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Understanding the Pharmacokinetics of Atrazine in Cynomolgus Monkeys:
Implications for Human Biomonitoring

By

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By
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University of Georgia
2010

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A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Global Environmental Health
2012

Abstract

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Implications for Human Biomonitoring
By: Elizabeth K. George

Background: Several studies suggest harmful effects caused by exposure to the widely used herbicide, atrazine (ATZ) however, little is known regarding the pharmacokinetics of ATZ.

Purpose: To understand the metabolism and elimination kinetics in an animal model by identifying a biomarker of exposure, determining the elimination half life of ATZ, an ideal serum sample collection time and inter- animal variation of the pharmacokinetics of atrazine.

Methods: Plasma samples were analyzed using a solid-phase extraction-high-performance liquid chromatography–tandem mass spectrometry method with isotope dilution quantification to measure four chlorinated atrazine metabolites in plasma; diaminochloroatrazine [DACT], desethyl atrazine [DEA], desisopropyl atrazine [DIA] and atrazine [ATZ].

Results: The metabolite diaminochloroatrazine [DACT] was identified as an appropriate biological marker for atrazine exposure due to its extended presence in serum as well as the fact that DACT proves to be present in the highest concentration relative to the other metabolites. The half live of DACT was determined to be approximately 17 hrs.

Conclusion: Based on the information determined through this project, a better understanding of the activities of ATZ post exposure will aid in laying a foundation for knowledge regarding the pharmacokinetics of ATZ.

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I. INTRODUCTION

The discovery and development of herbicides first began in the 1950's during a time when the country was faced with the challenge of meeting the increasing agricultural needs of its population (LeBaron, Homer, and McFarland). Over the years, the development of herbicides that consistently and cost-effectively kill both grasses and broad-leaf weeds has enabled farmers to adopt effective farming practices on millions of hectares of land around the world (LeBaron, Homer, and McFarland). Thus, herbicide use had proven extremely beneficial to the agricultural sector of the United States. However, by the 1960's the environmental movement, sparked by the release of Rachel Carson's *Silent Spring,* had begun drawing much attention to the negative effects of pesticides on the environment and human health. The past few decades have been spent assessing the relative safety of many pesticides, including herbicides, that are widely used in the United States and around the world as the battle continues between the economic benefits and health related drawbacks of the application of herbicides. The most prominent class of herbicides in use today is known as the triazine class. The triazine compound is a cyclic structure analogous to the six-membered benzene ring but with three carbons replaced by nitrogens. Among this class of herbicides is the most well known of the triazines, atrazine (ATZ).

Atrazine, (2-chloro-4-ethylamine-6-isopropylamino-s-triazine), a chlorotriazine herbicide, has the structure of the traditional triazine but in addition, a chlorine atom is attached to the primary ring structure (Figure 1). ATZ is widely used and regularly found in the environment with approximately eighty million pounds applied yearly in the United States alone (Lin, Fisher et al. 2011). This is more than any other agricultural herbicide (Sathiakumar, MacLennan et al. 2011). ATZ has been used worldwide primarily for the control of broadleaf and grassy weeds in an effort to increase crop production and for

residential lawn maintenance (Jiang, Adams et al. 2005). Contamination of environmental media by ATZ and its byproducts has been reported in surface waters, ground waters, and soil in North America (Edginton and Rouleau 2005). The mode of action of this compound involves inhibition of photosynthesis (ATSDR). Its widespread use has led to increased concerns due to ATZ's relative persistence in soil and its ability to migrate from the upper soil surface to deeper soil layers and eventually enter groundwater (ATSDR). ATZ and its byproducts are some of the most frequently detected in ground and surface waters (LeBlanc and Sleno 2011). In 2004, the European Union banned the use of ATZ citing its persistence in groundwater. Even so, it is employed in over seventy different countries worldwide. The World Health Organization (WHO) provides some physical and chemical properties of ATZ that are relevant to its environmental fate. These properties are summarized in Table 1.

Previous toxicological animal studies have identified reproductive and developmental abnormalities. These effects include delayed puberty, delayed mammary gland development, disruption of ovarian cycles, suppression of luteinizing hormone surge, neurotoxicity in dopaminergic systems, and developmental immunotoxicity (Dooley, Hanneman et al. 2007). A study performed by Edginton et. al. in 2005 assessing toxicokinetics using ^{14}C-labelled ATZ concluded that although ATZ does not appreciably accumulate in stage-66 X. laevis larvae, elimination of all the metabolites was substantially slower than ATZ, suggesting that, during constant exposure, their accumulation potential is greater than that of ATZ. Authors also included that toxicokinetics of the *X. laevis* frog species has similar behavior to that observed in fish. Due to its physicochemical properties, ATZ is considered stable in the environment so its use can cause damage to biota (Jacomini et al., 2003). According to the results obtained in a study performed by Nogarol et. al. assessing the effects of exposure to ATZ of the *D. expansus* fish species, ATZ presents a dose-dependent response to exposure

in the *D. expansus* fish species. The highest concentrations of this pesticide were able to induce alterations to the gill filaments of the Diplodon expansus fish species found in Brazil. Induced alterations to the respiratory mechanisms of this species can be life threatening to the species itself, and consequently, the surrounding ecosystem. Various other animal model studies have been performed however their implications on human health have been somewhat varied and controversial. There remains a need for more in-depth scientific studies in order to draw a more complete picture of the potential impact of ATZ on human health. For a thorough study of ATZ's toxicology, the formation of its metabolites and the result of their exposure need to be assessed (LeBlanc and Sleno 2011). This study was initially undertaken to quantify a variety of metabolites including both chlorinated and mercapturate metabolites with the eventual goal of establishing a pharmacokinetic model for ATZ. However, based on the current understanding of the metabolism of ATZ, mercapturic metabolites are found predominantly in urine (Chevrier, Limon et al. 2011) while their glutathione adduct form and chlorinated form can be found in serum.

Nevertheless, glutathione derivatives will not be focused on for this particular project. Consequently the focus of this project (which analyzes serum samples) will be on ATZ and its chlorinated metabolites in serum. The chlorinated metabolites include diaminochloroatrazine [DACT], desethyl atrazine [DEA], desisopropyl atrazine [DIA]. Figure 2 demonstrates the currently understood version of the metabolic pathway of ATZ with respect to the various metabolites. This study is performed with the understanding that the non-human primate model provides a solid foundation for knowledge regarding the pharmacokinetics of ATZ in humans.

The objectives met throughout this project will allow the foundation to be laid for a better understanding of how ATZ and its chlorinated metabolites behave in the human

body. Firstly, the best biological marker for ATZ will be identified among the various metabolites mentioned earlier. A biomarker is a characteristic chemical that can be objectively measured and evaluated as an indicator of either exposure or of a response (The Handbook of Biomarkers, Jain). Having an appropriate biomarker to correctly represent an exposure to ATZ will aid in future exposure assessments involving this herbicide. The metabolite whose concentration reaches the highest level in the body can be determined through extractions and subsequent high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) analysis. Consequently, the metabolite that best represents an exposure to ATZ can be identified.

In addition, the elimination half-life of ATZ and its metabolites will be determined based on the plasma concentration time profiles of each of the metabolites. By assessing the period of time in which ATZ and each of its metabolites are reduced to half the original concentration (assuming first order kinetics), one can begin to gain a better understanding of how long the herbicide resides in the body and the amount of time in which it is able to take its effect. Previous animal model studies performed by Edginton et. al. have shown a half life of forty eight minutes for the parent compound of ATZ and a half life of seventy two hours for the metabolite compound DIA in the Xenopus laevis larvae, however, these organisms are known to have a high metabolic capacity. According to the WHO after oral administration to rats, ^{14}C-labelled ATZ was rapidly and almost completely absorbed, independent of dose and sex. The elimination half-life of radiolabeled ATZ from the whole body was 31.3 h in rats however rat metabolism of ATZ varies from humans due to binding of ATZ to cysteine sulfhydryl groups in rodent hemoglobin. A study similar to the current study was done to assess the oxidative metabolism of atrazine. This particular study, however, evaluated absorption in rats. The study, undertaken by McMullin et. al., assessed

atrazine and metabolites including DACT through physiologically based pharmacokinetic (PBPK) models. Authors found that "the kinetics of the [chlorinated] compounds were found to be controlled by complex absorption processes, extensive oxidative metabolism in the liver and presumably intestines" (McMullin, Hanneman et al. 2007). This theory helped to explain the rapid clearance of ATZ and the extended presence of DACT in the system. Although animal studies such as this help one to gain a basic understanding of the activities of ATZ in the body, a better assessment is needed. Employing a model that can be extrapolated more accurately to humans should be performed. The non-human primate model used in this project can be used as a better tool for a variety of reasons particularly, "the close phylogenic relationship to humans, and greater similarities with regard to anatomy, physiology, biochemistry and organ systems, as compared to rodents" (Takayama, Thorgeirsson et al. 2008). In addition to assessing the elimination half-life, the objective to determine the ideal time to collect serum samples after exposure to ATZ will also be achieved. By building concentration-time curves of each metabolite of ATZ, one can identify the time points in which each individual metabolite peaks. Thus, an ideal collection time can be determined in conjunction with building these pharmacokinetic curves. The future goal will be to use the knowledge gained from this study when assessing ATZ exposure in humans.

II. METHODS

a. Experimental Subjects and Dosing Scheme

The experimental subjects involved in this project are a group of six female cynomolgus monkeys of varying ages. The monkey colony consists of one species, *Macaca fascicularis* (cynomolgus monkey). The project involves three different phases, which varied based on the vehicle of administration or target dose level. A total of four compounds were evaluated; ATZ, DEA, DIA, and DACT. Phase 1 involved the administration of the test compound, ATZ, to all six monkeys with equal target dose levels of 0.5 mg/kg through 1% carboxymethylcellulose (CMC) in water with a total of 114 plasma samples collected. The vehicle for Phase 2 administrations was a 5% ethanol (EtOH) in water solution with an identical target dose level as Phase 1 with a total of 113 samples collected. Phase 3 had an identical vehicle of administration as Phase 1, 1% CMC to water, however the target dose level was increased five fold from 0.5 mg/kg to 2.5 mg/kg with 113 samples collected after dosing. The dosing scheme for all three phases is summarized in Table 2.

b. Sample Collection

Plasma sample collection time points for all three phases were relatively consistent however certain time points were missing throughout the phases for various monkeys. Each phase contained an ample number of time points to build an accurate plasma concentration time profile for each individual monkey. Each phase followed the usual pattern, with slight variations, of sample collection at the following time points (hr); 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 spanning a total of seven days.

c. Sample Preparation and Analysis

During sample preparation, a 200-μL aliquot of plasma sample was mixed with isotopically labeled internal standards prior to protein precipitation with 1mL of methanol.

Sample was then vortexed and centrifuged at 1,200 rpm for 15 minutes. The supernatant was collected and dried using a TurboVap LV Evaporator. Water temperature was set to 45 ºC and nitrogen pressure to 18 psi. Dried residue was reconstituted with 2mL of 2% formic acid in deionized water and loaded onto a Strata X-C solid phase extraction cartridge (cation exchange) that had been conditioned with methanol and water, respectively. Cartridge was washed with 1mL of 2% formic acid in 80:20v/v water:methanol twice. Vacuum was used to dry the cartridge before elution with 2mL of 2% ammonium hydroxide in methanol. Elution was then repeated with an additional 2mL of the same solvent. The eluate was then brought to dryness and reconstituted with 100 uL of 0.1% formic acid in deionized water right before the injection. Included in each run was a full 9-pt calibration set per analytic run (0.5-200 ng/mL).

The chromatographic separation of ATZ and its metabolites was performed on an Agilent 1200 system (Agilent Tech., Waldbronn, Germany) consisting of a binary pump, degasser, an auto sampler, and a temperature-stable column compartment. All the HPLC modules were programmed and controlled using Mass Hunter Workstation software version B.03.01 SP2 (Agilent Tech., Waldbronn, Germany). The analytical column was C6-Phenyl (100x4.6mm, 3 µm particle size, 110-0A pore size, Phenomenex, CA, USA) and placed in column compartment that set the temperature for 45 °C during the analysis. The gradient elution using 0.2% formic acid in deionized water and 0.2% formic acid in methanol was used for the optimum separation. The total run time was approximately eighteen minutes. An HPLC-MS/MS (Agilent Tech., Waldbronn, Germany) equipped with a positive mode jet-stream electrospray chemical ionization (J-ESI) interface was used to perform sample analysis. Target analytes were optimized to achieve their highest intensity. Multiple segments containing specific multiple reaction monitoring experiments were created and used during

MS/MS acquisition. Mass spectrometer was programmed and controlled Mass Hunter Workstation software (Agilent Tech., Waldbronn, Germany).

d. Data Analysis

Plasma concentration time profiles for ATZ and its metabolites were built through available software using information on dosing scheme, average concentrations of ATZ and its metabolites found in plasma of Cynomologous monkeys, and time fashion records. Results were used to identify a biomarker that best represents exposure to ATZ herbicide as well as calculate the biological half-life of the biomarker of choice. Half-lives were calculated using the slope of the terminal log-linear portion of the plasma concentrations. Results were also used to determine the ideal time frame to collect plasma samples. These results can then be used as a basis for human biomonitoring following ATZ exposure.

III. RESULTS

A total of 340 plasma samples were extracted and analyzed throughout all three phases. From Phase 1, 114 samples, and 113 samples from both Phase 2 and Phase 3 were extracted and analyzed. Samples were taken from dosed Cynomolgous monkeys at time points spanning seven days. Time points in day 1 include 5 min., 10 min., 15 min., 1 hr., 6 hr., 12 hr., 18 hr. and 24 hr. Time points in day 2 include 30 hr., 42 hr. and 48 hr. Time points in day 3 include 60 hr. and 72 hr. Time points in days 4, 5, 6 and 7 are as follows respectively; 96, 120, 144, and 168 hrs. Chromatographic separation in plasma sample was performed as shown in Figure 3.

a. Identify a biomarker

An elevation of any of the chlorinated metabolites of atrazine; DACT, DEA, DIA and ATZ, can be used as a biological marker of exposure to atrazine. Plotting the extracted concentration of each metabolite from the serum samples included in Phase 1, 2 and 3 allows one to visually identify the metabolite that peaks at the highest concentration in all three phases. One can also identify the metabolite that is consistently at a higher concentration throughout the sample collection period. This metabolite will prove to be the best biological marker of atrazine exposure because it will be the easiest to detect in serum post-exposure. Through examination of concentration vs. time plots of all metabolites of interest, it is clear that DACT proves to be the best biomarker of exposure for atrazine in plasma. Figure 4 shows the built plasma concentration time profiles for ATZ from Phases 1, 2 and 3 with DACT representing the highest peak in all three phases. It is clear that of the four chlorinated metabolites present in serum post-exposure, DACT comprises the predominant proportion as compared to DEA, DIA and ATZ and is consistently more prominent relative to the other metabolites throughout the sample collection period.

b. Determine the elimination half-life

Phase 2 varied from Phase 1 and 3 by the vehicle of administration. As mentioned earlier Phase 2 was administered with 5% EtOH in water while Phase 1 and 3 are administered with 1% CMC in water. Due to the fact that human exposure to ATZ in conjunction with ethanol is unlikely, Phase 2 data were not taken in to consideration when calculating the half-life of ATZ, DEA, DIA and DACT. It was also hypothesized that due to an identical vehicle of administration, the half-life found in Phase 1 and 3 should be relatively similar. For both Phase 1 and 3, concentrations for each time point, for each

metabolite, were averaged for each monkey. The log of the concentrations of DACT extracted at each time point was graphed against the time points and the slope was found. A half-life for each metabolite was calculated for both phases. Calculated half lives between Phase 1 and 3 were similar, as expected. The half-life for the metabolite of interest, DACT was found to be 17.7 hrs for Phase 1 and 16.5 hrs for Phase 3. The half-lives for the remaining metabolites for Phase 1 and 3 (ATZ, DEA and DIA) can be found in Table 3.

c. Determine ideal serum sample collection time

If the level of exposure during biological monitoring is known, the half life can be used to extrapolate how much of the target metabolite will remain in the body over time. However this information surrounding exposure is not always known when samples are being collected. For situations such as these where the level of exposure is unknown, it would be prudent to collect a serum sample at a time where one can be sure that at least half of the initial concentration of the metabolite remains. In order to do this, collection must occur within the first half-life. Because DACT provides the longest window of collection time as well as the highest concentration relative to the other chlorinated metabolites, it proves to be the best biomarker for exposure to atrazine in serum. Therefore, the half-life of DACT will be used as a reference to reflect the parent compound during biomonitoring. In order to collect a sample that can reflect exposure, a sample must be taken within the first 16 to 18 hours following exposure. This range reflects the half-life of DACT, which can be referenced in Table 3.

d. Identify inter- animal variation

In determining typical measures of pharmacokinetics such as half-life, it is important to assess any variation that may occur between experimental subjects. For Phase 1, the range

of calculated half-lives of DACT was 9.71-22.80 for all six monkey. For Phase 3 the range of calculated half-lives of DACT was 10.64-13.62. These ranges reflect the variation among Monkey 1 through Monkey 6 regarding the half-life of DACT from the point of administration to the last collection time point. However, the average for each phase is quite similar as seen in Table 3 showing consistency when factoring in different dosing schemes.

Figure 5 shows the plotted concentration of DACT for each time point, averaged between all six monkeys, for each phase. The peak for each phase in indicated in red in each graph. When comparing the peak concentrations for each phase (Phase 1: 178.86 ng/mL ± 28.77, Phase 2: 50.98 ng/mL ± 9.77 and Phase 3: 776.66 ng/mL ± 164.80) it can be seen that the variation is approximately the same across each dosing scheme. This pattern of the inter-animal variation seen with DACT follows first-order elimination kinetics. The assumption of first order kinetics has been assumed throughout this study. When examining the plotted concentrations, although variability is seen for each time point represented by the error bars, the peak point and elimination pattern is the same for each monkey. Figure 6 shows the plasma concentration of DACT at three distinct time points (1-hr, 6-hr and 12-hr) across all six monkeys.

IV. DISCUSSION

a. *Major findings*

The results of this study help to lay a better foundation regarding the way in which ATZ behaves once it enters the body. The results confirm the idea that DACT is the primary metabolite found in serum following ATZ exposure. A variety of studies have shown this to be the case. These results allow for DACT to be employed as a biological

marker for ATZ exposure in serum and have been seen in previous animal studies involving Sprague-Dawley rats. It is important that these results can be replicated in a model more similar to a human such as a monkey model. This study has begun to fill this knowledge gap.

A biomarker can be broadly defined as a measurable change in a biological system that is caused by exposure to an exogenous chemical (Metcalf and Orloff, 2004). Biomarkers are often divided into three categories: biomarkers of exposure, effect, and susceptibility (NRC, 1987). A schematic of the three different types of biomarker and their role in environmental/biological monitoring can be seen in Figure 7 (Bernard, 2008). DACT was identified as suitable biological marker of ATZ exposure in serum due to its ability to meet a variety of criteria. According to Metcalf and Orloff, ideally, a biomarker should be sensitive, specific, biologically relevant, and practical among other factors. The particular method of solid-phase extraction-high-performance liquid chromatography–tandem mass spectrometry employed in this study has proven to be a sufficient method of detecting DACT in plasma, making this method sensitive to the biomarker of choice. In addition, based on the accepted metabolic pathway of ATZ, as seen in Figure 2, as well as the similar structural makeup of DACT to the parent compound as seen in Figure 1a and 1b, DACT is capable of representing exposure to ATZ. This fact also makes DACT specific to ATZ because it is neither produced endogenously nor is it formed after exposure to other compounds.

In addition to sensitivity and specificity, the biomarker for ATZ should be also be biologically relevant to the exposure-disease continuum seen in Figure 6. Although DACT has not been shown to directly affect target organs and tissue adversely, DACT represents exposure to a parent compound, which can lead to an endpoint of altered structure or function and even clinical disease as previously mentioned. Therefore, discovery of DACT in plasma can allow for the opportunity to halt this continuum and play a role in a potential

therapeutic or preventive strategy.

Practicality, the last of the mentioned criterion, may be the most important. Although it is vital that the biomarker that is chosen is sensitive to the contaminant after exposure, selective for the target toxicant, and biologically relevant to human health, these factors can become secondary if the biological sample necessary for analysis is not readily available. According to Metcalf and Orloff, this requirement usually limits biological monitoring to easily obtained samples, such as urine or blood. Relative to other matrices such as adipose tissue, placenta or meconium, blood is much more readily available. DACT has been successfully detected in blood throughout this study.

Identifying a biomarker of ATZ in plasma was a distinct objective of the study, however, to continue to study the pharmacokinetics of a toxicant, one must determine the duration at which this toxicant and its metabolites are present in the body. This will then set the stage for the fate of the toxicant as well as its effect on health. Because herbicides are toxic by design, it is important to understand how long ATZ and its main metabolites are present in the system. In the current study, it was determined that the half-life of DACT in plasma is 17.08 hours or approximately 0.7 day (averaged between Phase 1 and 3). Because ATZ is an herbicide that is used quite often in the agricultural sector, it is critical to note that exposure may occur on a regular basis for those who are applying the chemical. Therefore, levels of ATZ, and its metabolite DACT, will reach a steady state from low-level residue exposure. Awareness of DACT's presence in plasma close to 18 hours post exposure would allow those who are biomonitoring a larger window of time to collect samples (i.e. evening collection following morning exposure). This fact relates directly to the next objective in the present study; determining ideal serum sample collection time.

As mentioned earlier, if the level of exposure for the study population is know, it would be fairly easy to extrapolate how much of the target metabolite, in this case DACT, would remain in the body after a given period of time. However, attaining these specific details of exposure may prove difficult and potentially unreliable when dealing with a large study population, particularly while taking in to account multiple factors such as direct exposure versus secondary exposure, use of personal protective equipment etc. It is in situations such as these, knowledge of a target metabolite's half-life can be useful and sample collection can occur accordingly.

The last objective of the study was to determine inter-animal variation of DACT among experimental subjects (six, female, cynomolgus monkeys). Understanding the inter-subject variability of ATZ pharmacokinetics will aid in developing therapeutic strategies and well as policies that can be applied widely regarding exposures. If extensive pharmacokinetic variability among experimental subjects did exist, it would be difficult to assess toxicity across a large population. In addition, developing a treatment strategy that was similar in efficacy for all those who have been exposed would be complicated. Because this study is the first of its kind in terms of looking at atrazine pharmacokinetics in a monkey model, no concrete assumptions can be drawn when assessing pharmacokinetic variability due to the lack of a comparison study or previous knowledge, therefore at this time, data will simply be presented for future elaboration.

b. Limitations

Because this study was done using a small sample group of six monkeys, the results may not be generalizable. However, this study was carried out for the purpose of understanding the way in which ATZ breaks down in the blood and to gather baseline data

on the pharmacokinetics of ATZ. Therefore, the sample group size is sufficient for this goal. In addition, because this study is novel in its use of ATZ and a monkey model, the variability data presented currently also has no baseline comparison information; therefore this data will become significant following future studies. This study assumes first order kinetics; a one-compartment model and all calculations are based on a single-dosing scheme. These assumptions can be considered a limitation however they are based on the results from a previous study done on a rat model when studying the pharmacokinetics of ATZ. According to McMullin et. al., plasma time-course concentrations of DACT after dosing…followed first-order absorption and elimination kinetics. The differences between a rat and monkey model and the pharmacokinetic variability between both species should be taken in to consideration. This study aims to present preliminary data in the hope that further research will be done to elaborate on the data presented.

V. CONCLUSION AND RECOMENDATIONS

Based on the results of this study, DACT, a chlorinated metabolite of ATZ, has been identified as biomarker of exposure in serum. In addition, a half-life of approximately 17.08 hours has been calculated for DACT. This half-life can serve as a reference time frame when collecting serum samples during human biological monitoring of ATZ exposure. Data has been presented regarding pharmacokinetic variability among the experimental subjects, however further research should be done to fully understand the consequences of this variability. Currently, the pharmacokinetics of atrazine is being studied in greater detail employing physiologically based pharmacokinetic (PBPK) modeling in a different laboratory setting in the United States. Those results will further build upon the foundation set by this

study regarding how ATZ is processed through the body. As knowledge of ATZ grows, the United States Environmental Protection Agency continues to evaluate the safety of one of the most widely used agricultural herbicides in the country. EPA concluded its last evaluation of ATZ in 2003, however, given the significant new body of scientific information as well as the documented presence of atrazine in both drinking water sources and other bodies of water, further evaluation is has been deemed necessary. On June 12th-15th of 2012, there will be a 4-day meeting of the Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel (FIFRA SAP) to consider and review the reassessment of ecological risks from the use of ATZ. Due to the continued assessment of the safety of ATZ use, it is important that studies such as this one are carried out to gain baseline knowledge on this commonly used herbicide.

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VII. TABLES AND FIGURES

Fig. 1a: Structure of Atrazine

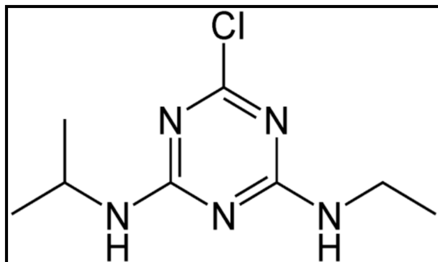

Fig. 1b: Structure of DACT

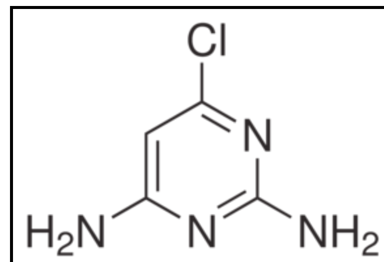

Fig. 2: Atrazine Metabolism

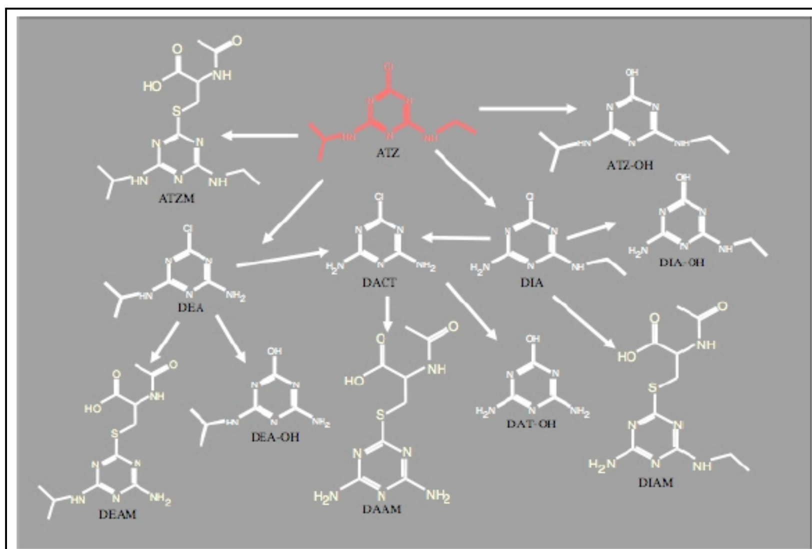

Fig. 3: Plasma Sample Chromatographic Separation

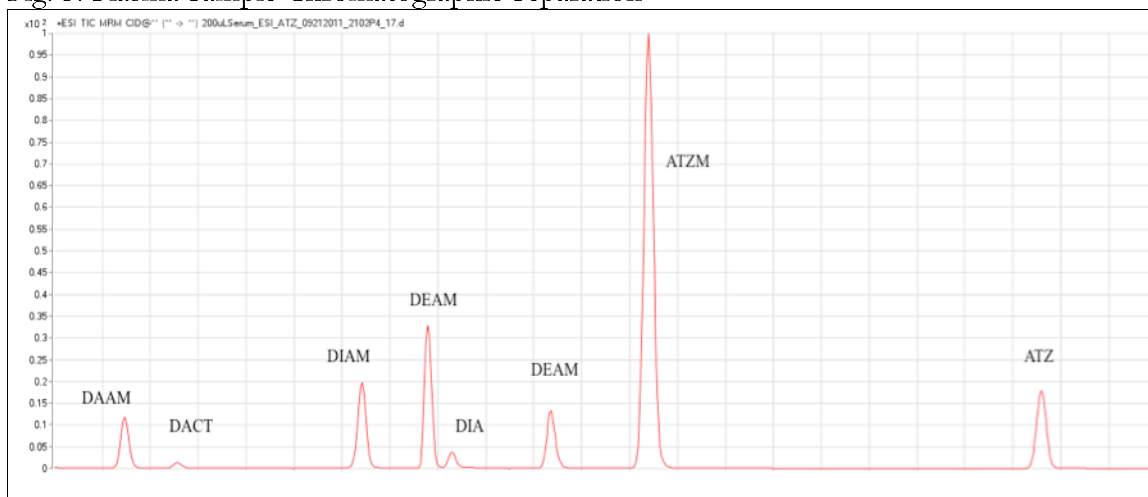

Fig. 4: Average Plasma concentration time profiles for Phases 1, 2 and 3.

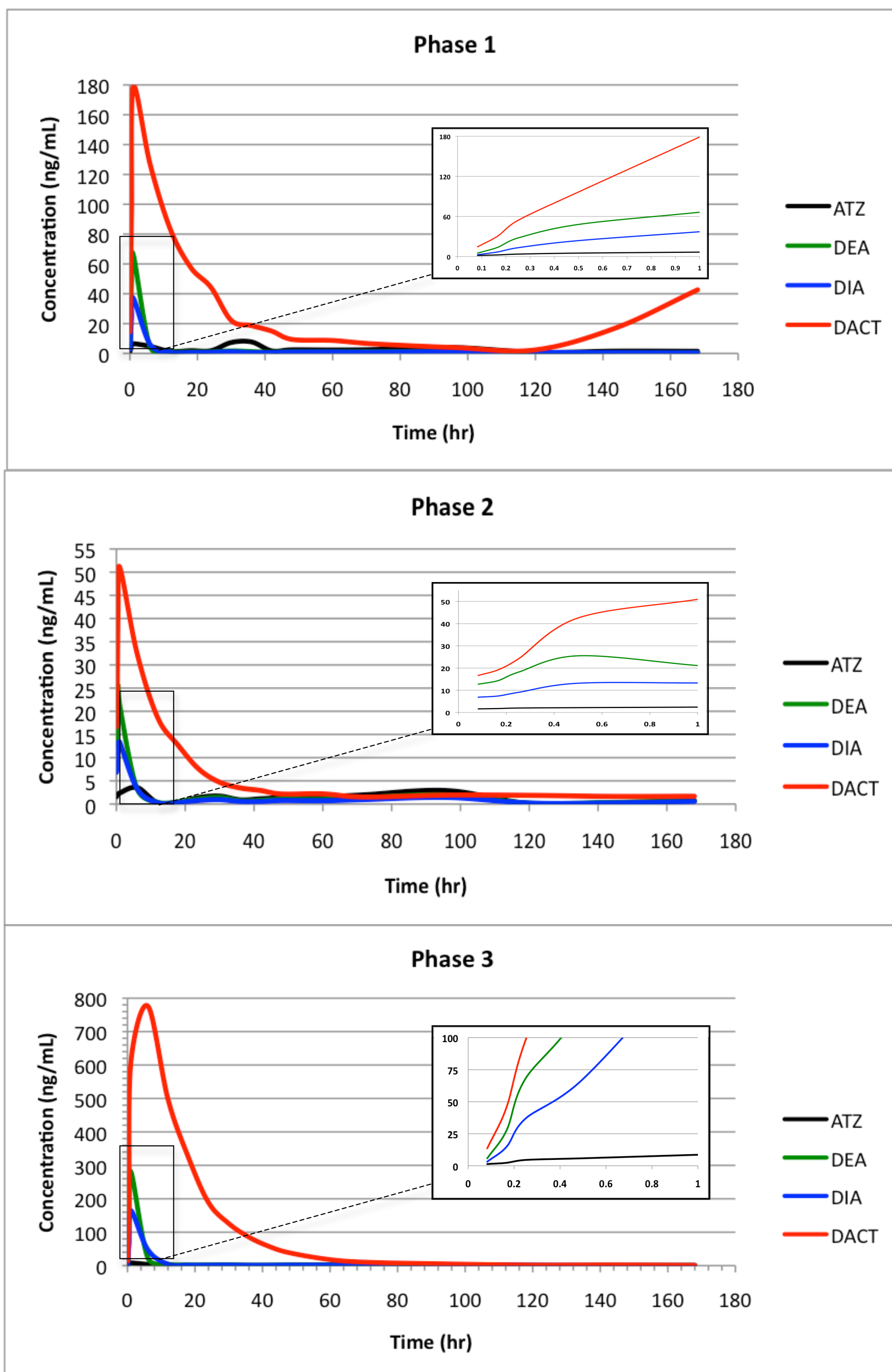

Figure 5: DACT Variability

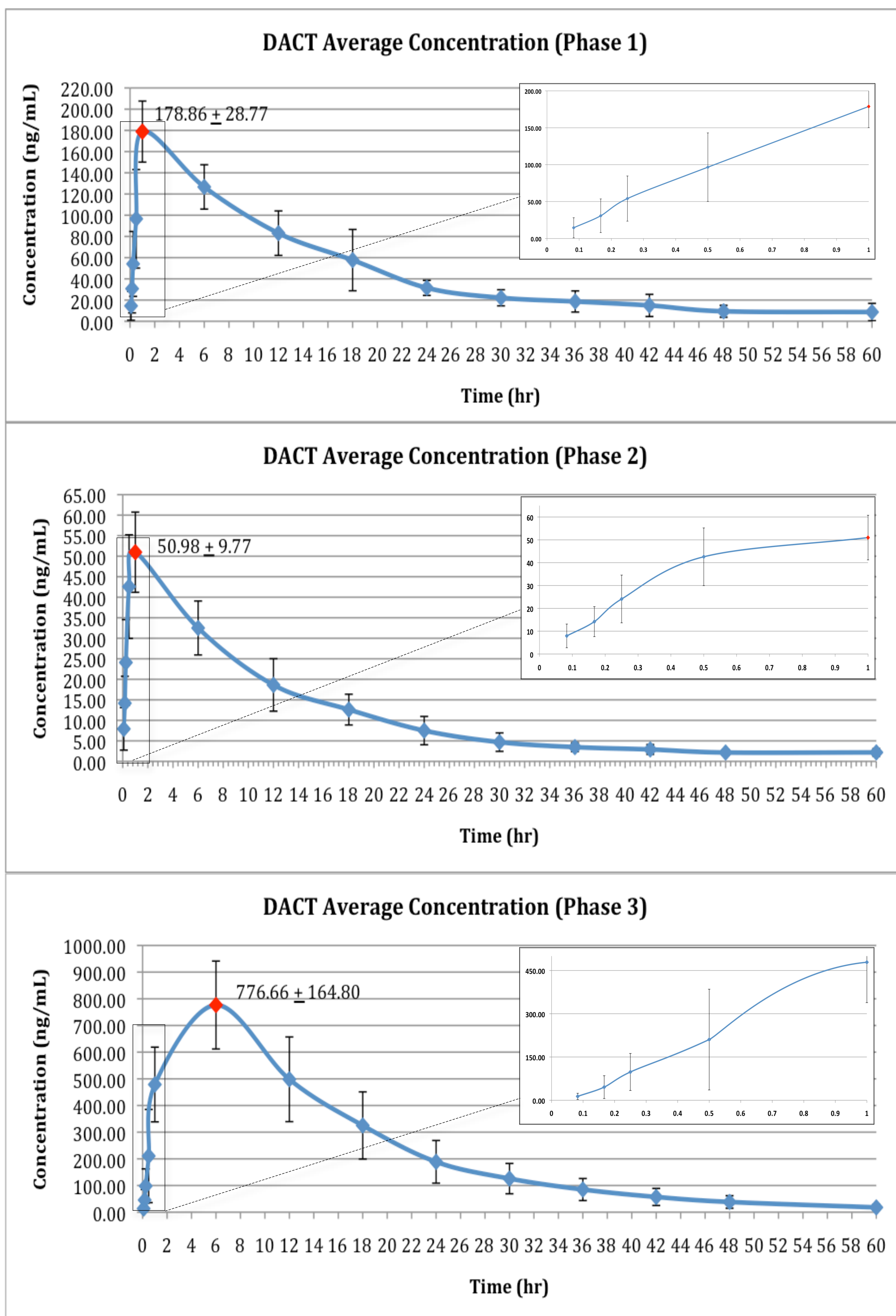

Figure 6: Inter-animal Variation

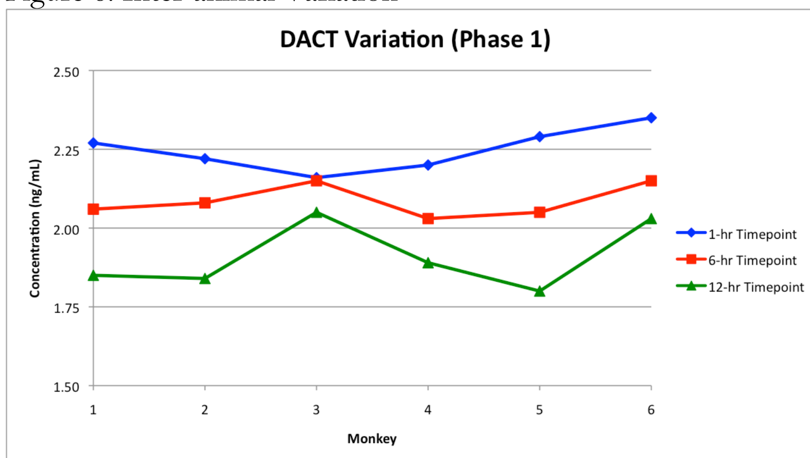

Figure 7: Type of Biomarkers

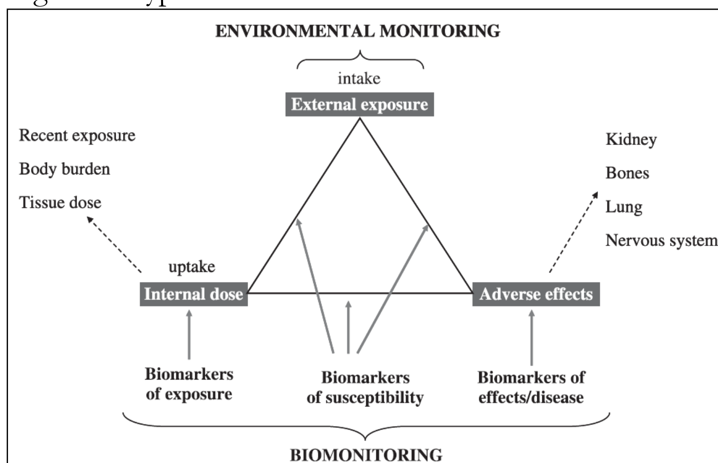

Figure 8: Exposure-disease Continuum

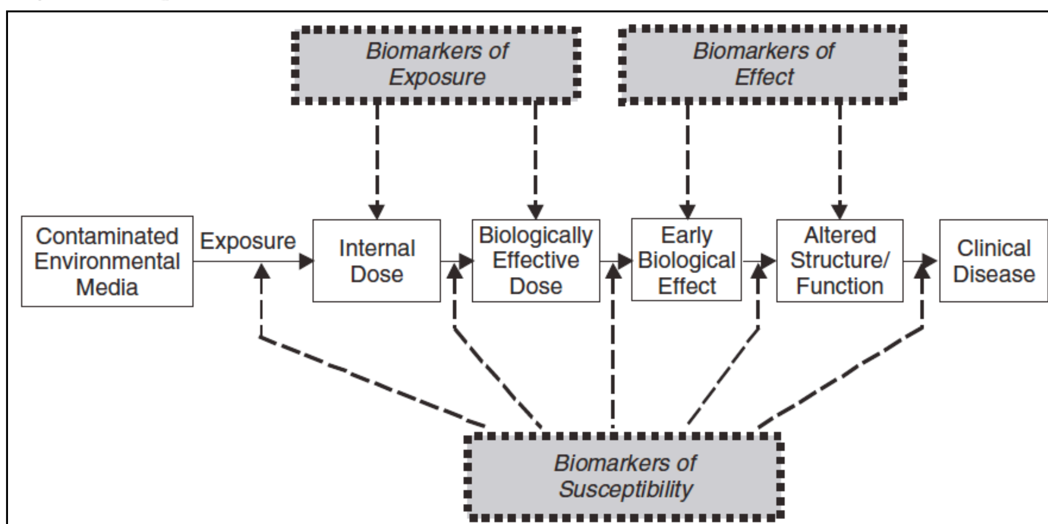

Table 1: Physical and Chemical Properties of Atrazine

Property	Value
Melting point	175–177 °C
Density	1.187 g/cm^3 at 20 °C
Water solubility	30 mg/l at 20 °C
Log octanol–water partition coefficient	2.3
Vapour pressure	40×10^{-6} Pa at 20 °C

[a] From Meister (1989); Royal Society of Chemistry (1991); Worthing (1991).

Table 2: Monkey Dosing Scheme

Phase	# of Female Animals	Test Article	Vehicle	Dose Route	Target Dose Level (mg/kg)
1	6	Atrazine	1% CMC	Oral	0.5
2	6	Atrazine	5% EtOH	Oral	0.125
3	6	Atrazine	1% CMC	Oral	2.5

CMC Carboxymethylcellulose (1%, CMC:water, w/v)
EtOH Ethanol (5%, EtOH:water, v:v)

Table 3: Half Lives for ATZ, DEA, DIA and DACT (Phase 1 and 3)

	Half-Life (hr)		
Metabolite	**Phase 1**	**Phase 3**	**Average**
ATZ	1.77	4.80	3.29
DEA	6.62	6.40	6.51
DIA	1.83	2.99	2.41
DACT	17.71	16.45	17.08