on measuring what was understood to be the best urinary metabolite of atrazine, atrazine mercapturate (AM) (Chevrier et al. 2011). Relatively recent work has demonstrated that AM might not be the best biomarker for this purpose. When researchers at the National Center for Environmental Health at the

Centers for Disease Control and Prevention compared the urinary atrazine metabolite profile (the relative concentrations of each measured atrazine metabolite in urine), they found that the metabolite profiles varied among exposure scenarios (Barr et al 2007). Atrazine mercapturate is only excreted in an appreciable amount when the exposure is to pure atrazine (e.g., occupational exposures), but real exposures are a mix of atrazine and its environmental degradates – primarily its dealkylated products diaminochlorotriazine (DACT), desethylatrazine (DEA), and desisopropylatrazine (DIA). That study determined that urinary DACT and desethylatrazine, rather than AM, are the most important metabolites to measure to assess exposure to atrazine (Barr et al. 2007). These degradates have been demonstrated to have similar modes of biologic action as atrazine, so they are important to consider in studies that assess exposure, especially those that aim to investigate exposures related to a health outcome (Donaldson, Kiely, and Grube 2002).

Atrazine is considered a non-persistent organic pollutant. Elimination begins almost immediately after exposure occurs, so biomarkers of exposure are indicators of fairly recent exposure; the biologic half-life of atrazine is around one day. Effective biomonitoring techniques can address developing concerns about human exposure to atrazine, but the properties of atrazine including its relatively short biological half-life present challenges to studies of exposure to atrazine. This study utilizes a non-human primate model to examine atrazine metabolism within the first few half-lives after exposure in ways that could inform assessment of human exposure to the pesticide by biomonitoring.

Here, we examine variation atrazine metabolism: (1) in a single individual subject over time and among subjects in one dosing group; (2) variation in the metabolic

profile by exposure vehicle; and (3) a comparison of biomonitoring matrices with a look at the elimination of atrazine and its metabolites from plasma and their appearance in urine.

Hypotheses:

1. Intra-subject variation is lower than inter-subject variation.
2. The DACT serum elimination rate constant that resulted from exposure via intraperitoneal injection is different from the DACT serum elimination rate constant that resulted from the oral dose pathway.
3. DACT is detectable in urine for more time after exposure and at higher concentrations.

II. METHODS

Experimental subject dosing and sample collection

Experimental subjects included six female *Macaca fascicularis* (cynomologus) monkeys. Sample collection for the data in this study was completed in five phases, each defined by the atrazine dosing vehicle and dose. The dosing scheme and the number of samples per phase are detailed in Table 1.

Table 1. Dosing scheme

Phase	Vehicle and dose	Plasma (N)	Urine (N)
1	Oral 0.5 mg/kg in 1% carboxymethyl cellulose (CMC)	114	69
2	Oral 0.125 mg/kg in 5% EtOH	113	74
3	Oral 2.5 mg/kg in 1% CMC	113	75
4	Single IV dose of 0.125 mg/kg in 5% EtOH at a volume of 1.25 mL/kg	126	69
5	Gavaged with 10 ml/kg or 25 ppm atrazine in water	126	73

N = number of temporally resolved samples collected.

Plasma samples were collected at 21 time points over seven days (0.083, 0.167, 0.25, 0.5, 1, 2, 4, 6, 12, 18, 24, 30, 36, 42, 48, 60, 72, 96, 120, 144, 168 hours after dose administration). Plasma sampling time points were consistent across each dosing phase,

although some plasma samples missing in some phases across subjects. Urine samples were collected at 1-2, 2-4, 4-6, 6-12, 12-18, 18-24, 24-30, 30-36, 36-42, 42-48, 48-60, 60-72, 72-96, 96-120, 120-144, and 144-168 hours, though some urine samples were pooled to establish adequate volume for analysis.

Sample preparation and analysis

The concentrations of atrazine and selected metabolites were determined using solid-phase extraction (SPE) sample preparation and high performance liquid chromatography- tandem mass spectrometry (HPLC-MS/MS) analysis with isotope dilution quantification. Prior to SPE, a 500 μL aliquot of each urine sample was combined with isotopically labeled internal standards and 2 mL of a solution of 2% formic acid in water. To prepare plasma samples for SPE, a 200 μL aliquot of each sample was combined with the internal standard solution, and then serum protein precipitation was carried out on the mixture with 1 mL of methanol. Samples were vortex mixed for 10 minutes at 1000 rpm then centrifuged at 1500 rpm for 15 minutes at 4°C. The resulting supernatant liquid was collected and dried using a TurboVap LV Evaporator with a water temperature of 45°C and nitrogen pressure of 18 psi. The residue was reconstituted with 2 mL of 2% formic acid in water. Each batch of samples was prepared concurrently with a 9-point set of calibration standards ranging from 0.5-200 ng/mL and two high- (QCH; target 50 ng/mL) and two low- (QCL; target 10 ng/mL) quality control samples. Two method blanks (negative controls) were included in each sample batch as well.

After preparation, samples were then loaded onto Strata X-C cation exchange SPE cartridges that had been conditioned with methanol followed by deionized water.

Each cartridge was then washed twice with 1 mL of 2% formic acid in 80:20 (v/v) water:methanol before vacuum drying. Elution of the analytes from the dried sorbent was performed twice with 2 mL of 2% ammonium hydroxide in methanol. Each sample eluate was brought to near dryness in the evaporator, then residues were washed down the sample tube with 1 mL of methanol, and samples were brought to dryness. Samples were reconstituted with 0.1% formic acid in water immediately prior to HPLC-MS/MS injection.

Chromatographic separation of atrazine and the selected metabolites was carried out on an Agilent 1200 system (Agilent Tech, Waldbronn, Germany) using a C6-Phenyl analytical column (100x4mm, 3 μm particle size, 100-0A pore size; Phenomenex, CA, USA). The instrument was programmed and controlled using Mass Hunter Workstation software version B.03.03 SP2 (Agilent Tech, Waldbronn, Germany). The column temperature was maintained at 45°C in a temperature-stable column compartment. The mobile phases were 0.2% formic acid in water and 0.2% formic acid in methanol, which were controlled in a gradient elution for optimal separation. Sample run time was approximately 18 minutes. Tandem MS/MS (Agilent Tech, Waldbronn, Germany) via positive-mode jet-stream electrospray ionization (J-ESI) was used for analyte detection and was also controlled and programmed by Mass Hunter Workstation software. Several multiple reaction monitoring time segments were optimized for analyte intensity.

Data processing was performed using Agilent Tech Mass Hunter Workstation software for mass spectrometry, and preliminary data analysis was performed using Microsoft Excel. All statistical analysis of variation in the biomarker among individuals,

groups, exposure pathways, and analytical matrices were performed in SAS 9.3 (SAS Institute Inc., Cary, NC).

Analysis

To compare elimination of metabolites within subjects and among subjects and dosing schemes, an estimate of the elimination rate constant of DACT was calculated for each subject in each experimental phase. Assuming that elimination occurs through first-order kinetics (equation 1), the elimination rate constant, K_e, was calculated by subtracting the natural log of the concentration of DACT at each time point (C_t) from the natural log of the peak concentration (C_0) and regressing the difference over time, t without an intercept (equation 2).

$$C(t) = C_0 e^{-K_e t} \qquad \text{eq. 1}$$

$$\ln[C(t)] = \ln[C_0] - K_e t$$

$$\ln[C(t)] - \ln[C_0] = -K_e t$$

$$\ln[C_0] - \ln[C(t)] = K_e t \qquad \text{eq. 2}$$

The slope of the line that best describes that relationship is the estimated elimination rate constant (equation 2). The difference in equation 2 was regressed over time between the time points at which DACT was at its peak concentration and had reached the limit of detection (LOD). Regression was performed using the REG procedure in SAS 9.3.

To test the assumption of first order kinetics, the data were plotted over the model generated by inserting K_e estimates into equation 1, and the differences between the data and the models' predictions were plotted as residuals for each phase. Residual was expressed as a percent to allow comparison over the elimination period since DACT concentrations are much higher at the beginning than the end of the elimination curve.

Half-lives were calculated to allow for comparison in the rate of elimination in terms of hours (equation 3).

$$t_{1/2} = \frac{\ln[2]}{K_e} \qquad \text{eq. 3}$$

Variation within subjects

Intra-subject variation of the rate of elimination of atrazine was examined to inform about potential differences in metabolic transformation of atrazine and its degradation products and to monitor for potential contamination and/or degradation in during preservation of the samples. Variation in the subjects' rates of elimination was examined by generating an average percent standard deviation of the estimated rate of elimination for each subject where percent standard deviation equaled the standard error of the elimination rate as calculated by the REG procedure in SAS divided by the estimated elimination rate constant multiplied by 100 (equation 4).

$$\frac{Standard\ Error_{Ke}}{K_e} \times 100 = \%SD \qquad \text{eq. 4}$$

Percent standard deviation is expressed as a percentage of the elimination rate constant to make standard deviations within a subject comparable across phases where the dosing scheme varies.

Variation among subjects

Like intra-subject variation in atrazine biomarkers, inter-subject variation in metabolite elimination is an important part of biomonitoring atrazine exposure. To test for significant variation in the rates of elimination between subjects, an analysis of variance among each subject's five K_e estimates within each phase was performed using the ANOVA procedure in SAS. The examination of intra-subject and inter-subject

variation in DACT elimination was performed using the hypothesis that intra-subject variation in the metabolite elimination would be lower than the inter-subject variation.

Variation of the metabolic profile by exposure vehicle

The third objective was to examine variation of the metabolic profile of atrazine by exposure vehicle. This analysis included two exposure pathways, allowing for an examination of whether the exposure pathway matters in the metabolism of atrazine to test whether DACT's serum elimination rate constant resultant from exposure via intraperitoneal injection is different from the elimination rate constant resultant from oral exposure. This objective was achieved through a paired t-test of estimated DACT elimination rate constants. Rate constants were matched by subject and compared between exposure vehicles.

Variation between plasma and urinary metabolite concentrations

The fourth objective was to examine variation between plasma and urinary metabolite concentrations in order to look at any potential biomonitoring implications of a difference between the two matrices and the compare serum elimination of metabolites to their urinary appearance. Serum and urine elimination rate constants were compared across subjects and experimental phases through a paired t-test.

RESULTS

In serum and urine ATZ, DEA, DIA, and DACT were quantified above method limits of detection of 1.53 ng/mL, 1.27 ng/mL, 1.02 ng/mL, and 2.23 ng/mL, respectively. Atrazine mercapturate, diaminoatrazine mercapturate (DAAM), and desethylatrazine mercapturate (DEAM) were quantified in urine, where method limits of detection were

0.8 ng/mL, 0.4 ng/mL, and 1.1 ng/mL. The accuracy of the measurement of all analytes in serum and urine was 100±20% with relative standard deviations < 12%

The serum elimination model developed using each estimated elimination rate constant was plotted against the data for each subject by phase in Figures 1-5 (see Section VII for figures). The model curves fit the data on average, supporting the assumption of first-order elimination. Serum curves had correlation coefficients greater than 0.92 except for subject 4 in phase 4 ($R^2 = 0.75$) and subject 3 in phase 1 ($R^2 = 0.83$). Median R^2 for serum curves was 0.968. Plots of percent residual between the serum models and data show residuals approximately evenly distributed about the model in phases 1, 4, and 5 and further support first-order elimination (Figures 6-10). Serum residuals are generally below 100%. The residual around the serum model for phases 2 and 3 appear curves and may suggest unequal variance.

Plots of the individual subject urine elimination models against data for each phase also demonstrate a fit between the data and a curve generated with an estimated elimination rate constant under an assumption of first-order elimination (Figures 11-15). Urine curves had correlation coefficients greater than 0.92 except for subject 1 in phase 4 ($R^2 = 0.81$) and subject 5 in phase 3 ($R^2 = 0.87$). Median R^2 for urine curves was 0.962. Residuals are generally a greater percentage of the estimated urine DACT concentration than they were for serum, suggesting that these urine curves may be less predictive than the serum models (Figures 16-20). Urine residuals are generally below 200%.

Table 2 summarizes estimated average serum and urine elimination rate constants and half-lives for each experimental phase. Tables 3 and 4 provide complete lists of

elimination rate constants for each subject in serum and urine (see Section VII for additional tables).

Table 2: Average Serum and Urine DACT Elimination Rate Constant and Half-life				
Phase	Mean Serum DACT K_e	Mean Serum DACT Half-life (hr)	Mean Urine DACT K_e	Mean Urine DACT Half-life (hr)
1	0.0686	10.38	0.0579	12.20
2	0.0682	10.20	0.0483	14.37
3	0.0646	10.94	0.0578	12.00
4	0.0482	15.57	0.0414	16.90
5	0.0654	10.62	0.0499	13.94

Urine half-lives were about two hours longer than serum half-lives. Half-lives in phase 4, where the dose was administered intravenously, were about three hours longer than the half-lives in the oral dose vehicle phases.

Variation within subjects

The average percent standard deviations (%SD) of the estimated serum elimination rate constants for subjects 1, 2, 3, 4, 5, and 6 were 4.24%, 4.26%, 8.19%, 9.25%, 8.57%, and 3.63%, respectively. Subject 4 had the highest average %SD in the elimination rate constant, and it also had the highest individual %SD, 22.39% for Phase 4. This probably results from a low number of samples (three) for this individual between the time of dose administration and the time when the DACT concentration reached the LOD. Overall %SD for the total of 30 estimates of DACT elimination rate constants was between 1.57% and 9.91% except for the high value for subject 4 and the high values for subject 5 and subject 3, each having a maximum of 17.57%. Four of the six subjects had their maximum %SD in phase 4.

The average %SD of the estimated urine elimination rate constants for subjects 1, 2, 3, 4, 5, and 6 were 8.01%, 5.67%, 6.16%, 6.47%, 6.87%, and 5.67%, respectively. Subject 1 also had the highest average %SD in the urine elimination rate constant, and it had the highest individual %SD, 14.42 % for Phase 4. Overall %SD for the total of 30 estimates of urine DACT elimination rate constants was between 3.05% and 14.42%.

Variation among subjects

An analysis of variance among the estimated serum DACT elimination rate constants grouped by subject produced an F-statistic of 1.99 with p=0.1167 (Figure 21). The F-statistic, the ratio of the variance among the subject means to the variance within the subjects, is greater than one, suggesting that inter-subject variation in the serum elimination rate constant is greater than intra-subject variation. The high p-value, however, does not support accepting this hypothesis. An analysis of variance on the estimated urine DACT elimination rate constants grouped by subject produced an F-statistic of 0.45 and p=0.8122 (Figure 22). These analyses suggest that the group means of the estimated elimination rate constants among subjects are not significantly different from each other. Variation among subjects is acceptably low.

Variation of the metabolic profile by exposure vehicle

Phase 2 and Phase 4 doses were administered at the same dose in the same solvent. Phase 2 was administered orally, and phase 4 was administered intravenously. A paired t-test of serum DACT phase 2 and phase 4 elimination rate constants where elimination rate constants were matched by subject returned a p-value of 0.0068. This supports the hypothesis that the rate of elimination of serum DACT is different when atrazine is administered intravenously versus orally. A paired t-test of urine DACT phase 2 and

phase 4 elimination rate constants where elimination rate constants were matched by subject returned a p-value of 0.0225, suggesting the same conclusion for the rate of urine DACT elimination.

Additionally, analyses of variance were performed among the orally administered exposure vehicles, which vary by target dose and solvent. An ANOVA among estimated K_e grouped by exposure vehicle produced an F-statistic of 6.70 with p=0.0026 (Figure 23). ANOVA among the estimates urine DACT elimination rate constants grouped by exposure vehicle produced an F-statistic of 10.01 with p=0.0003 (Figure 24). These results suggest that the average elimination rate constants for each phase are not equal. The F-statistics suggests that inter-phase variation in estimated K_e was greater than intra-phase variation for both urine and serum.

Variation between plasma and urinary metabolite concentrations

A paired t-test of all DACT serum and urine half-life estimates, matched on phase and subject, resulted in a t-value of 4.0 with p=0.0004. This result indicates that serum and urine elimination are significantly different. The mean difference between urine and serum half-life was 2.34 hours, 95% CI [1.15 , 3.53] with an estimated standard deviation of 3.20 hours (137% SD).

Table 5 summarizes the average maximum DACT concentrations, average time at maximum concentration, and average time at which the DACT concentration in samples fell below the limit of detection for serum and urine samples. The maximum DACT concentration was at least an order of magnitude higher in urine than in serum in every phase. The average time of peak serum concentration was earlier than time of peak

concentration in urine, and the time at which samples fell below the LOD was earlier. This study may lack the temporal resolution in urine necessary to indicate at what point

Table 5: Temporal Summary of DACT Elimination from Serum and Urine

Phase	Mean Maximum Serum Conc (ng/mL)	Mean Time of Max Serum Conc (hr)	Mean Time of Serum LOD (hr)	Mean Maximum Urine Conc (ng/mL)	Mean Time of Max Urine Conc (hr)	Mean Time of Urine LOD (hr)
1	358.10	6.8	78	5120.71	9.3	144
2	53.82	0.9	47	558.98	8.00	100
3	854.11	4.3	106	14208.51	10.7	168
4	25.34	2.5	48	358.89	12.7	112
5	98.43	1.8	58	1148.40	11.3	120

urine concentration peaks in the early hours after a dose is received. Tables 6 and 7 (see Section VII) show that a majority of peak urine concentrations were observed at the first time point. Urine samples were pooled when volumes were inadequate. Even when rejecting the hours before the first urine sample could be taken, the window of time over which DACT was detectable was longer for urine samples than for serum samples by an average of 54.2 hours.

IV. DISCUSSION

First-order DACT elimination models developed on the data from this study describe elimination of the most abundant atrazine metabolite from serum and urine. High correlation coefficients suggest that change in log DACT concentration does vary in the way that the model describes and that the error around the model is normally distributed. Serum phase 2 and 3 percent residual plots may indicate non-normal variance

through the range of the model, though at opposite ends of the elimination curves. Urine phase 2 and phase 3 percent residual plots may also indicate non-normal variance. Measurement error is not likely to be a factor in the appearance of non-normal variance as error in the analytical method is small and should be normal.

Elimination rate constants were calculated from experimental data for each subject in each experimental phase and were used to compare concentrations of DACT in serum and urine after exposure to atrazine. Average intra-subject percent standard deviation in serum elimination rate constants was between 3.63% and 9.25%. In urine average intra-subject %SD was 5.67% and 8.01%. An inter-subject comparison of experimental serum and urine elimination rate constants indicates that differences in the six subjects' DACT elimination rates are not statistically significant. The analyses of variance did not support the conclusion that inter-subject variation is greater than intra-subject variation in rates of DACT elimination. Urine samples were pooled across varying spans of time, and no adjustment for variation in urinary volumes was performed, potentially limiting the utility of this comparison (Barr et al 2006). Inter-subject variation was likely affected by study design, as the group of subjects was selected to be as homogenous as possible on demographic and genetic factors. This makes comparisons of metabolite concentrations among individuals appropriate, but comparisons of variation among subjects probably underestimate the inter-subject variation that would be observed in humans.

Estimated DACT elimination rate constants were significantly different when atrazine was administered intravenously rather than orally. Among oral administrations at different doses and in different solvents, DACT elimination rate constants also were not

equal. The average highest DACT serum concentration in phase 4 occurred after the time of average peak serum DACT in phase 2, and it was less than half the peak concentration of the phase 2 peak concentration. Phase 4 DACT concentrations fell below LOD earlier than phase 2.

These observations are in line with our understanding of absorption, distribution, metabolism, and excretion (ADME) of nonpersistent pesticides in the body (Klassen 2010). While a portion of the phase 2 atrazine dose was presumably not absorbed and was partitioned into feces, the absorbed portion of the dose would be passed from the gastrointestinal tract straight through portal blood to the liver where it would undergo N-dealkylation by the cytochrome P450 system (Panuwet et al. 2010). The phase 4 dose, where administration bypassed the gastrointestinal tract, takes a less direct route through the body to the liver where it is metabolized into DACT, so DACT concentrations peak later and with less intensity, as atrazine would be removed from serum and excreted through urine starting at the time of dose administration.

Serum and urine DACT elimination rates were significantly different across subjects and experimental phases. Serum concentrations peaked earlier and were detectable for less time than urine concentrations. This is also in line with expectations of nonpersistent pesticides based on ADME. Higher DACT concentrations and longer DACT half-lives in urine make it a more useful biological matrix for measuring DACT as a biomarker of atrazine exposure. The window to take samples to observe atrazine exposure is longer. It is also easier to collect, store, and analyze urine than serum, and urine sample collection is less of a burden on subjects. However, the higher percent residuals around the urine models and slightly higher %SD of the elimination rate

constant estimate would make it more difficult to build a model of biomonitoring utility out of these data. In this study the median width for a 95% confidence interval for urine DACT half-life is 3.5 hours. Extrapolating that CI in practice leads to a 21-hour window between the minimum and maximum lengths of time it would take the DACT concentration to reach 1.5% of its original concentration (6 half-lives).

The necessity of pooling urine samples to meet analytical volume requirements removed temporal resolution from many of the urine DACT elimination sample sets. Due to lack of volume correction, urine DACT concentrations are less comparable over the sample period and are an adequate but not ideal fit to predicted elimination curves. This made it so that error around the elimination curve is up to 200%. Figures 11-15 indicate that individual subjects' data was a better fit in some experimental phases than others, limiting comparability across phases.

In serum and urine data, some individuals experienced spikes in DACT concentrations at time points that could indicate sample contamination (e.g. Figure 1 subject 3, Figure 13 subject 1). It would be difficult to determine if urine samples were contaminated due to pooling and no volume corrections. Serum sample contamination in Figure 1 subject 3 is unlikely because more than one sample is higher than average, and internal standard quantification was used alongside quality controls.

The study results indicate that K_e may be partially dependent on the magnitude of the dose. This may limit the applicability of the estimates of variation among and within subjects and the predictive utility of the elimination models where doses are likely to be much lower than the doses administered in this study.

In studies of atrazine exposure in a population, DACT itself may not be a unique biomarker of atrazine exposure because DACT and other dealkylated metabolites of atrazine can result from metabolism of other chlorotriazine pesticides. DACT in serum or urine could also represent exposure to DACT, DEA, or DIA rather than atrazine because the dealkylated metabolites of atrazine can also reside in the environment from breakdown of the herbicide.

The data in this study represent a response to an acute exposure to atrazine. Many of the population of interest who are exposed to atrazine regularly (e.g. farm workers, residents where atrazine is measureable in drinking water) are likely to have steady-state concentrations of atrazine biomarkers or concentrations of biomarkers that represent episodic exposures. Where this is true an assumption of first-order elimination will not result in accurate estimations of received dose.

V. CONCLUSIONS AND RECOMMENDATIONS

This study assessed variation in a biomarker of atrazine exposure in a nonhuman primate model in serum and urine. In the study group inter-individual variation and intra-individual variation in elimination rate constants were low. Rates of elimination of doses administered via oral exposure pathways were different from each other and from an intravenous exposure pathway. Urine and serum biomarker concentrations were different as were rates of elimination of the biomarker. These conclusions can inform planning for future studies that involve biomonitoring of atrazine and its most abundant biomarker, especially where estimation of the magnitude of received dose or exposure is a goal.

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VII. TABLES AND FIGURES

Table 3: Estimated Elimination Rate Constants

Table 3: Estimated Elimination Rate Constants							
Phase	Subject	Serum K_e	Mean Serum K_e	95% C.I. Mean Serum K_e	Urine K_e	Mean Urine K_e	95% C.I. Mean Urine K_e
1	1	0.0706	0.0686	[0.0596 : 0.0777]	0.0602	0.0579	[0.0496 : 0.0662]
	2	0.0703			0.0673		
	3	0.0784			0.0653		
	4	0.0577			0.0454		
	5	0.0830			0.0502		
	6	0.0517			0.0590		
2	1	0.0674	0.0682	[0.0583 : 0.0780]	0.0461	0.0483	[0.0399 : 0.0566]
	2	0.0627			0.0507		
	3	0.0717			0.0487		
	4	0.0752			0.0471		
	5	0.0674			0.0478		
	6	0.0647			0.0492		
3	1	0.0618	0.0646	[0.0609 : 0.0683]	0.0568	0.0578	[0.0518 : 0.0638]
	2	0.0610			0.0593		
	3	0.0645			0.0607		
	4	0.0656			0.0585		
	5	0.0831			0.0564		
	6	0.0516			0.0550		
4	1	0.0539	0.0482	[0.0368 : 0.0597]	0.0448	0.0414	[0.0342 : 0.0487]
	2	0.0507			0.0377		
	3	0.0437			0.0476		
	4	0.0628			0.0408		
	5	0.0525			0.0354		
	6	0.0259			0.0422		
5	1	0.0650	0.0654	[0.0566 : 0.0742]	0.0504	0.0499	[0.0437 : 0.0561]
	2	0.0669			0.0467		
	3	0.0661			0.0502		
	4	0.0635			0.0458		
	5	0.0712			0.0566		
	6	0.0599			0.0499		

Table 4: Estimated DACT Half-lives

Table 4: Estimated DACT Half-lives							
Phase	Subject	Serum Half-life	Mean Serum Half-Life	95% C.I. Mean Serum Half-Life	Urine Half-Life	Mean Urine Half-life	95% C.I. Mean Urine Half-Life
1	1	9.81	10.38	[8.92 : 11.63]	11.52	12.20	[10.47 : 13.97]
	2	9.85			10.30		
	3	8.83			10.61		
	4	12.01			15.26		
	5	8.35			13.79		
	6	13.41			11.75		
2	1	10.28	10.20	[8.88 : 11.89]	15.04	14.37	[12.25 : 17.35]
	2	11.05			13.67		
	3	9.67			14.22		
	4	9.22			14.72		
	5	10.28			14.50		
	6	10.72			14.08		
3	1	11.21	10.94	[10.14 : 11.39]	12.20	12.00	[10.86 : 13.39]
	2	11.37			11.69		
	3	10.75			11.41		
	4	10.56			11.84		
	5	8.34			12.28		
	6	13.44			12.61		
4	1	12.86	15.57	[11.61 : 18.83]	15.47	16.90	[14.24 : 20.29]
	2	13.68			18.39		
	3	15.86			14.57		
	4	11.03			16.99		
	5	13.20			19.57		
	6	26.77			16.41		
5	1	10.66	10.62	[9.34 : 12.24]	13.74	13.94	[12.34 : 15.84]
	2	10.36			14.84		
	3	10.49			13.80		
	4	10.91			15.14		
	5	9.74			12.24		
	6	11.57			13.88		

Figure 1: Phase 1 Observed Serum DACT and Model-Predicted Serum DACT

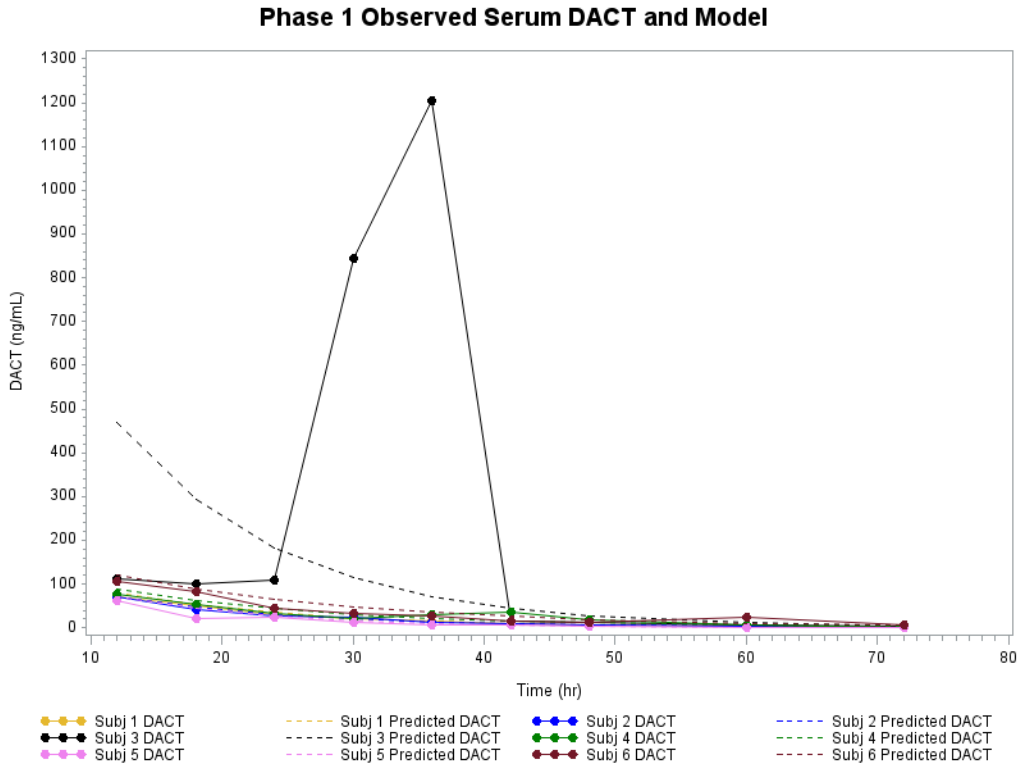

Figure 2: Phase 2 Observed Serum DACT and Model-Predicted Serum DACT

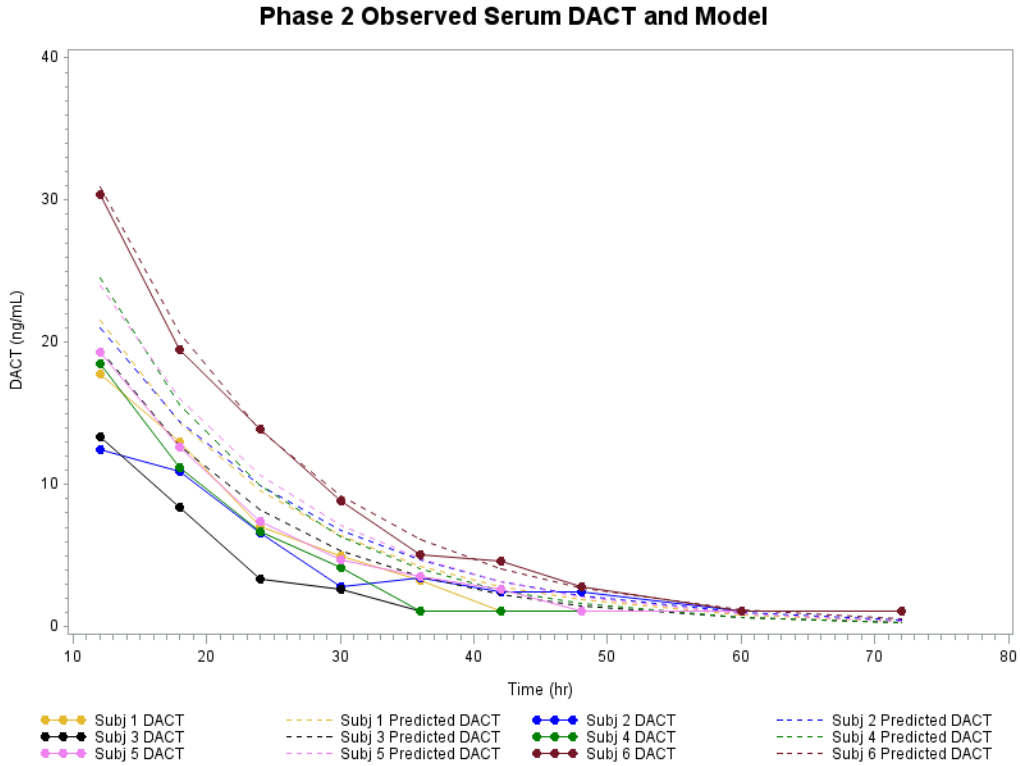

Figure 3: Phase 3 Observed Serum DACT and Model-Predicted Serum DACT

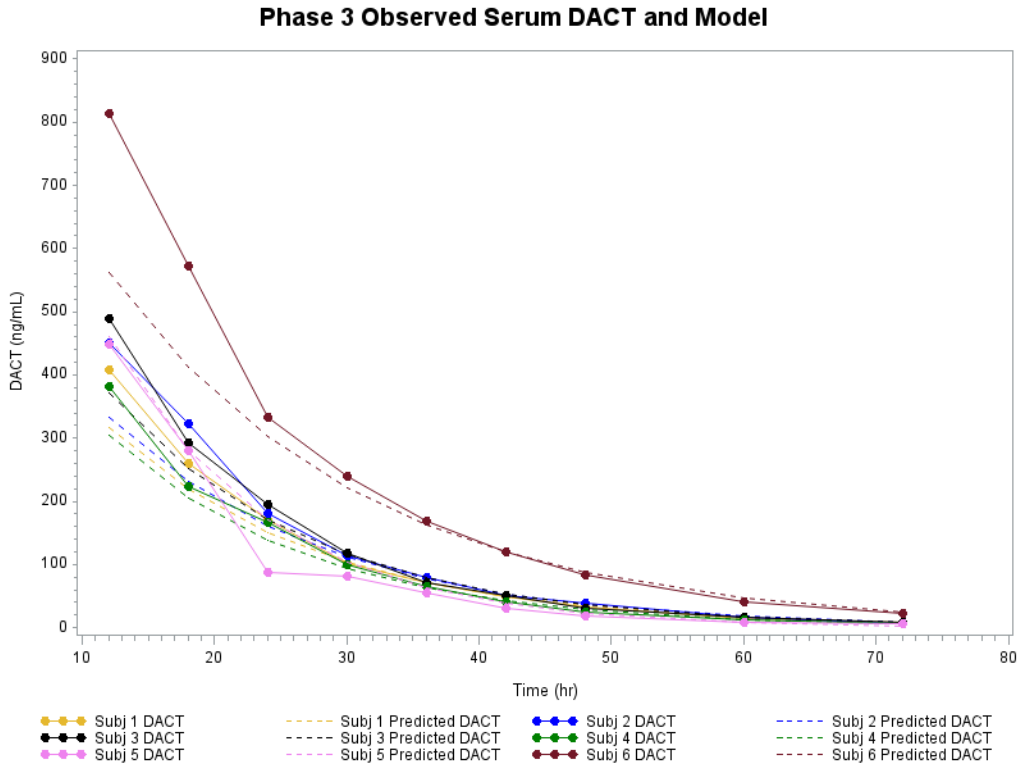

Figure 4: Phase 4 Observed Serum DACT and Model-Predicted Serum DACT

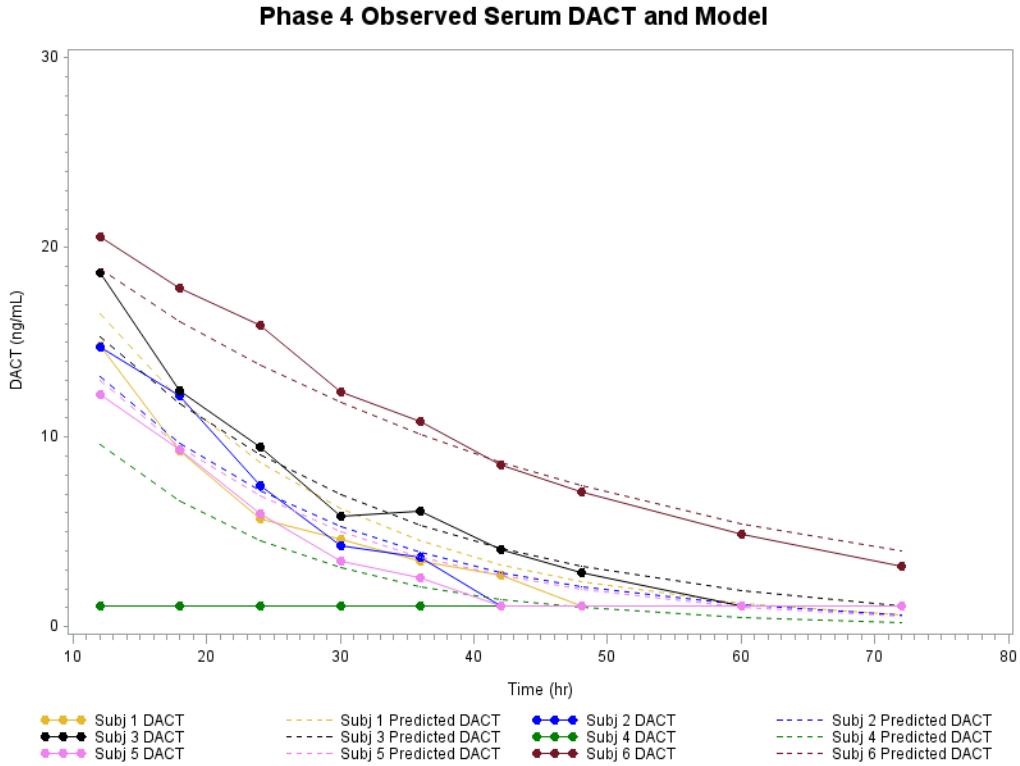

Figure 5: Phase 5 Observed Serum DACT and Model-Predicted Serum DACT

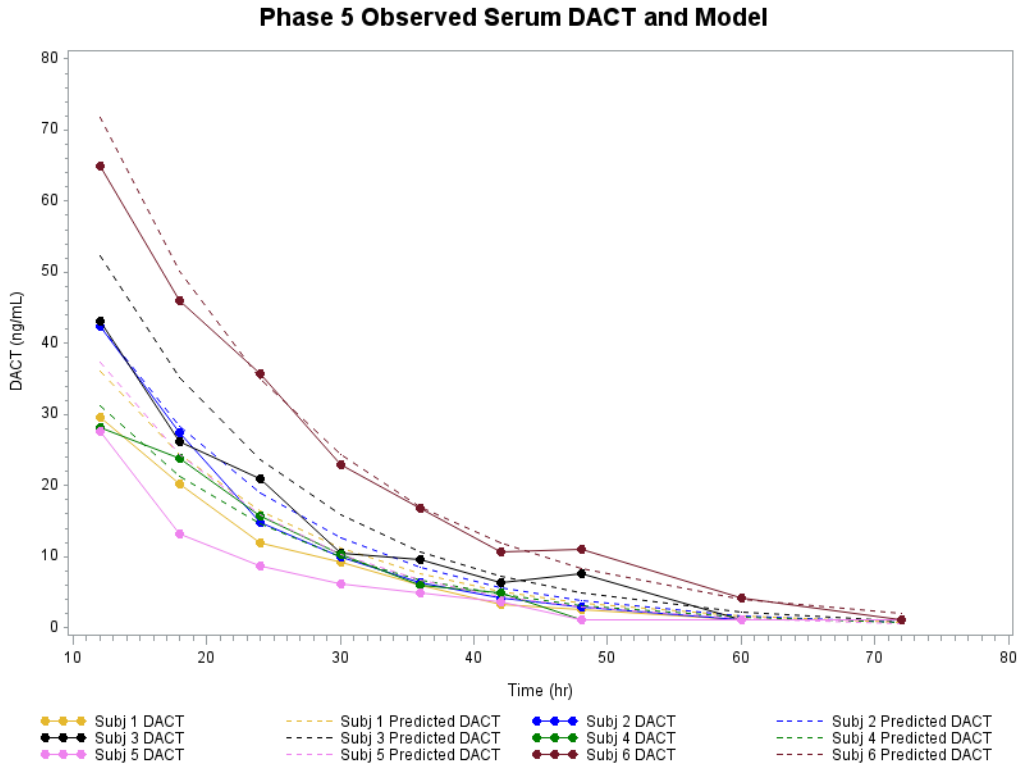

Figure 6: Phase 1 Percent Residual of Model-Predicted Serum DACT

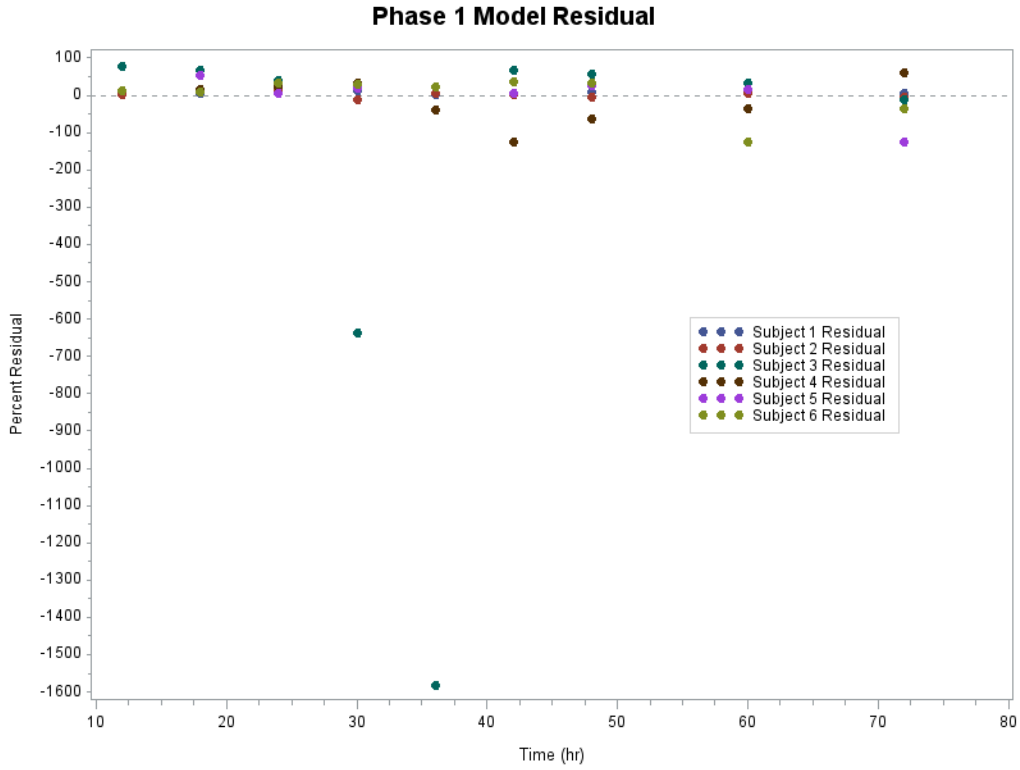

Figure 7: Phase 2 Percent Residual of Model-Predicted Serum DACT

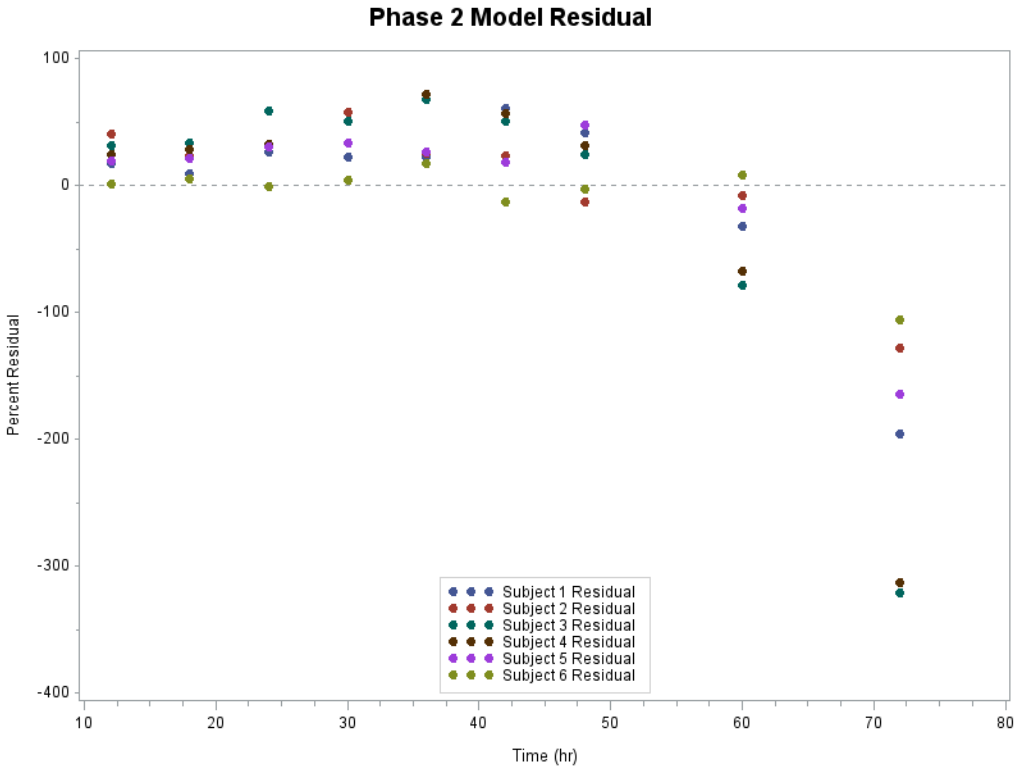

Figure 8: Phase 3 Percent Residual of Model-Predicted Serum DACT

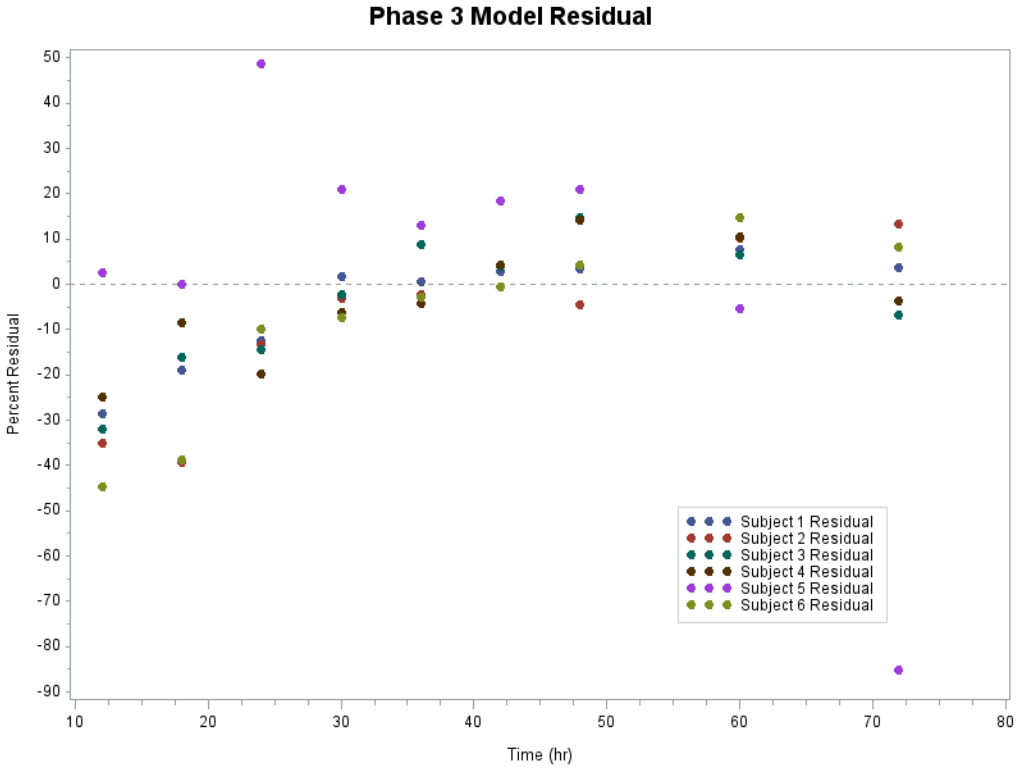

Figure 9: Phase 4 Percent Residual of Model-Predicted Serum DACT

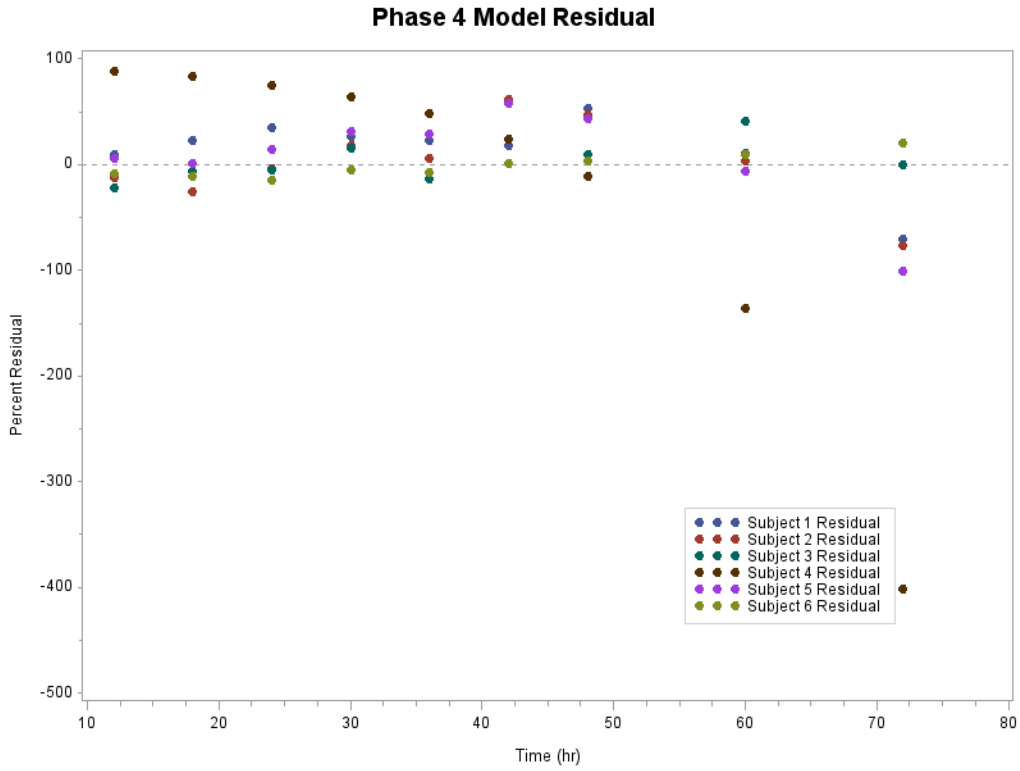

Figure 10: Phase 5 Percent Residual of Model-Predicted Serum DACT

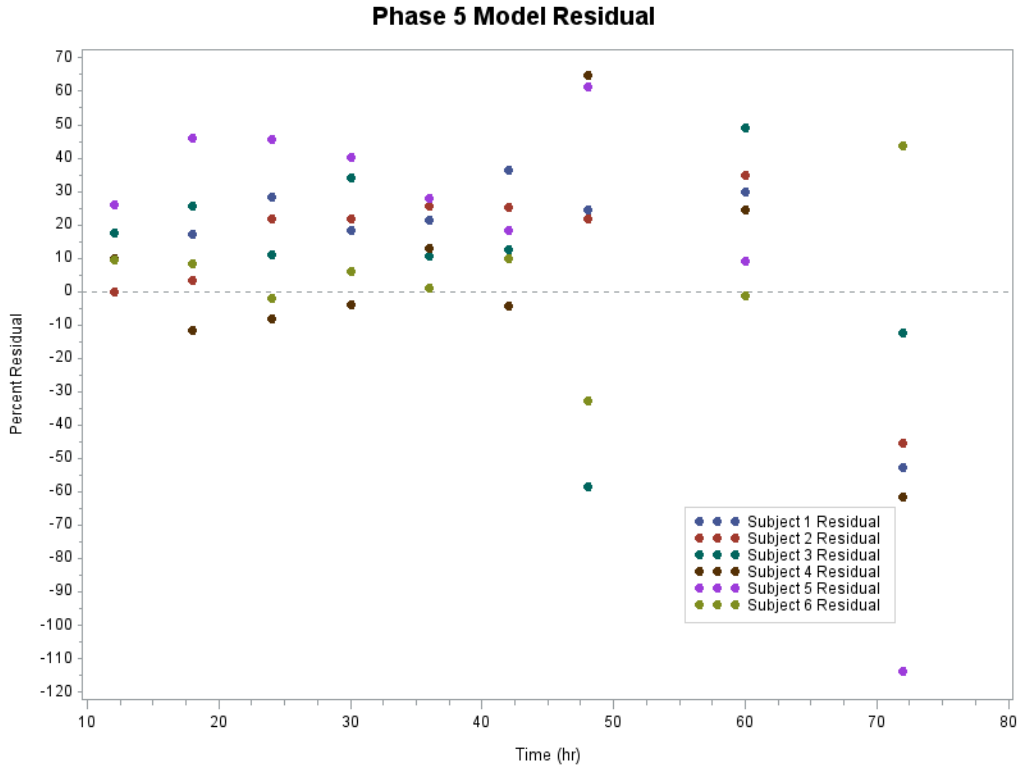

Figure 11: Phase 1 Observed Urine DACT and Model-Predicted Urine DACT

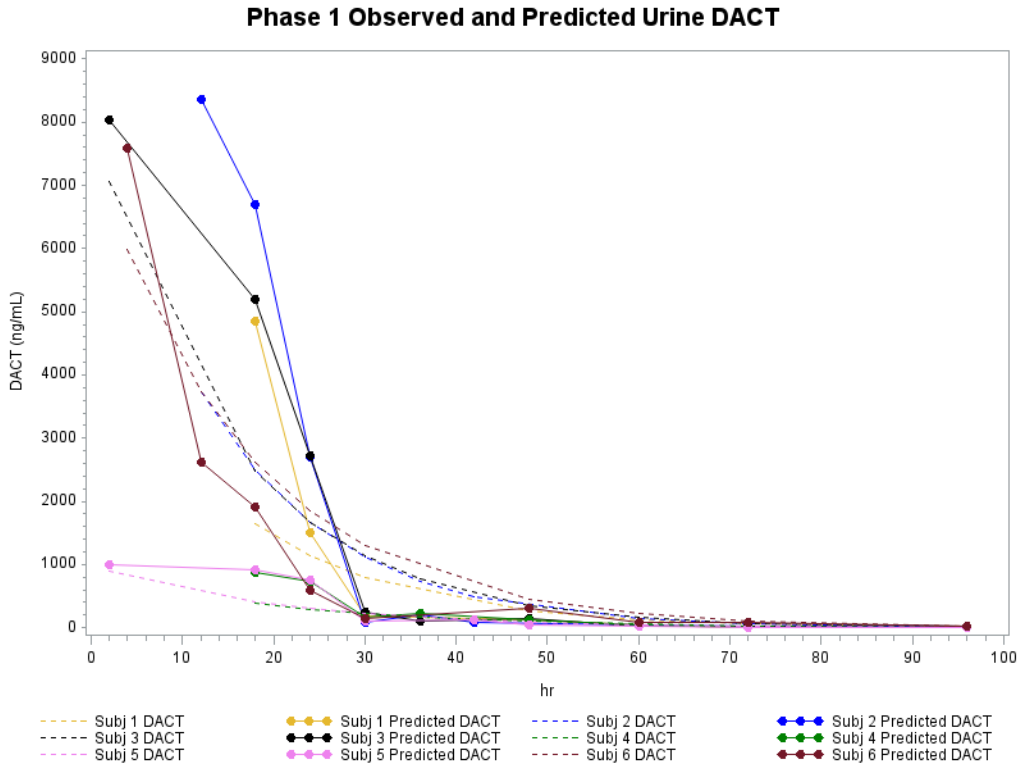

Figure 12: Phase 2 Observed Urine DACT and Model-Predicted Urine DACT

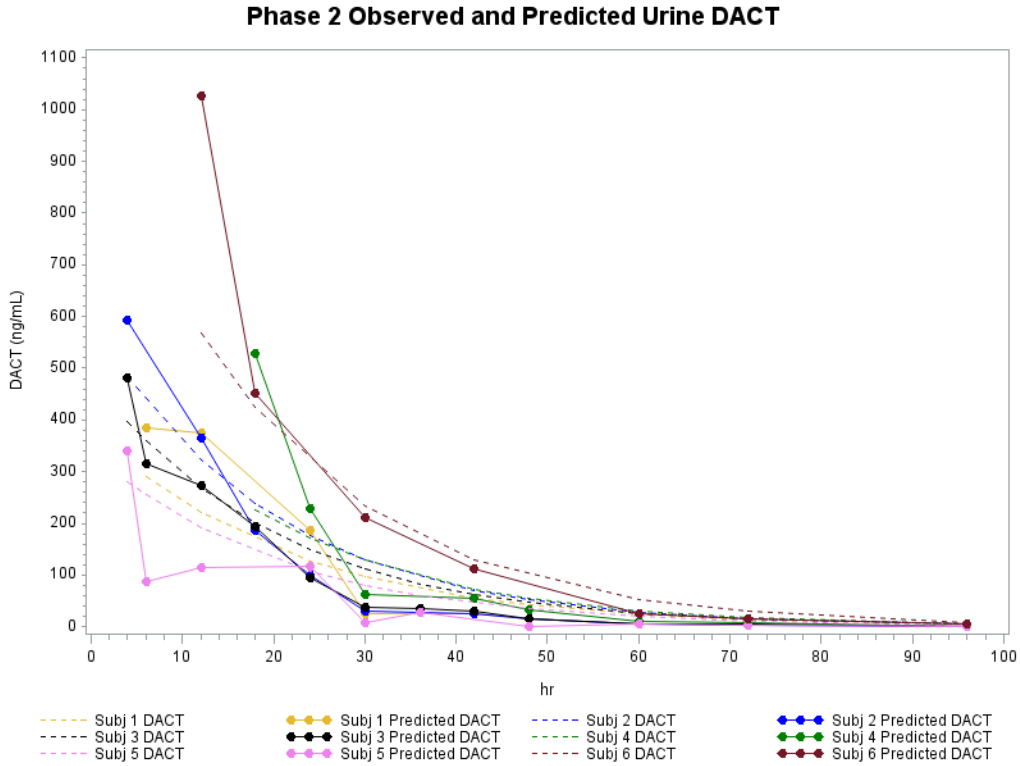

Figure 13: Phase 3 Observed Urine DACT and Model-Predicted Urine DACT

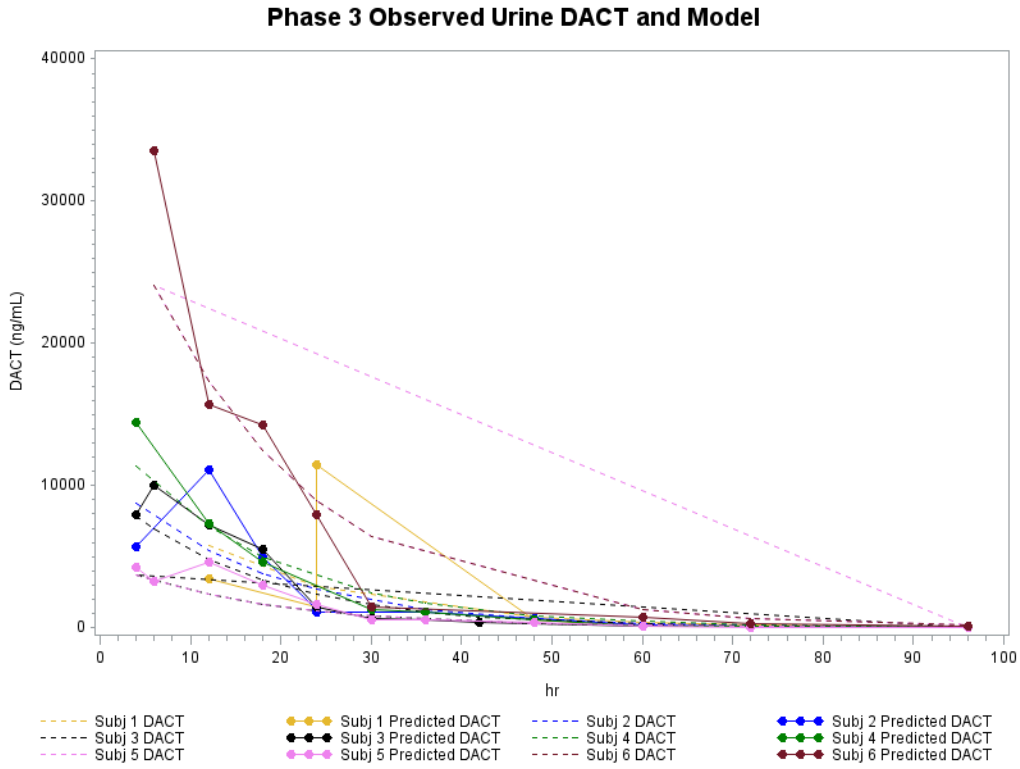

Figure 14: Phase 4 Observed Urine DACT and Model-Predicted Urine DACT

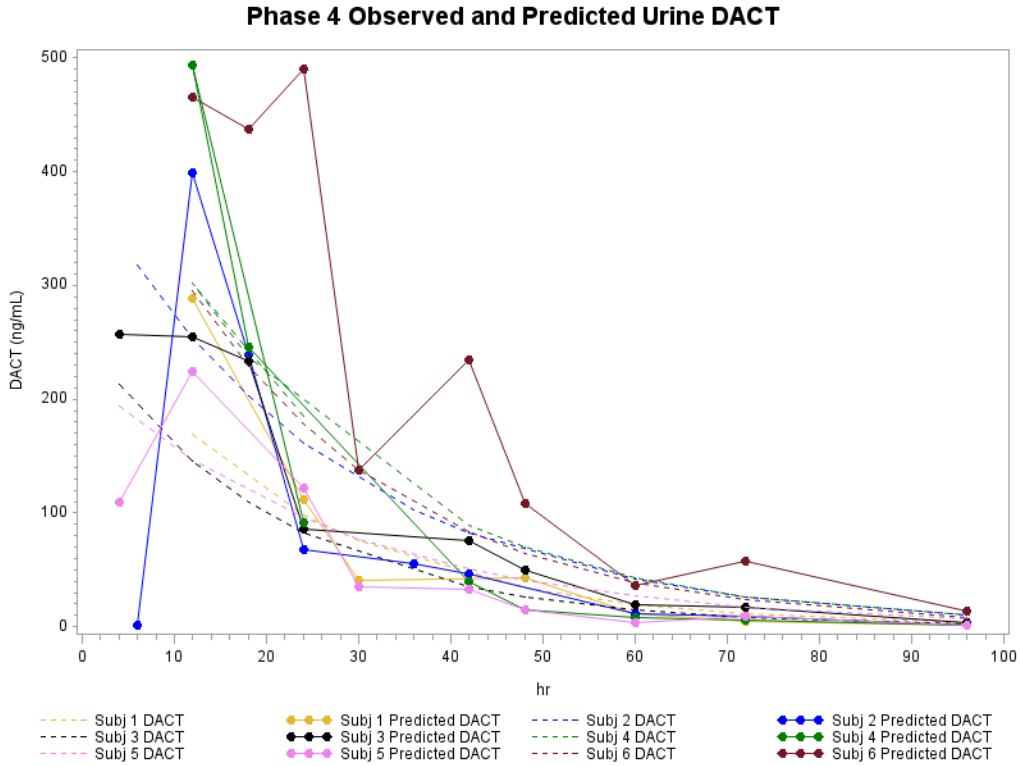

Figure 15: Phase 5 Observed Urine DACT and Model-Predicted Urine DACT

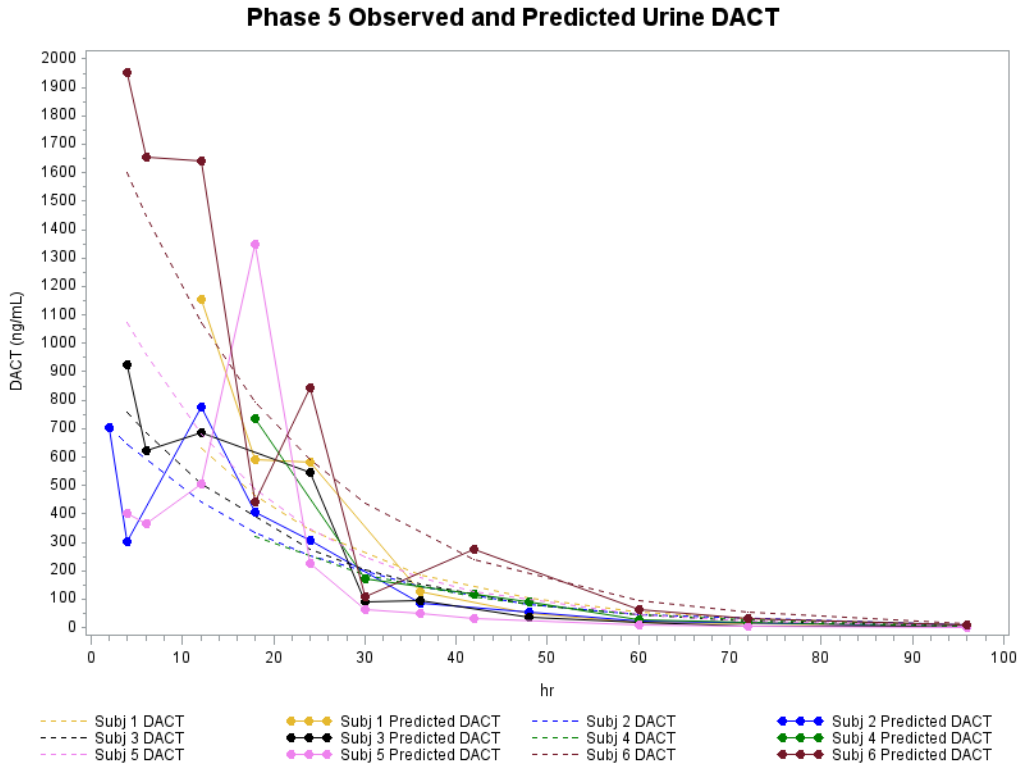

Figure 16: Phase 1 Percent Residual of Model-Predicted Urine DACT

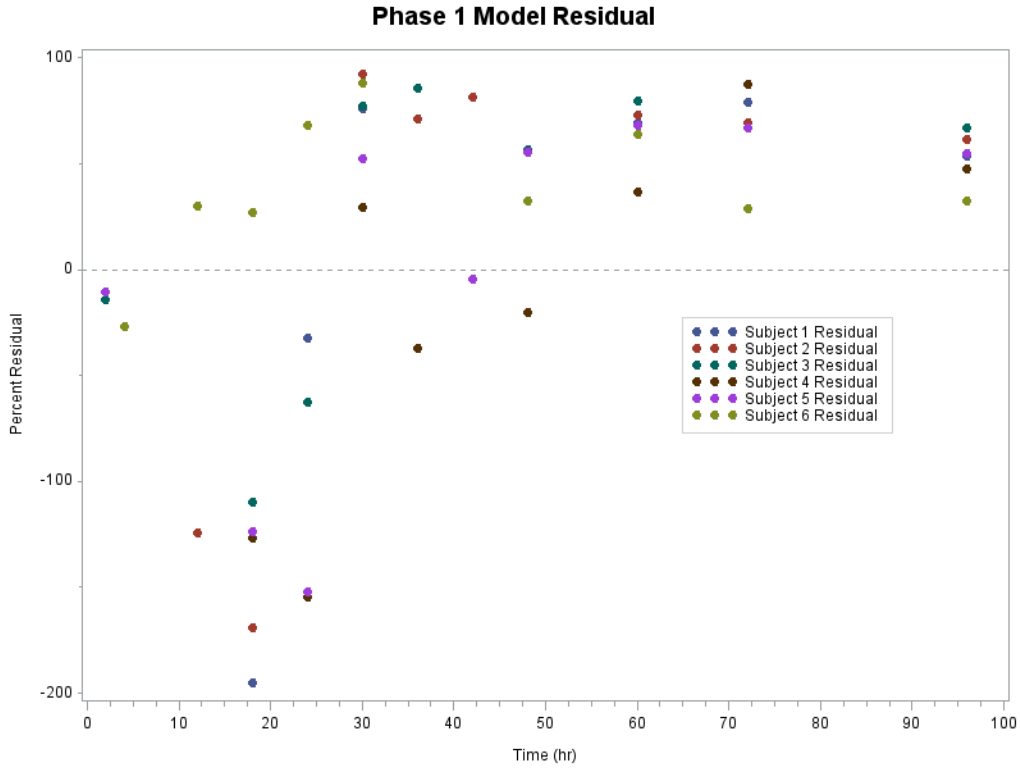

Figure 17: Phase 2 Percent Residual of Model-Predicted Urine DACT

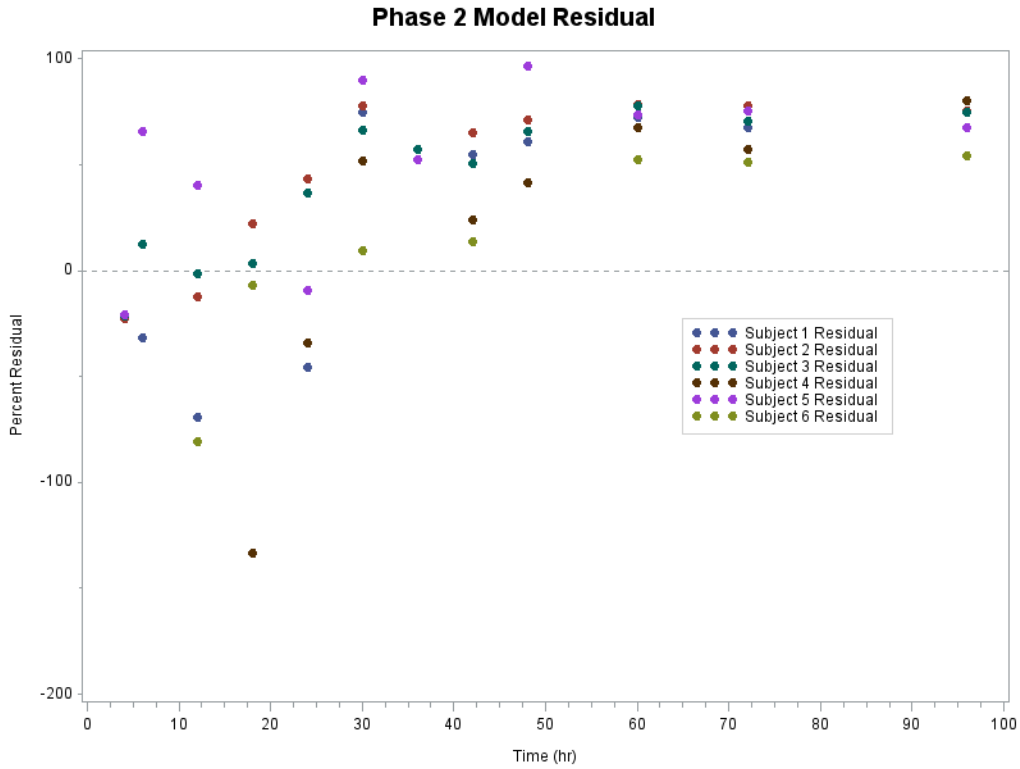

Figure 18: Phase 3 Percent Residual of Model-Predicted Urine DACT

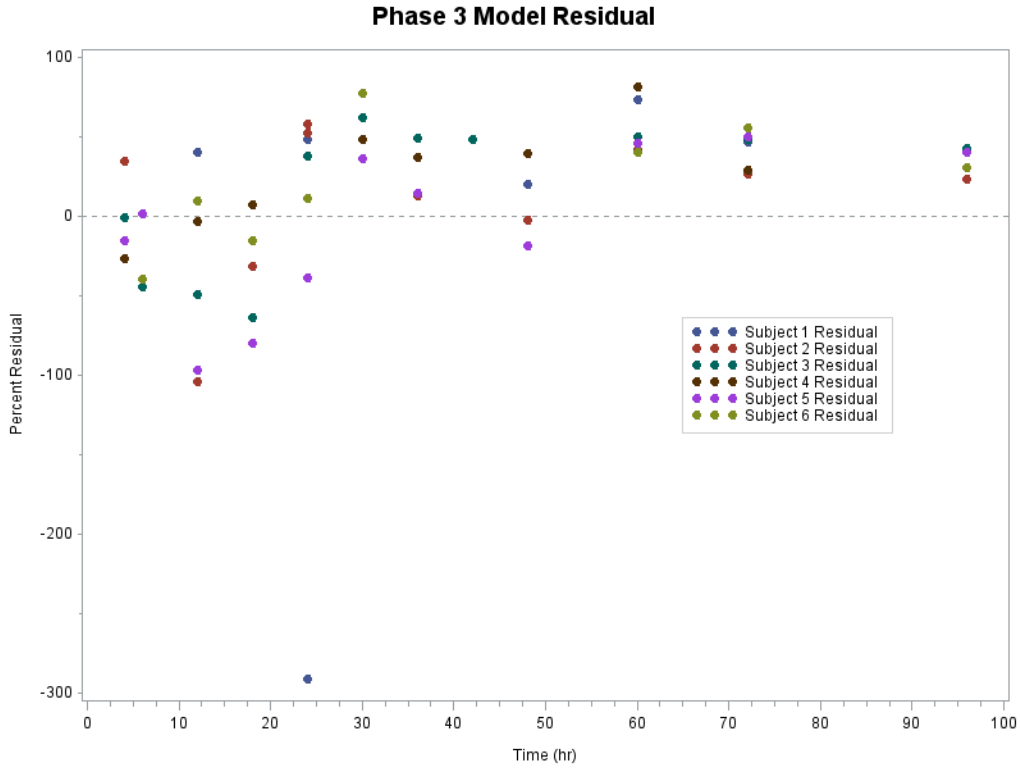

Figure 19: Phase 4 Percent Residual of Model-Predicted Urine DACT

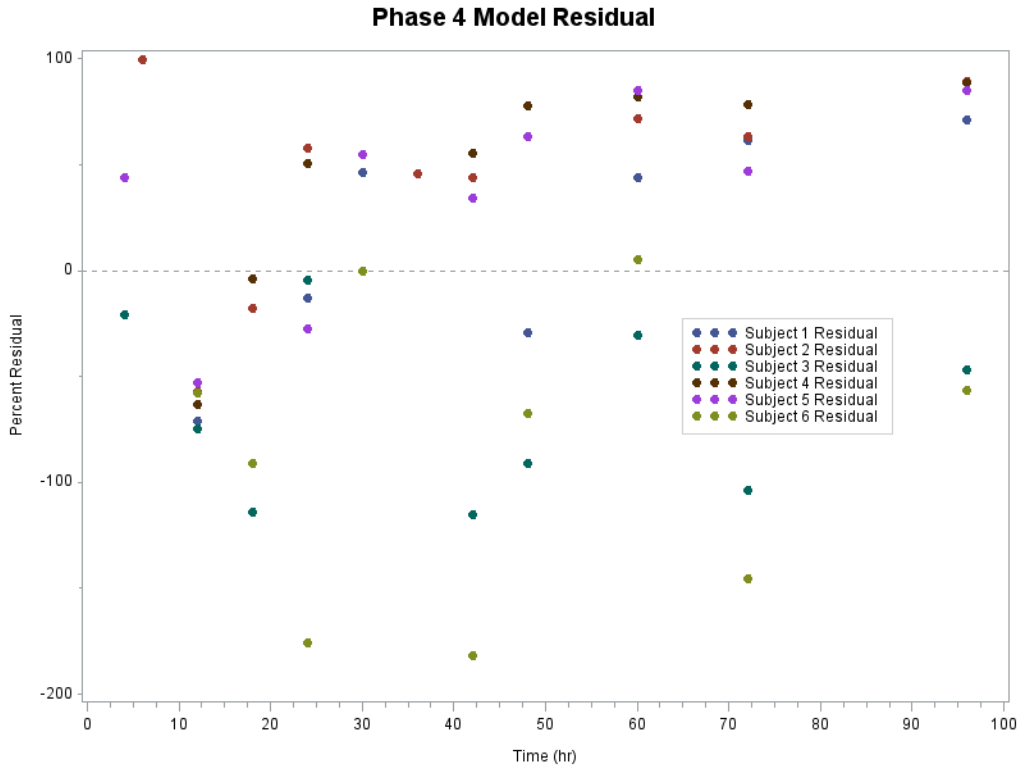

Figure 20: Phase 5 Percent Residual of Model-Predicted Urine DACT

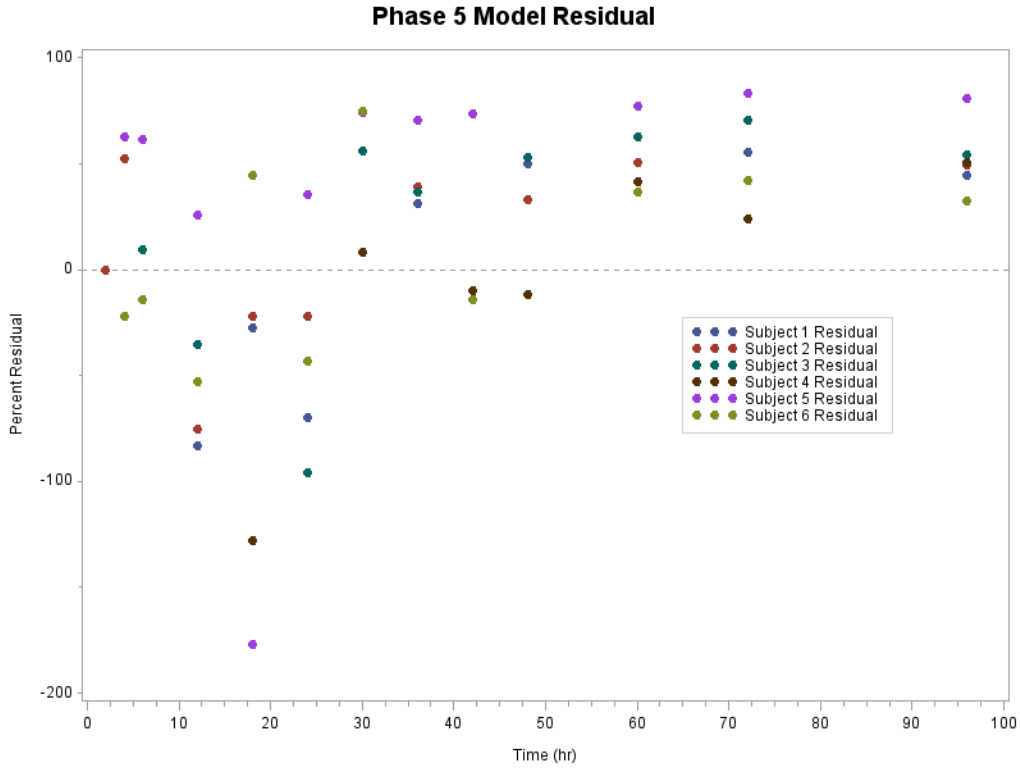

Figure 21: One-way ANOVA Among Subject Serum DACT Elimination Constants

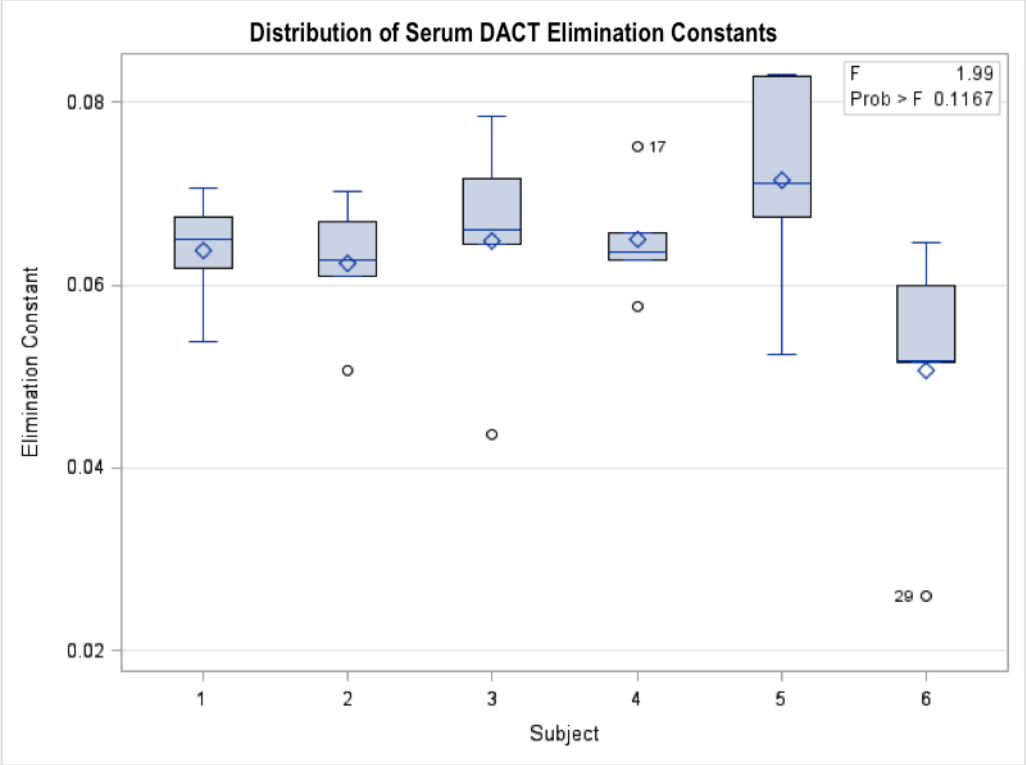

Figure 22: One-way ANOVA Among Subject Urine DACT Elimination Constants

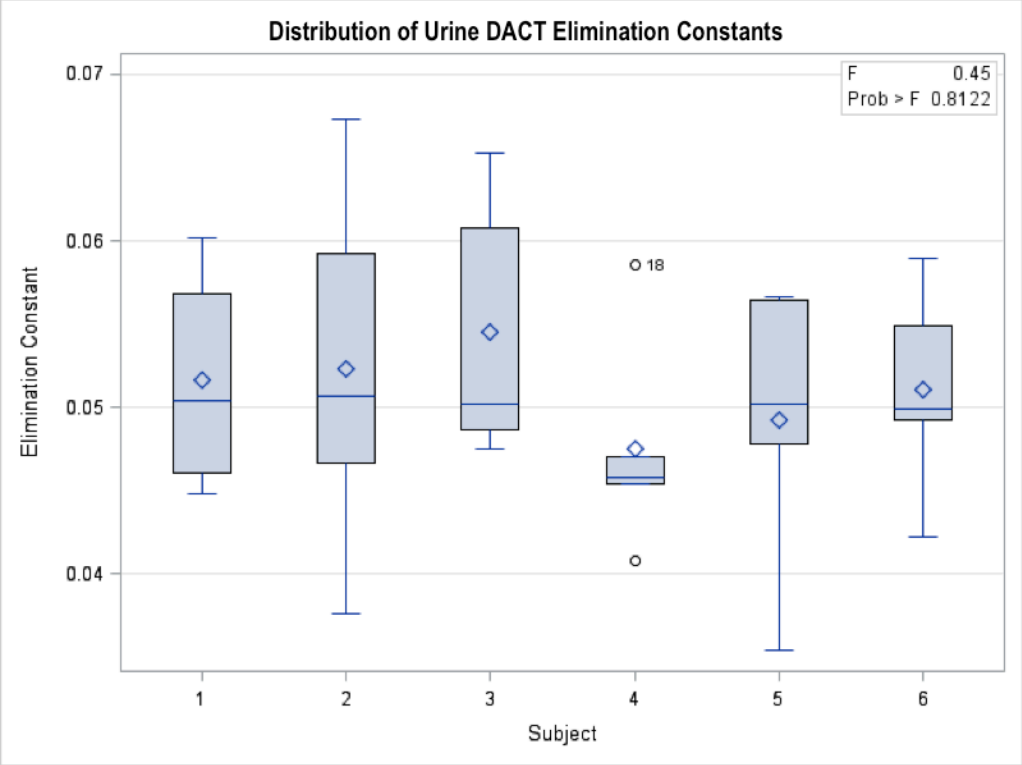

Figure 23: One-way ANOVA Among Phase Serum DACT Elimination Constants

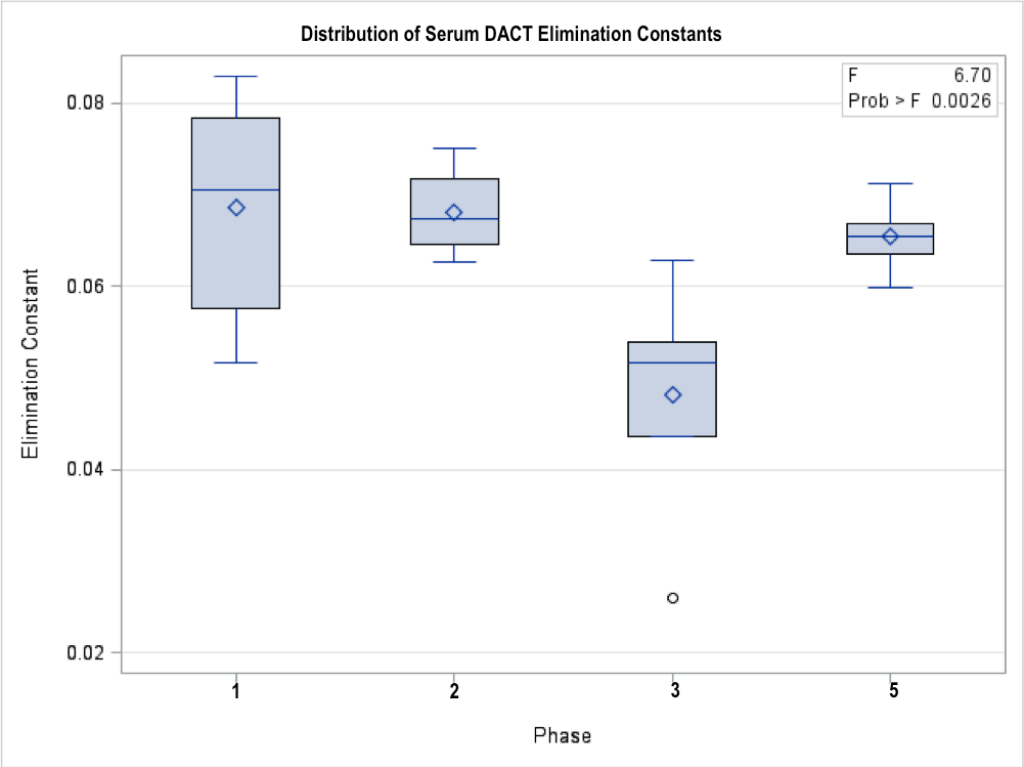

Figure 24: One-way ANOVA Among Phase Urine DACT Elimination Constants

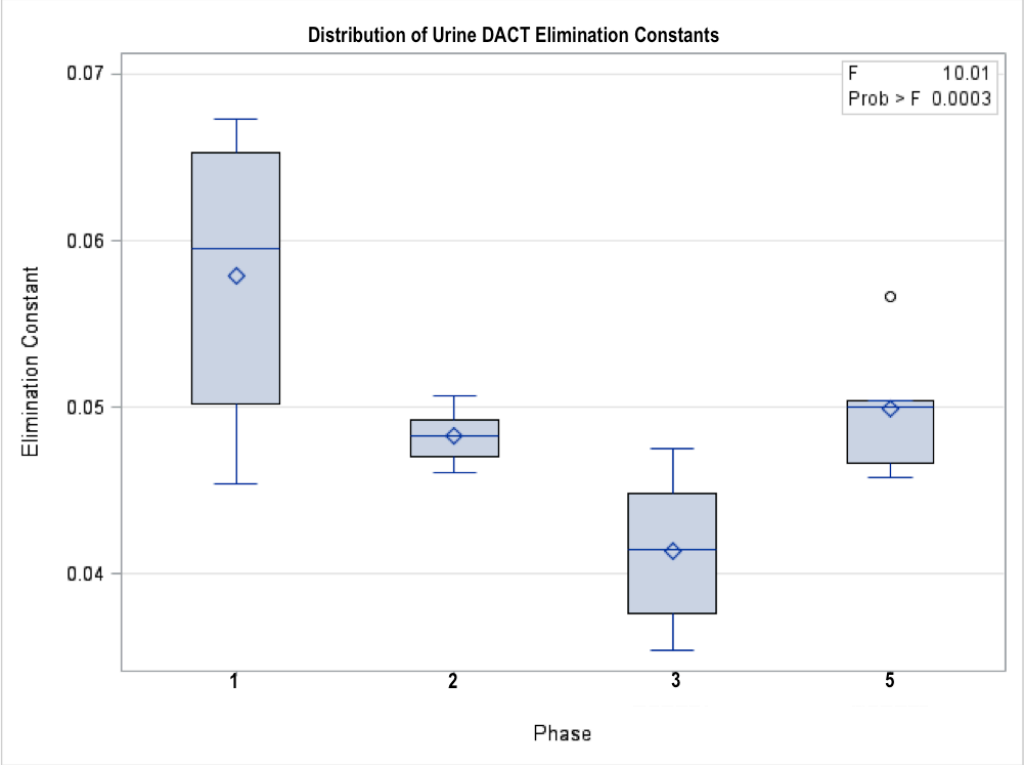

Table 6: Maximum Serum DACT Concentration and Time Points

Maximum Serum DACT Concentration and Time Points							
Phase	Subject	Maximum DACT Conc (ng/mL)	Mean Maximum DACT Conc (ng/mL)	Time of Max DACT Conc (hr)	Mean Time of Max DACT Conc (hr)	Time of LOD (hr)	Mean Time of LOD (hr)
1	1	186.96	358.10	1	6.75	72	78.00
	2	166.49		1		72	
	3	1203.39		36		72	
	4	174.14		0.5		72	
	5	192.96		1		60	
	6	224.68		1		120	
2	1	48.43	53.82	1	0.85	42	47.00
	2	44.56		1		60	
	3	46.14		1		36	
	4	60.50		***0.083***		36	
	5	53.87		1		48	
	6	69.44		1		60	
3	1	666.34	854.11	6	4.33	120	106.00
	2	692.55		6		120	
	3	803.59		6		120	
	4	670.59		1		96	
	5	1247.37		1		120	
	6	1044.20		6		60	
4	1	31.52	25.34	2	2.50	48	48.33
	2	24.18		4		42	
	3	25.81		4		60	
	4	20.50		1		2	
	5	24.35		2		42	
	6	25.71		2		96	
5	1	78.64	98.43	4	1.83	60	58.00
	2	94.50		1		60	
	3	115.51		2		60	
	4	66.89		1		48	
	5	87.72		1		48	
	6	147.34		2		72	

Where time of Max DACT is italicized and bold – ***hr*** – maximum DACT conc occurred at first time point measured

Table 7. Maximum Urine DACT Concentration and Time Points

Maximum Urine DACT Concentration and Time Points							
Phase	Subject	Maximum DACT Conc (ng/mL)	Mean Maximum DACT Conc (ng/mL)	Time of Max DACT Conc (hr)	Mean Time of Max DACT Conc (hr)	Time of LOD (hr)	Mean Time of LOD (hr)
1	1	4857.43	5120.71	***18***	9.33	144	144.00
	2	8369.58		***12***		120	
	3	8040.03		***2***		144	
	4	867.36		***18***		168	
	5	1002.02		***2***		120	
	6	7587.82		***4***		168	
2	1	384.37	558.98	***6***	8.00	96	100.00
	2	594.06		***4***		96	
	3	481.27		***4***		96	
	4	528.62		***18***		96	
	5	339.50		***4***		96	
	6	1026.06		***12***		120	
3	1	11507.31	14208.51	24	10.67	168	168.00
	2	11136.74		12		168	
	3	10024.72		6		168	
	4	14394.24		***4***		168	
	5	4648.72		12		168	
	6	33539.32		***6***		168	
4	1	289.07	358.89	***12***	12.67	96	112.00
	2	399.17		12		96	
	3	257.59		4		120	
	4	493.34		***12***		96	
	5	223.88		12		96	
	6	490.31		24		168	
5	1	1153.53	1148.40	***12***	11.33	120	120.00
	2	775.82		12		120	
	3	926.84		***4***		120	
	4	734.82		***18***		120	
	5	1347.55		***18***		96	
	6	1951.81		***4***		144	

Where time of Max DACT is italicized and bold – ***hr*** – maximum conc occurred at first time point measured

VIII. APPENDICES

Appendix i: List of Tables

Table **Page**

Appendix ii: List of Figures

Figure **Page**