TOXICOKINETICS OF DELTAMETHRIN IN RATS: VEHICLE, DOSAGE, AND AGE DEPENDENCY

by

CHEN CHEN

(Under the Direction of JAMES V. BRUCKNER)

ABSTRACT

Deltamethrin (DLM) is a pyrethroid pesticide. The objective of this study was to characterize the toxicokinetics (TK) of DLM in rats. Serial blood samples were taken from adult rats following oral/i.v. administration. Serial sacrifice was performed on rats in three age groups. Plasma and tissue samples were analyzed for DLM concentration. The time-course data were analyzed to estimate TK parameters. Absorption of DLM was protracted and incomplete. The vehicle influenced the plasma TK profile. Plasma time-courses of different dosages paralleled one another, with TK generally linear over the evaluated range, typically exhibiting a progressive increase, a plateau, and a slow terminal elimination phase. Age was inversely related to the extent of systemic exposure given the same dosage. Brain concentrations were lower than plasma levels. Plasma and tissue profiles showed inconsistency among the age groups. This indicates age-difference of DLM kinetics, which may be important in risk assessments.

INDEX WORDS: Adult Rats, Deltamethrin, GC-MS, I.V. Dosing, Juvenile Rats, Oral Dosing, Tissue Distribution, Toxicokinetics, Weanling Rats

TOXICOKINETICS OF DELTAMETHRIN IN RATS: VECHILE, DOSAGE, AND AGE DEPENDENCY.

by

CHEN CHEN

B.M. Peking University, China, 2011

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2015

TOXICOKINETICS OF DELTAMETHRIN IN RATS: VEHICLE, DOSAGE, AND AGE DEPENDENCY

by

CHEN CHEN

Major Professor: JAMES V. BRUCKNER

Committee: CATHERINE A. WHITE
RANDALL L. TACKETT

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
August 2015

DEDICATION

Dedicate to my family, especially to my grandmother, Dianhua, my mother, Hua, and my wife, Yan.

ACKNOWLEDGEMENTS

I cordially thank my major advisor, Dr. James Bruckner, for his self-giving guidance during these years of my graduate study. It was an unforgettable period in my life working in his lab on this project. This will have a great impact on my future career. I would also give my gratitude to Dr. Catherine White and Dr. Randall Tackett for giving me great suggestions and guidance on toxicokinetics and physiology. My special thanks go to Mr. Srinivasa Muralidhara, for his kindly assistance in all the animal experiments. I also sincerely appreciate all my colleagues in the lab, Dr. Darren Gullick, Dr. Jing Pang, Mr. Manoj Amaraneni, and Mr. Tanzir Mortuza, for supporting my research and providing a friendly atmosphere.

I am grateful to my parents and parents-in-law, who have given unconditional assistance from family. Thanks to my wife, Yan, who always loves me and inspires me for both working and life. Without her, nothing would have come true. Thanks to our son, Theodore, who gives us happiness and teaches us how to love.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

1.1. Pyrethroids

Pyrethroids, synthetic derivatives of naturally-occurring pyrethrins, are a major class of insecticide. Utilization of pyrethroids has increased dramatically over the last decade in the U.S., Canada, and the European Union, due to the restrictions on organophosphates (Power and Sudakin, 2007; Williams et al., 2008). Pyrethroids are applied to a variety of crops, livestock, stored foods, as well as residential and commercial settings. Permethrin, the most commonly-used insecticide in the U.S., is even prescribed for scabies, mites and lice infestations of pets and humans. The majority of the general population is exposed to pyrethroids, albeit at quite low levels (Barr et al., 2010; Fortin et al., 2008; Heudorf et al., 2004). The chemicals are found in minute amounts in some fruits, leafy vegetables and grains (Lu et al., 2010). Thus, data from low-level exposure studies will be of greatest utility in the health risk assessments currently being conducted by the U.S. EPA (2014).

All pyrethroids share some common physiochemical properties. They are all insoluble in water but soluble in organic solvents. Pyrethroids are traditionally divided into two types based on the structure. Type I pyrethroids do not have a cyano group, and the most common signs of toxicity in mammalian species are tremors and skin parathesias (T syndrome). Type II pyrethoids do have a cyano group, and the most common signs of toxicity in mammalian species are choreoathetosis and salivations (CS syndrome) (Lawrence et al., 1982; Ray et al., 2000). The basic mechanism of pyrethroids' toxicity is interference with nerve membrane voltage-sensitive

sodium channels in the central nervous system (CNS), leading to prolonged depolarization or depolarization block resulting in nerve inexcitability (Narahashi, 1996).

1.2. Deltamethrin (DLM)

Deltamethrin (DLM), [(S)-α-cyano-3-phenoxybenzyl – (1R, cis) -2, 2-dimethyl-3-(2, 2-dibromovinyl) – cyclopropanecarboxylate] (Figure 1), is classified as a type II pyrethroid, which contains an α-cyano moiety. It is a widely used pyrethroid in veterinary products and agricultural formulations. Unlike most pyrethroids with at least two isomers manufactured, such as permethrin, DLM is marketed as only a single isomer. DLM is also one of the most neurotoxic pyrethroids by delaying sodium channel closure and prolonging the depolarization of neurons (Tabarean et al., 1998).

1.3. Toxicokinetic (TK) studies

Toxicokinetic (TK) studies are playing an increasingly important role in reducing uncertainties inherent in risk assessments. Toxicity is a dynamic process in which the degree and duration of adverse effects are dependent upon the amount of bioactive moiety reaching and remaining at the site of action. Pyrethroids are accute neurotoxicants. The parent compounds are the toxic moieties. The target tissue (i.e., brain) dose is dependent upon the net effect of absorption, plasma protein binding, transportation, tissue deposition, metabolism, and elimination. Gaining understanding of these processes and how they differ with dose, exposure route and vehicle, age, gender, species, etc. can substantially reduce assumptions made in assessments of human risks.

1.4. Previous studies on DLM TK

Definitive TK data for most pyrethroids are lacking for humans and quite limited for laboratory animals. Comprehensive profiles are necessary to accurately estimate essential TK parameters, including half-life and measures of internal dosimetry (e.g., AUC). One of the first studies was conducted by Gray and Rickard (1982), who monitored the elimination of radioactivity from the blood and several tissues of rats following i.v. injection of 1.75 mg/kg of ^{14}C- DLM. Anandon et al. subsequently investigated the TK of permethrin (1991), DLM (1996) and lambda-cyhalothrin (2006), using high performance liquid chromatography (HPLC) to monitor the parent compounds in plasma and selected tissues. In each instance, the doses were so high that they elicited frank neurotoxicity. Kim et al. (2008) employed a refined HPLC method to define the time-course of DLM in plasma and tissues of the orally-dosed adult male rats. The highest dose, 10 mg/kg, elicited mild toxicity. It was not possible to obtain complete plasma profiles in rats given 0.4 or 1.0 mg/kg for them, due to lack of analytical sensitivity. There are fewer TK data for pyrethroids in immature animals. Kim et al. (2010) administered oral doses (0.4, 2.0, and 10.0 mg/kg) of DLM to 10-, 21-, and 40-day-old rats and reported plasma and tissue profiles. Plasma and brain DLM concentrations and AUCs were inversely related to age. The 2 and 10 mg/kg doses were much more neurotoxic to the youngest rats. Thus, it was not possible to obtain a complete time-profile for these pups due to their failure to survive long enough. The 0.4 mg/kg dose was not neurotoxic in young rats, but the insensitivity of the HPLC analytical method precluded measurement of plasma or tissue levels beyond 12 hours of dosing. A dose of 1 mg/kg DLM is 350-1,700 fold higher than the estimated total daily exposure of U.S. residents to the insecticide (EPA, 2014). Thus, it is important to obtain complete time-course data from experiments with even lower doses.

1.5. Objectives

The route and mode of exposure in TK studies should be as near to those in "real life" as possible, in order to maximize the relevance of the findings. The overall objective of the current investigation was to assess the TK of a wide range of relatively low oral doses of DLM, a representative pyrethroid, in rats for plasma and various tissues. A new GC-MS analytical method with increased sensitivity was employed (Gullick et al., 2014). This allowed characterization of the TK of a low dosage range more relevant to actual environmental exposures. TK data sets at a wide range of low doses of orally-administered pyrethroids are necessary to calculate the essential TK parameters and compare their dose-dependency. Thus, 5 dosages in a 100-fold range were selected to determine the dose-dependency of DLM. A comparable i.v. dosage was given to acquire information on bioavailability.

Consumption in drinking water is modest relative to ingestion in foods and hand-to-mouth activity by children (EPA, 2011; Lu et al., 2006, 2010). Vegetable oils have been used as dosing vehicles for many oral toxicity studies of lipophilic chemicals. Ingestion of such agents in an oil diluent or vehicle is known to retard their gastrointestinal (GI) absorption and significantly influence their TK and ensuing toxicity (Kim et al., 1990 a,b; Coffin et al., 2000). Thus, it is important to determine the effect of some commonly-used vehicles on the systemic absorption and TK of DLM, which is another objective of this project. Experiments were designed to assess differences in systemic absorption and disposition of DLM from corn oil and glycerol formal, as well as the influence of the volume of the oil on the TK of this pyrethroid.

Age differences play a major role in pediatric toxicology. It is widely known that mature and immature animals differ in body fat content, plasma protein profile, enzyme activity and metabolism, blood brain barrier (BBB) permeability, etc. Previous studies have shown that

neonates and immature rats are more susceptible to DLM acute toxicity represented by higher brain levels (Kim et al., 2010). The question of whether this age-dependency still exists at a lower dosage of DLM remains unanswered. Thus, experiments were designed to assess the plasma and tissue age-dependency of TK of DLM from a range of low orally administrated dosages dissolved in corn oil.

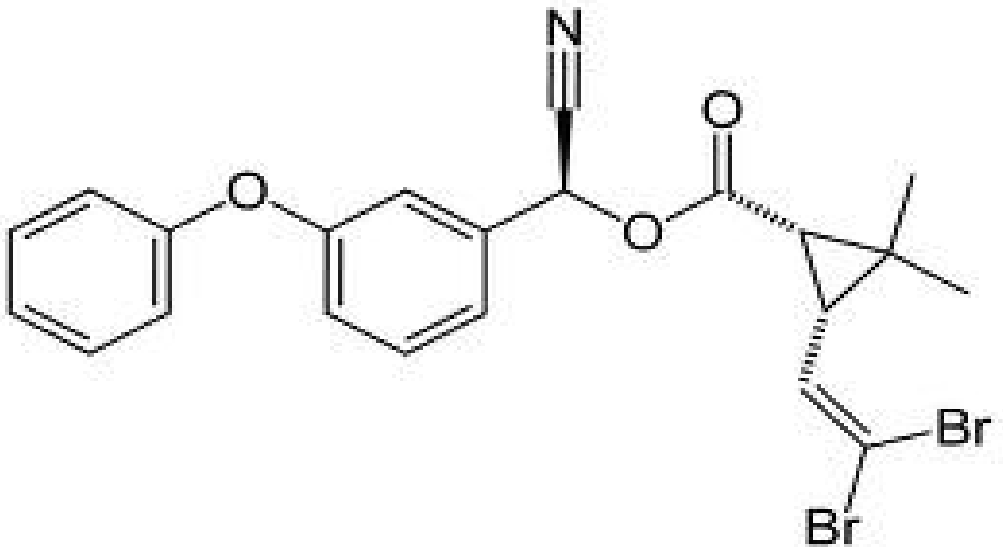


Figure 1. Chemical structure of deltamethrin (DLM) [(*S*)-α-cyano-3-phenoxybenzyl – (1R, *cis*) - 2, 2-dimethyl-3-(2, 2-dibromovinyl) – cyclopropanecarboxylate]

CHAPTER 2

MATERIALS AND METHODS

2.1. Testing subject and vehicles

Two batches of deltamethrin (DLM) [(S)-α-cyano-3-phenoxybenzyl – (1R, cis) -2, 2-dimethyl-3-(2, 2-dibromovinyl) – cyclopropanecarboxylate] (Figure 1, purity, 99.9% and 99.4%, respectively), were supplied by Bayer CropScience AG (Monheim, Germany). Their batch numbers were 0902200301 and ABKBDCK008, respectively. Glycerol formal (GF) and corn oil (CO) were obtained from Sigma Aldrich (St. Louis, MO). All other solutions were of the highest grade commercially available.

2.2. Animal maintenance.

Adult Male Crl:CD Sprague-Dawley (S-D) rats were purchased from Charles River Labs (Raleigh, NC). Upon receipt, all animals were inspected by a qualified animal technician. The rats were quarantined and their health monitored for one week at the University of Georgia's (UGA) AAALAC-accredited central animal facility. The rats were then transferred to the College of Pharmacy Animal Care Facility or Biological Sciences Animal Care Facility at least 2 days before initiation of experiments to allow adequate time for acclimation. Food and tap water were provided *ad libitum*. A 12-h light/dark cycle (light 6 AM - 6 PM) was maintained. All animal procedures were approved by UGA's Institutional Animal Care and Use Committee.

For TK experiments on immature rats, pregnant female Crl:CD S-D rats were purchased from Charles River Labs (Raleigh, NC). Upon receipt, all animals were inspected by a qualified

animal technician. The rats were quarantined and their health monitored for 7 days at UGA's AAALAC-accredited central animal facility and delivered. The females with pups were then transferred to the College of Pharmacy Animal Care Facility or Biological Sciences Animal Care Facility, and the pups allowed to reach 15 or 21 days of age before being used for experiments. Food and tap water were provided *ad libitum*. A 12-h light/dark cycle (light 6 AM - 6 PM) was maintained. All animal procedures were approved by UGA's Institutional Animal Care and Use Committee.

2.3. Animal experiments preparation.

For the serial sampling experiments on adult rats, one day before the experiment, groups of rats were cannulated via the left carotid artery under anesthesia, so that serial blood collection could be performed. The cannula was tunneled subcutaneously to exit at the nape of the neck so the animals could move freely upon recovery. For the group receiving an i.v. injection, another cannula was implanted into the right jugular vein. After the surgery, animals were caged individually and fasted during a 12- to 18-hour recovery period before dosing. Food was provided 4 h after dosing, while water was available *ad libitum* throughout the experiment.

For the serial sacrifice experiments on adult or young rats, no surgery was performed prior to the onset of all serial sacrifice experiments. Adult rats were group caged and fasted for approximately 12 hours before dosing. Post natal day (PND) 21 rats were weaned and fasted since the midnight of 20 days of age, thus fasted for approximately 10 hours before dosing. Solid food was provided 4 h after dosing for these two age groups of rats. PND 15 rats were not fasted and kept with mothers through the whole experiment with food provided to the mother. Water

was available *ad libitum* throughout the experiment for all three groups of rats throughout the experiment.

2.4. DLM dosages preparation

Appropriate amounts of DLM were diluted in GF or CO, so that desired dosage could be administered in a total volume of 0.5 ml GF/kg (for i.v. studies), 1 ml GF/kg, 1 ml CO/kg, or 5 ml CO/kg (for oral studies). There were 4-8 rats in each group for these experiments.

DLM was administered in a dosage of 0.5 mg/kg to the i.v. group to delineate the plasma DLM profiles.

Five dosages ranging from 0.05 to 5 mg/kg were given to other groups of rats by gavage in 5 ml CO/kg to delineate the dose-dependency of plasma DLM profiles.

Two additional groups of rats were given 1 mg DLM/kg in 1 ml GF/kg or 1 ml CO/kg to delineate the vehicle-dependency of plasma DLM profiles.

Three dosages (0.1, 0.25, and 0.5 mg/kg) were each given to 3 age-groups of rats (adults, PND21, and PND15) at 5 ml CO/kg for serial sacrifice experiments to delineate plasma and tissue DLM profiles.

2.5 Plasma sampling

For serial sampling experiments, groups of rats were dosed around 10:00 AM. A total of 10 to 20 blood samples were taken from each rat, with sampling times ranging from 5 min to 96 h post dosing. At each sampling time, a heparinized 3-way stopcock was connected to the cannula and ~200 μl of blood withdrawn into a heparinized syringe. Lost blood volume was replaced with heparinized saline. All animals were monitored for the primary clinical signs of

acute type II pyrethroids poisoning, including salivation, choreoathetosis and tremors until 8 h after dosing. Blood samples were centrifuged for collecting plasma right after collection. Plasma samples were stored at -20 or -80 ℃ until analysis.

2.6. Blood and tissue collection

For serial sacrifice experiments, groups of rats were dosed around 10:00 AM. All rats were monitored for the primary clinical signs of acute type II pyrethroids poisoning, including salivation, choreoathetosis and tremors until sacrifice or 8 hours after dosing, whichever was sooner. A total of 8-11 time points were chosen. Serial sacrifices (cervical dislocation with exsanguinations following cardiac puncture) were conducted with groups of 4-5 rats per time-point, in order to collect blood and selected tissues (brain, liver, and abdominal muscle). For adult rats, the liver was perfused with 10 ml of saline before collection. All blood samples were centrifuged for separation of plasma immediately after collection. All tissues were flash frozen in liquid nitrogen right after collection. Plasma and tissue samples were stored at -20 or -80 ℃ until analysis.

2.7 Analysis of DLM.

Plasma samples were processed, and DLM was quantified by the gas chromatography-negative chemical ionization-mass spectrometry (GC-NCI-MS) method of Gullick et al. (2014). Briefly, 100 μl of plasma were added to centrifuge tubes containing 10 μl of sodium fluoride, which was used to inhibit the activity of carboxylesterases (CaEs). Five hundred microliters of acetonitrile containing 1% phosphoric acid and 50 ng/mL cis-permethrin as the internal standard were added. These tubes were then vortexed and centrifuged. Supernatants were transferred into

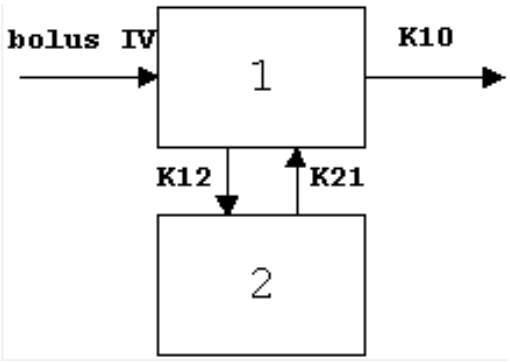
new tubes and dried in an oven, following by reconstitution with 100 µl of tolune and transferred to glass vials. The glass vials were dried and reconstituted with 10 µl of tolune for analysis. The limits of detection and quantification (LOD and LOQ) were 0.1 and 0.3 ng/mL, respectively.

Tissue samples were processed, and DLM was quantified by the GC-NCI-MS method (Gullick et al., unpublished). Briefly, 2 mL (or 3 mL for muscle) of saline were added into each gram of tissue prior to homogenation. Three hundred microliters (or 200 µL for liver) of tissue homogenate were spiked into centrifuge tubes. Eight hundred microliters of hexane saturated acetonitrile containing 50 ng/mL cis-permethrin as internal standard were added. These tubes were then vortexed and centrifuged. Supernatants were transferred into Agilent QuEcHERS tubes ("Meaty" tubes for brain and muscle, or "Fatty" tubes for liver). Those tubes were then vortexed and centrifuged. Supernatants were transferred into filter tubes. After filtration, filtrates were dried in an oven, followed by reconstitution with 100 µl of tolune and transferred to glass vials. The glass vials were dried and reconstituted with 10 µl of tolune for analysis. The limits of detection and quantification were 0.17 and 0.5 ng/mL, respectively, for brain homogenates; and 0.33 and 1 ng/mL, respectively, for liver and muscle homogenates.

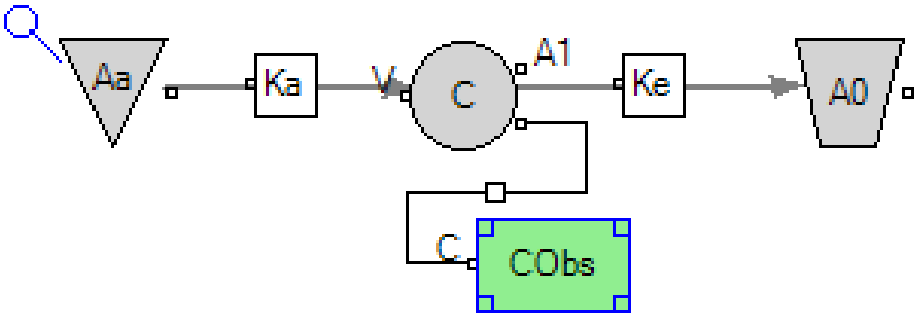
2.8. Data analysis.

Means and standard deviations (SDs) were calculated by Microsoft Excel (Microsoft Co., Redmond, WA). A measured concentration between LOD and LOQ was marked as half of the LOQ, and one below LOD was marked as half of the LOD. Toxiokinetic (TK) parameters of the i.v. study, including alpha half-life (HL), beta HL, central and peripheral volume of distribution (V_1 and V_2), clearance (Cl), and area under the plasma concentration versus time curve (AUC) were calculated using Pharsight WinNonLin (ver 6.4) 2-compartmental analysis (Pharsight, Cary,

NC) (Figure 2(a)). TK parameters for oral study, including maximum plasma concentration (Cmax), observed time of maximum concentration (Tmax), effective elimination HL, and area under the plasma concentration-time curve (AUC_0^{24} and AUC_0^{∞}) (by linear up/log down trapezoidal rule) were calculated using WinNolin (ver 4.1) non-compartmental analysis (Pharsight, Cary, NC). Bioavailability was measured as the dose-normalized AUC of an oral dosage divided by the AUC of the i.v. reference. In order to get the absorption rate constant (Ka), multiple efforts was tried. First, a WinNonLin build-in 2-compartment model was used. However, the simulated curve did not correlate with the actual data very well and the variations of the parameters were extremely high. Then, a Pharsight model was used, which had more flexibility on the absorption scenario. Zero-order absorption was tried due to the slow release from dosing vehicle to absorption site, but large variability was still found for the terminal phase on the two compartment model. Eventually, TK parameters for oral study, including Ka and apparent clearance (Cl/F), were calculated using Phoenix Pharsight (ver 6.4) 1-compartmental analysis (Pharsight, Cary, NC) with data of 0-24 hours post dosing by a model assuming zero order administration to the absorption apartment (Figure 2(b)). The statistical significance of dose- and vehicle-dependent differences in mean values (Cmax and AUCs normalized by dosage) was assessed with a one-way ANOVA, followed by Tukey's honestly significant difference Test using Prism (3.03) (GraphPac Software, Inc., San Diego, CA). The statistical significance of Cmax from young animal to adult in the corresponding dosage was also assessed with a one-way ANOVA, followed by Tukey's honestly significant difference Test using Prism (3.03).



(a)



(b)

Figure 2. Kinetics models used to analyze TK data.

(a) An i.v. administration 2-compartment kinetics model used for analyze plasma DLM concentration-time data of individual animals after i.v. administration of DLM.

(Note: 1. central compartment; 2. peripheral compartment.)

(b) An oral administration 1-compartment kinetics model used for analyze plasma DLM concentration-time data of individual animals after oral administration of DLM

(Note: Aa. absorption compartment; C: central compartment; CObs: observation site; A0: elimination compartment.)

CHAPTER 3

RESULTS

3.1. IV study of DLM TK

A dose of 0.5 mg/kg DLM was given i.v. in GF, so that oral bioavailability could be calculated (Figure 2, Table 1). The elimination of DLM in plasma shows two phases. Plasma DLM levels decreased very rapidly for the first 12 hours post injection with the alpha half-life of 0.95 hr. The initial rapid drop was followed by a slow, prolonged decline for the later part of 96 hrs, with beta half-life of 62.4 hours. Central volume of distribution is also much smaller than the volume of the terminal phase. The AUC of DLM following its i.v. injection was calculated as 5297 μg *hr/L, which was used for calculating the bioavailability of oral doses.

3.2. Vehicle-dependency of DLM TK

Plasma DLM concentration-time profiles for adult rats gavaged with 1.0 mg /kg DLM in 1 ml GF/kg; 1 ml CO/kg; and 5 ml CO/kg are illustrated in Figure 3. The time-profiles during the initial 12 hours post dosing can be more readily distinguished in the figure insert. This demonstrates that the vehicles, in which DLM is administered orally, as well as the volume of corn oil, slightly affect the absorption and disposition of the parent compound, as represented by TK parameters (Table 2). It is evident from the figure that DLM is absorbed most rapidly from GF as reflected by the significantly high Ka (1.476 /hr) and shortest Tmax (2.8 hours). With 1 mg/kg, the Cmax for the 1 ml GF/kg group (245.7 μg/L) exceeds those of the 1 or 5 ml CO/kg groups. It is apparent that CO delays GI absorption of DLM. Absorption is somewhat slower

from the larger volume of CO, shown as a shorter Tmax in the 1 ml CO/kg compared to the 5 ml CO/kg group. The larger amount of corn oil also diminishes the peak plasma DLM concentration. DLM is eliminated most rapidly in the GF-treated animals, with an effective half-life of 1.8 hours. As might be anticipated, effective half-lives are longest in animals given DLM in the larger volume of CO. The 1 mg GF/kg group also exhibited a larger AUC. However, except for the case of Ka, none of these parameters differs significantly among the 3 groups, which suggest similar exposure and toxicity of DLM given at the same dosage in these vehicles, although GF somehow fastened the absorption. Bioavailability, calculated based on AUC, is highest in 1 ml GF/kg group (11.9%), and followed by 1 ml CO/kg group, and 5 ml CO/kg group is the lowest (9.9%).

3.3. Dose-dependency of DLM plasma TK

DLM, given orally in 5 ml CO/kg, exhibited generally linear TK over a 100-fold range of doses (0.05-5.0 mg DLM/kg). This is reflected by the largely parallel plasma absorption and elimination profiles pictured in Figure 4. DLM at all doses exhibits absorption rate-limited kinetics, manifest as broader peaks with similar small Ka and prolonged Tmax values of 5.0-6.7 hrs (Table 3). Cmax is proportional to the dosage, with the exception of the highest dosage. Effective half-life does not vary significantly with dose. Effective half-lives of all oral studies given in 5 ml CO/kg are substantially longer than that of the i.v. group, reflecting prolonged gastrointestinal absorption of the lipophilic chemical from the large amount of corn oil vehicle. Increase in AUC_0^{24} is clearly dose-dependent, and shows linearity at the range of 0.05-1.0 mg/kg. Somewhat lower normalized Cmax and AUC_0^{24} are observed in the 5.0 mg/kg dosage group, indicating some degree of non-linear behavior. The increases in AUC_0^{inf} are dose-dependent,

though proportionally is less precise due to scatter sampling at the later time-points when plasma concentrations approached the limit of quantitation. As might be anticipated, all TK parameters, including Cmax/Dose, Tmax, effective half-life, and normalized AUCs, does not differ significantly among these groups. Bioavailability varies from 5.8 to 9.7% at this range of dosage, consistently quite low, indicating limited systemic uptake of DLM.

3.4. Plasma and tissue TK of DLM in orally dosed adult male rats

The time courses of DLM in plasma, brain, liver, and muscle, of orally-dosed adult rats was determined by a series of serial sacrifice experiments (Figure 5, Tables 4-7). The average concentrations of each time point were analyzed, instead of data from individual rats, due to the nature of terminal sacrifice. The time-courses of DLM in various tissues were delineated in the 0.1-0.5 dose range. It can be seen in Figure 5 that DLM concentrations peaked sooner and diminished more rapidly in plasma than in brain. DLM concentrations peaked in plasma at the same time as in liver, but also diminished faster in plasma than in liver. Peak DLM concentrations were markedly lower in the brain, liver, and muscle as compared to plasma. The brain, liver, and muscle time-courses with the 0.1 mg/kg dosage, as well as the muscle time-course with the 0.25 mg/kg dosage, were not detectable since most samples were below the limit of quantitation (LOQ). Note that the LOQs of plasma, brain, liver, and muscle samples were 0.3, 1.5, 3, and 4 ng/ml or ng/g, respectively. The plasma and tissue TK were generally linear among this dosage, with few exceptions. The AUC of plasma in 0.25 mg/kg dosage exceeded that in 0.5 mg/kg dosage, while the Cmax of brain and liver in 0.5 mg/kg dosage was 3-4 times higher than that in 0.25 mg/kg dosage. Plasma and tissue concentrations persist until 48 hrs at a low concentration, which is common for lipophilic compounds which tend to sequester in fat.

3.5. Plasma and tissue TK of DLM in orally dosed PND21 rats

Time courses of DLM in plasma and tissues after serial sacrifice of PND21 rats are presented in Figures 6 and Tables 4-7.The plasma levels appeared to rise at greater extents compared to that in adults at each dosage level, attaining their Cmax in usually less than 4 hours. This is generally sooner than in adults. Similar scenarios were noted in the tissues. Time courses of the tissues at the lowest dosage (0.1 mg/kg) could be delineated, which also suggests higher tissue exposure in young rats compared to adults, except for muscle in which DLM was not detected. In plasma, a more distinct peak was noted at the highest dosage at 0-1 hour post dosing. The Cmax and AUC for 0.25 and 0.5 mg/kg were proportional to dosage, but the rose to a disproportionally high peak and AUC for 0.1 mg/kg. At the lowest dose, the plasma concentrations declined at a slower rate compared to higher doses. Dose-dependency was noted in brain. Brain concentrations peaked in 2 hours at the highest dose, but 6-8 h with the other two doses. The brain time-course for the highest dose also diminished most rapidly with a short effective half-life, while for other dosages and ages, brain levels usually diminished slower than plasma. Brain Cmax is significantly higher than those of adults at both dosages (0.1 mg/kg cannot be compared due to lack of adult data), while no other tissues showed such significant age-dependency. For the PND21 group, brain, liver, and muscle exposures were lower than for the plasma, as reflected by the lower AUC. The same was true for adults.

3.6. Plasma and tissue TK of DLM in orally dosed PND15 rats

Time courses of DLM analysis in plasma and tissues after serial sacrifice of PND15 rats are presented in Figures 7 and Tables 4-7. For this age group, plasma, brain, and liver profiles for all dose groups followed a similar pattern. Plasma curves at the higher two doses showed

disproportionality. They did not differ so much, and were extremely high compared to the corresponding brain concentrations, suggesting a saturation of uptake processes. For the two high dosages, liver DLM levels increased disproportionately with dose, with the Cmax and AUC 4-5 times higher than that for the 0.1 mg/kg dose. This disproportionality corresponds to that in plasma, but in the opposite direction. Plasma and brain levels are slightly higher compared to the other two age groups at the same dosage, while liver levels were much higher compared to the other two age groups. Liver levels also exceeded the plasma levels in this age group, contrary to the case with the other two age groups. Muscle levels were above the LOQ for all 3 dosages, and higher compared to the other age groups, but still lower than but closer to the plasma levels. At this age group, almost all plasma and tissues showed significant age-dependency in Cmax, with PND15 values much higher compared to those of adults, with the exception of 0.1 mg/kg in plasma.

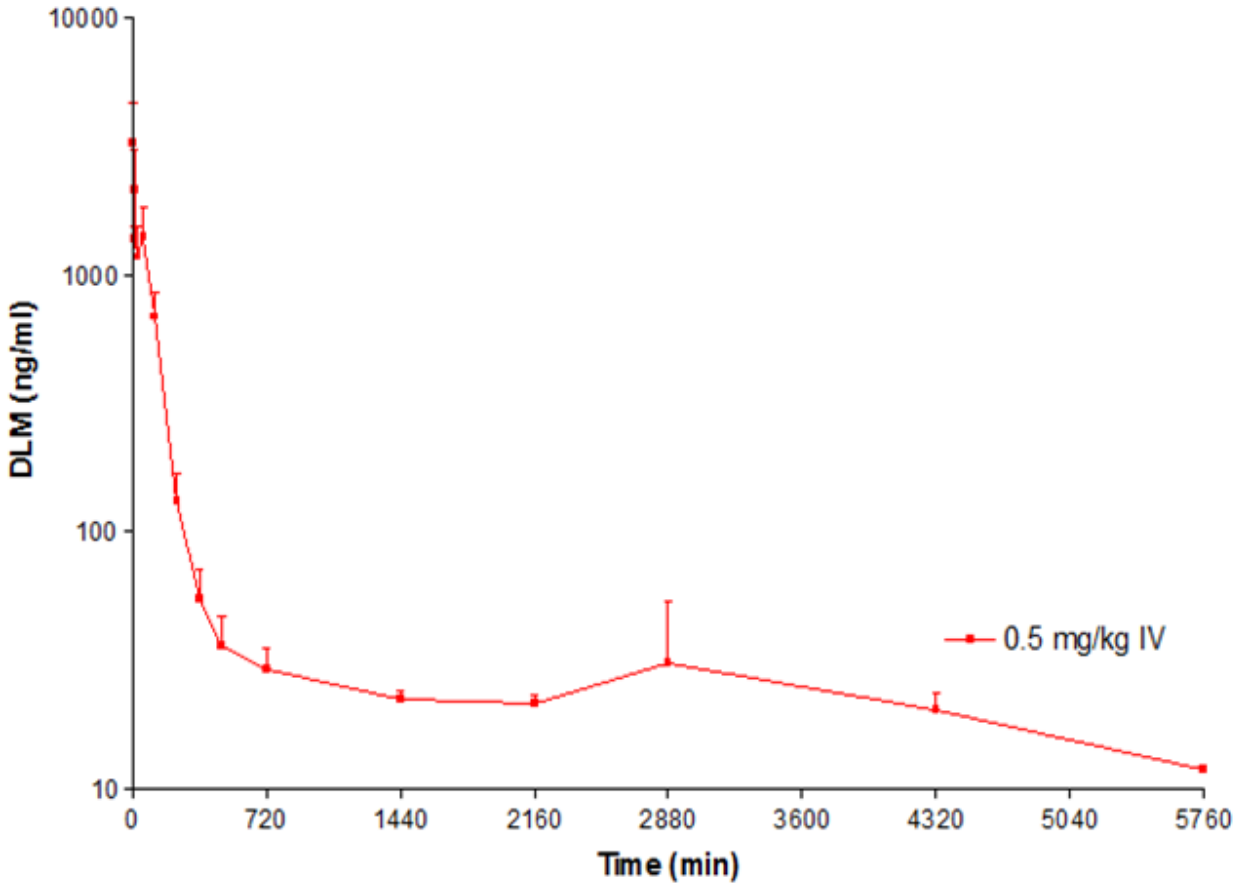


Figure 3. Plasma DLM concentration time-courses following i.v. administration of 0.5 mg/kg DLM in adult SD rats. Rats received a dosage of 0.5 mg DLM/kg i.v. dissolved in 0.5 ml/kg GF. Plasma DLM concentrations were measured in serially plasma samples taken as blood from a carotid artery cannula from 15 minutes – 96 hours post injection. Data points represent Means +/- SD of groups of 4 rats. Inset shows DLM levels during the first 12 hours.

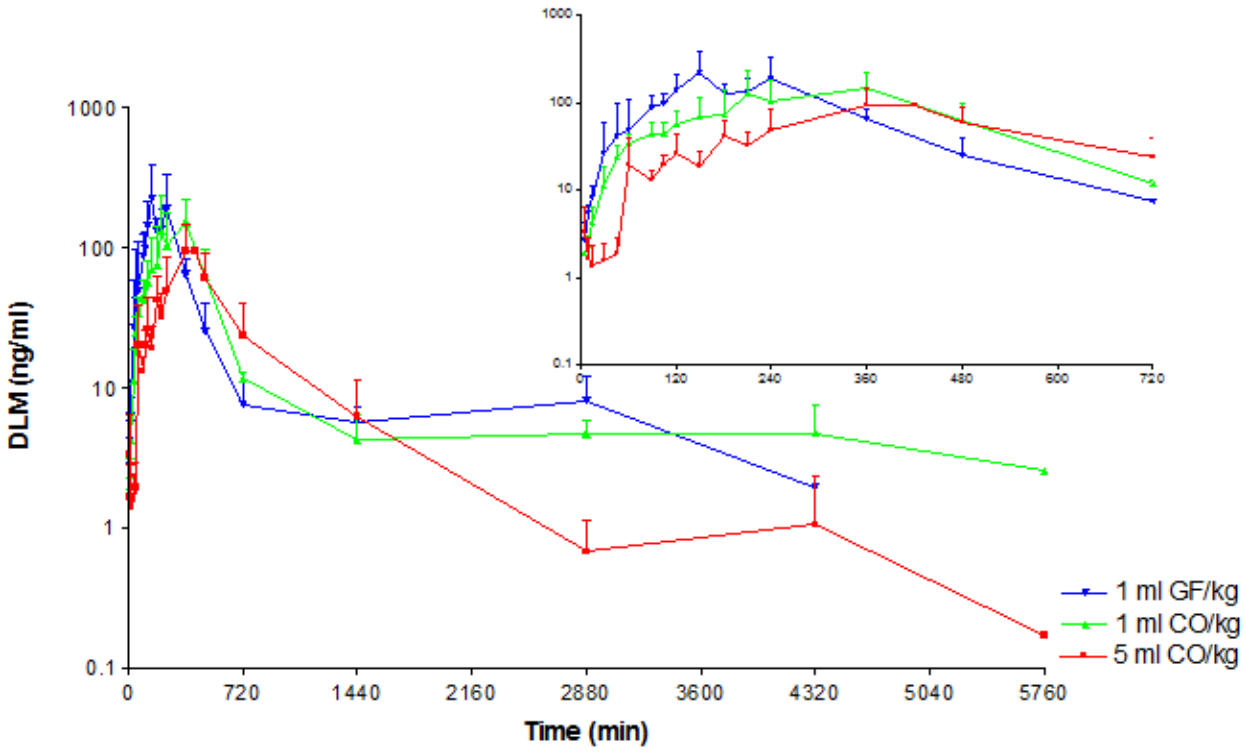


Figure 4. Plasma DLM concentration time-courses following oral administration of 1 mg/kg DLM in 1 ml GF/kg, 1 ml CO/kg, or 5 ml CO/kg in adult SD rats. Rats received a dosage of 1 mg DLM/kg p.o. Plasma DLM concentrations were measured in serially plasma samples taken as blood from a carotid artery cannula from 15 minutes – 96 hours post administration. Data points represent Means +/- SD of groups of 3-5 rats. Inset shows DLM levels during the first 12 hours.

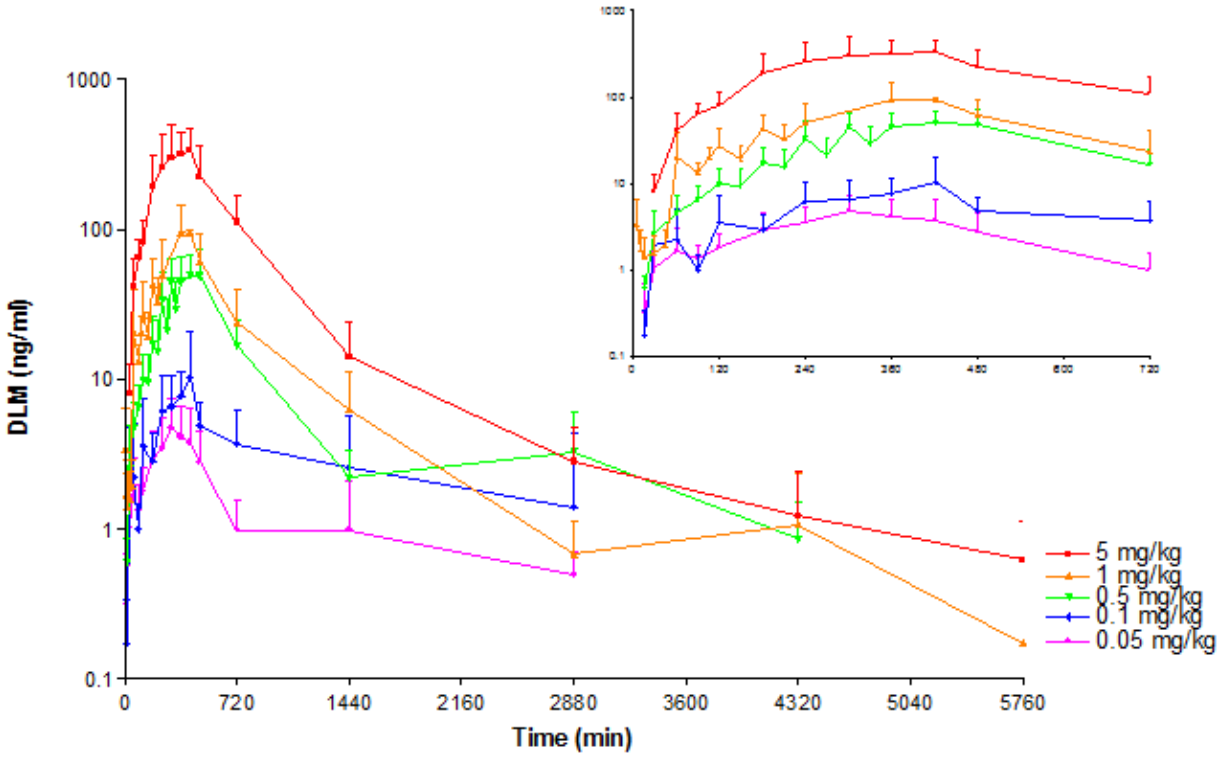
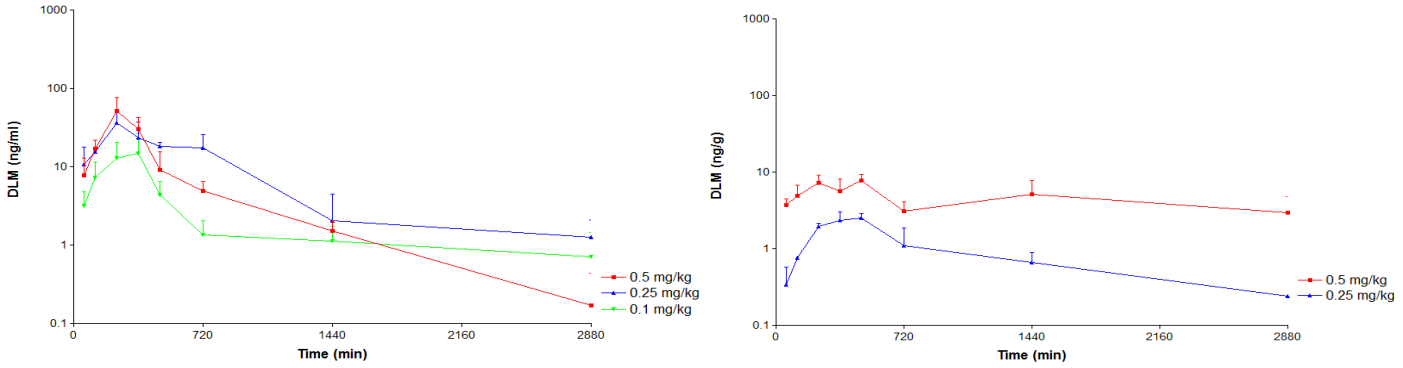
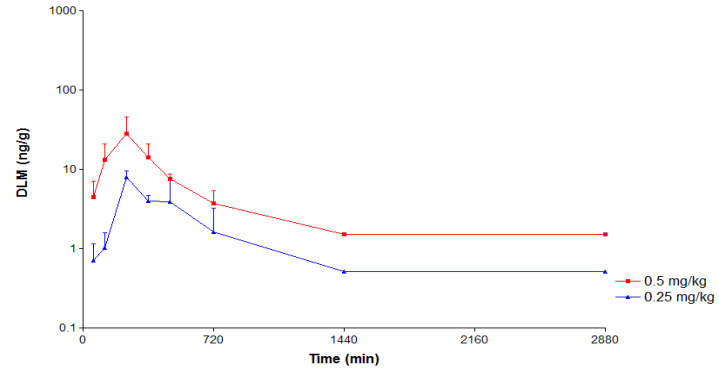


Figure 5. Plasma DLM concentration time-courses following oral administration of 0.05-5.0 mg/kg DLM in CO in adult SD rats. Rats received a dosage of 0.05-5 mg DLM/kg p.o. dissolved in 5 ml/kg CO. Plasma DLM concentrations were measured in serially plasma samples taken as blood from a carotid artery cannula from 15 minutes – 96 hours post administration. Data points represent Means +/- SD of groups of 4-6 rats. Inset shows DLM levels during the first 12 hours.

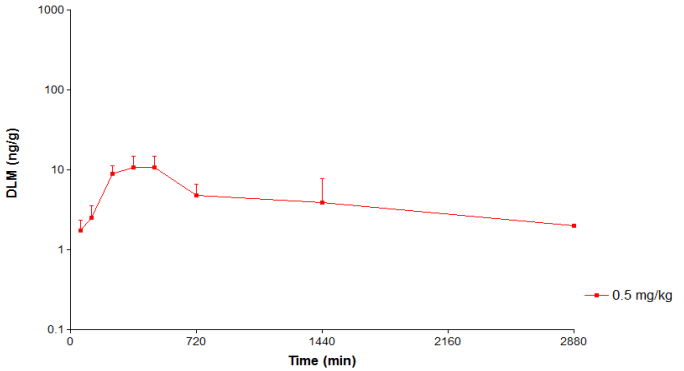


(a)

(b)

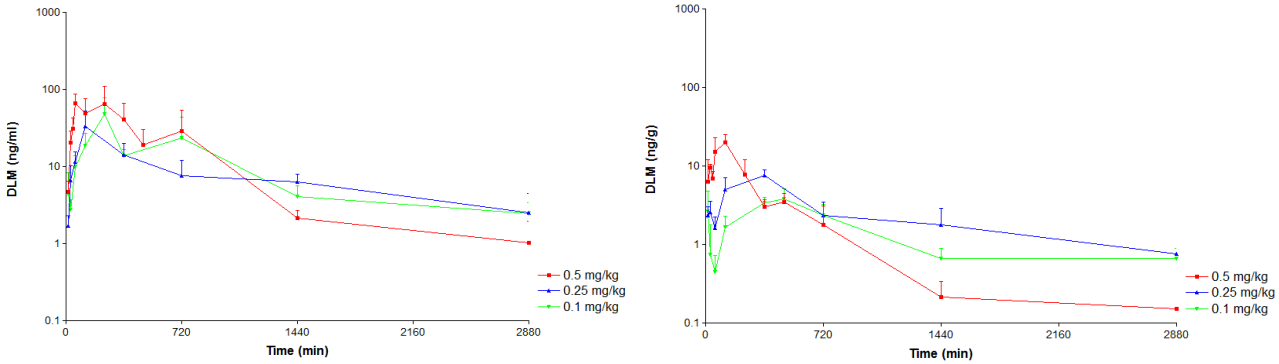


(c)



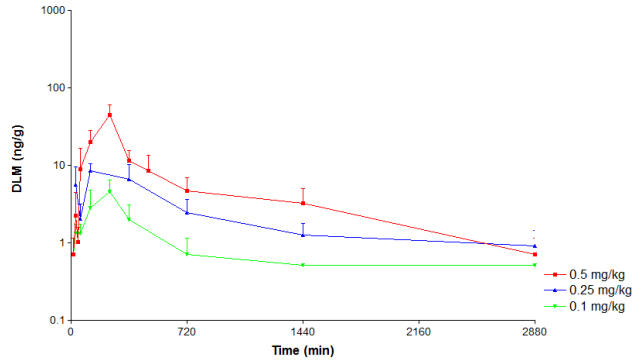
(d)

Figure 6. DLM concentration-time profiles of adult male S-D rats gavaged with 0.5 (red), 0.25 (blue), and 0.1 (green) mg DLM/kg. Serial plasma (a), brain (b), liver (c), and muscle (d) samples were collected and analyzed for DLM concentrations by GC-MS. Data points represent Mean +/- SD of groups of 5 rats.

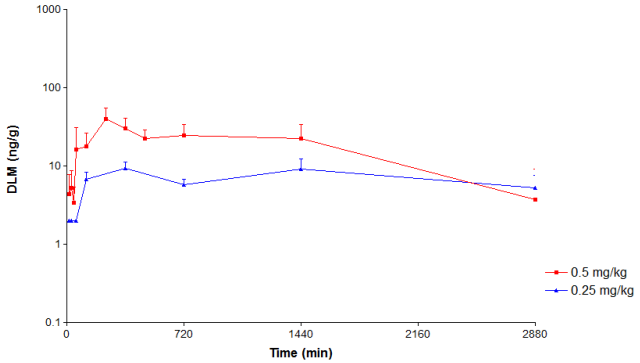


(a)

(b)

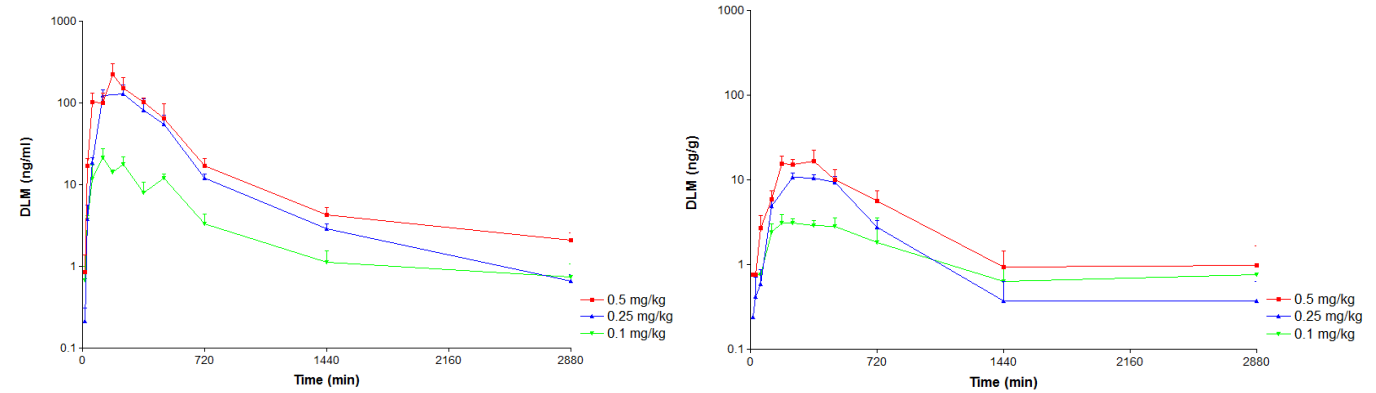


(c)



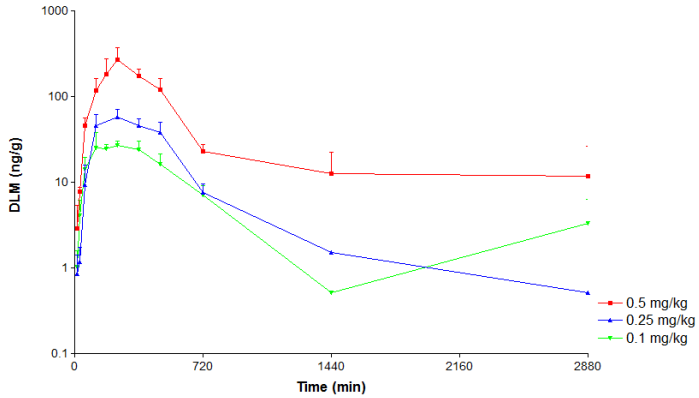
(d)

Figure 7. DLM concentration-time profiles of PND21 S-D rats gavaged with 0.5 (red), 0.25 (blue), and 0.1 (green) mg DLM/kg. Serial plasma (a), brain (b), liver (c), and muscle (d) samples were collected and analyzed for DLM concentrations by GC-MS. Data points represent Mean +/- SD of groups of 5 rats.

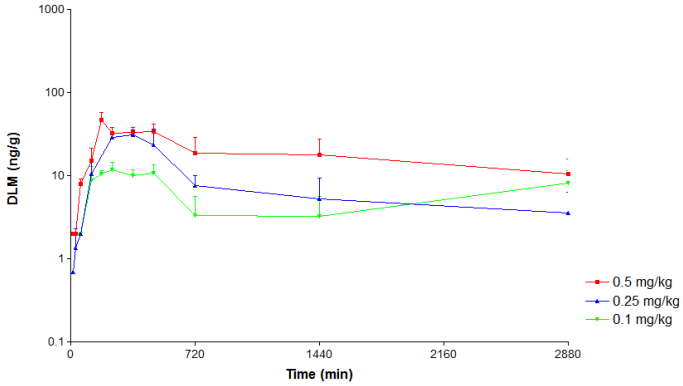


(a)

(b)



(c)



(d)

Figure 8. DLM concentration-time profiles of PND15 S-D rats gavaged with 0.5 (red), 0.25 (blue), and 0.1 (green) mg DLM/kg. Serial plasma (a), brain (b), liver (c), and muscle (d) samples were collected and analyzed for DLM concentrations by GC-MS. Data points represent Mean +/- SD of groups of 3-4 rats.

Table 1. Plasma TK Parameters for 0.5 mg DLM/kg Dosage Administrated i.v.

Dosage	**0.5 mg DLM/kg**
C_0 (µg/L)	1998 ±570
Alpha HL (hr)	0.95 ±0.17
Beta HL (hr)	62.4 ±26.6
V_1 (L/kg)	0.27 ±0.09
V_2 (L/kg)	6.70 ±2.74
Cl (L/hr/kg)	0.097 ±0.019
AUC_0^{∞} (µg*hr/L)	5297 ±955

Calculated from plasma DLM concentrations of adult male S-D rats following i.v. administration of 0.5 mg/kg DLM dissolved in 0.5 ml GF/kg. Plasma DLM concentrations were measured in serially plasma samples taken as blood from a carotid artery cannula from 5 minutes – 96 hours post administration.

Table 2. Plasma TK Parameters for 1 mg DLM/kg Dosage Administrated in 5 and 1 ml CO/kg and 1 ml GF/kg

Vehicle Used in the 1 mg DLM/kg Dosage	**5 ml CO/kg**	**1 ml CO/kg**	**1 ml GF/kg**
Ka (hr^{-1})	0.309±0.08	0.361±0.044	1.476±0.076*
T_{max} (hr)	5.0±1.4	4.8±1.4	2.8±0.6
C_{max} (µg/L)	99.0±46.7	191.7±75.6	245.7±152.4
Effective HL (hr)	4.2±1.8	2.1±0.7	1.8±0.5
Cl/F (L/hr/kg)	1.18±0.31	1.24±0.23	1.27±0.53
AUC_0^{24h} (µg*hr/L)	848.4±167.2	936.2±303	1023.6±74.3
AUC_0^{∞} (µg*hr/L)	1030.4±195.7	1161.2±242.7	1259.4±38.3
F (%)	9.9	11.0	11.9

* Significantly different from 5 ml and 1 ml CO/kg ($P<0.05$).

Calculated from plasma DLM concentrations of adult male S-D rats following oral administration of 1 mg/kg DLM dissolved in different vehicles. Plasma DLM concentrations were measured in serially plasma samples taken as blood from a carotid artery cannula from 5 minutes – 96 hours post administration.

Table 3. Plasma TK Parameters for the 0.05-5 mg DLM/kg Dosage Range.

DLM Dosage (mg/kg po)	**5**	**1**	**0.5**	**0.1**	**0.05**
Ka (hr^{-1})	0.565 ±0.208	0.309 ±0.080	0.321 ±0.137	0.592 ±0.173	0.527 ±0.325
T_{max} (hr)	6.1 ±1.4	5.0 ±1.4	6.1 ±1.4	6.7 ±2.1	5.9 ±1.6
C_{max} (µg/L)	394.7 ±159.6	99.0 ±46.7	59.4 ±19.4	13.4 ±9.1	5.5 ±2.0
Effective HL (hr)	3.8 ±1.1	4.2 ±1.8	3.7 ±0.8	3.8 ±1.4	2.4 ±1.0
Cl/F (L/hr/kg)	1.7 ±0.48	1.2 ±0.31	1.2 ±0.21	2.2 ±0.45	1.9 ±0.63
AUC_0^{24h} (µg*hr/L)	2881 ±871.7	848.4 ±167.2	429.9 ±119.7	81.6 ±47.7	40.1 ±12.2
AUC_0^{∞} (µg*hr/L)	3046.2 ±870.0	1030.4 ±195.7	499.6 ±165.7	84.6 ±48.0	46.2 ±14.1
F (%)	5.9	9.9	9.7	8.1	8.9

Calculated from plasma DLM concentrations of adult male S-D rats following oral administration of 0.05-5.0 mg/kg DLM dissolved in 5 ml CO/kg. Plasma DLM concentrations were measured in serially plasma samples taken as blood from a carotid artery cannula from 5 minutes – 96 hours post administration.

Table 4. TK Parameters for the 0.1-0.5 mg DLM/kg Dosage in Plasma.

DLM Dose (mg/kg po)	0.5 Adult	0.25 Adult	0.1 Adult	0.5 PND21	0.25 PND21	0.1 PND21	0.5 PND15	0.25 PND15	0.1 PND15
T_{max} (hr)	4	4	6	1	2	4	3	4	2
C_{max} (µg/L)	50.4 ±24.3	36.4 ±12.0	14.6 ±11.9	65.8 ±20.0	33.0 ±18.9	47.4 ±30.3	284 ±52*	128 ±36*	21.0 ±6.6
Effective Half-life (hr)	4.2	5.0	1.8	4.8	4.8	7.0	3.5	3.6	4.2
AUC_0^{24h} (µg*hr/L)	258.2	326.8	96.1	569.6	262.0	383.0	1172.1	857.5	148.1
AUC_0^{∞} (µg*hr/L)	274.0	374.4	119.4	612.6	377.1	463.2	1262.1	896.8	174.3

* Significantly different from adult's Cmax at the same dosage ($P<0.05$).

Calculated from plasma DLM concentrations of adult male, PND21, and PND15 S-D rats following oral administration of 0.1-0.5 mg/kg DLM dissolved in 5 ml CO/kg. Plasma DLM concentrations were measured in plasma samples taken from a serial sacrifice study.

Table 5. TK Parameters for the 0.1-0.5 mg DLM/kg Dosage in Brain.

DLM Dose (mg/kg po)	0.5 Adult	0.25 Adult	0.1* Adult	0.5 PND21	0.25 PND21	0.1 PND21	0.5 PND15	0.25 PND15	0.1 PND15
T_{max} (hr)	8	8	-	2	6	8	6	4	3
C_{max} (µg/L)	7.6 ±1.6	2.5 ±0.3	-	19.9 ±5.2**	7.5 ±1.5**	3.8 ±1.3	16.3 ±5.7**	10.7 ±1.1**	3.1 ±0.8
Effective Half-life (hr)	7.1	9.2	-	3.7	9.6	6.4	5.7	3.9	7.4
AUC_0^{24h} (µg*hr/L)	113.0	29.5	-	85.7	80.8	46.9	144.5	95.2	42.2
AUC_0^{∞} (µg*hr/L)	236.4	42.6	-	90.8	119.4	68.5	177.9	106.1	66.7

*Parameters cannot be calculated since most samples have concentrations below Limit of Quantitation.

** Significantly different from adult's Cmax at the same dosage ($P<0.05$).

Calculated from brain DLM concentrations of adult male, PND21, and PND15 S-D rats following oral administration of 0.1-0.5 mg/kg DLM dissolved in 5 ml CO/kg. Brain DLM concentrations were measured in brain samples taken from a serial sacrifice study.

Table 6. TK Parameters for the 0.1-0.5 mg DLM/kg Dosage in Liver.

DLM Dose (mg/kg po)	0.5 Adult	0.25 Adult	0.1 Adult	0.5 PND21	0.25 PND21	0.1 PND21	0.5 PND15	0.25 PND15	0.1 PND15
T_{max} (hr)	4	4	-	4	2	4	4	4	4
C_{max} (µg/L)	28.2 ±16.8	7.8 ±1.7	-	44.7 ±15.7	8.4 ±2.0	4.6 ±1.9	267 ±100**	57 ±13**	27 ±4
Effective Half-life (hr)	5.1	5.4	-	6.7	5.4	7.1	2.4	3.6	3.4
AUC_0^{24h} (µg*hr/L)	163.9	50.8	-	221.2	84.1	31.3	1762.9	437.0	238.5
AUC_0^{∞} (µg*hr/L)	211.0	63.0	-	267.6	116.8	48.8	2352.0	461.7	299.7

*Parameters cannot be calculated since most samples have concentrations below Limit of Quantitation.

** Significantly different from adult's Cmax at the same dosage ($P<0.05$).

Calculated from liver DLM concentrations of adult male, PND21, and PND15 S-D rats following oral administration of 0.1-0.5 mg/kg DLM dissolved in 5 ml CO/kg. Liver DLM concentrations were measured in brain samples taken from a serial sacrifice study.

Table 7. TK Parameters for the 0.1-0.5 mg DLM/kg Dosage in Muscle

DLM Dose (mg/kg po)	0.5 Adult	0.25 Adult	0.1 Adult	0.5 PND21	0.25 PND21	0.1 PND21	0.5 PND15	0.25 PND15	0.1 PND15
T_{max} (hr)	8	-	-	4	6	-	3	6	4
C_{max} (µg/L)	10.72 ±3.8	-	-	39.5 ±15.6**	9.3 ±1.8	-	46.7 ±10.5**	30.5 ±4.5	11.7 ±2.5
Effective Half-life (hr)	13.0	-	-	4.8	8.5	-	8.2	2.9	4.4
AUC_0^{24h} (µg*hr/L)	136.0	-	-	572.7	170.1	-	535.3	291.0	133.6
AUC_0^{∞} (µg*hr/L)	241.3	-	-	846.8	401.2	-	991.8	410.0	319.1

*Parameters cannot be calculated since most samples have concentrations below Limit of Quantitation.

** Significantly different from adult's Cmax at the same dosage ($P<0.05$).

Calculated from muscle DLM concentrations of adult male, PND21, and PND15 S-D rats following oral administration of 0.1-0.5 mg/kg DLM dissolved in 5 ml CO/kg. Muscle DLM concentrations were measured in brain samples taken from a serial sacrifice study.

CHAPTER 4

DISCUSSION

DLM appeared to be absorbed steadily, but incompletely from the GI tract of fasted rats. Bioavailability (F) of the pyrethroids generally ranged from 6 to 12%, irrespective of the dosage or vehicle. These values are comparable to the F value of 18% for DLM in fasted rats reported by Kim et al. (2008). These investigators administered this lipophilic chemical in GF, a dioxane alcohol that is miscible in water. DLM and other pyrethroids are extensively metabolized by liver cytochrome P450s and carboxylesterases (CaEs), as well as plasma CaEs (Anand et al., 2006; Ross et al., 2006; Scollon et al., 2009). First-pass metabolism might thus be expected to significantly reduce pyrethroid levels in the arterial circulation. It is, however, likely that the chemicals are absorbed largely via the lymphatics. Although there are apparently no data on pyrethroids, it has long been recognized that highly lipophilic chemicals are absorbed primarily via the lymphatics (Sieber, 1976), particularly when administered in an oil (Palin et al., 1982). Jandacek et al. (2009) demonstrated that lymphatic absorption of a series or organochlorine compounds increased in concert with their octanol: water partition coefficients. It might be anticipated that DLM would be well absorbed from the GI tract, due to its lipophilicity and lack of charge. Kim et al. (2008), however, reported 40% of a 10 mg /kg DLM oral dose was eliminated in the feces of rats. Upon consideration of other highly lipophilic compounds, it was surprising to learn they were not readily absorbed. Oral bioavailability was low for dioxin (Wang et al., 1997) and benzo(a)pyrene (Foth et al., 1988). Tanabe et al. (1981) noted that the efficiency of oral absorption of PCB isomers decreased with their increasing chlorine content (i.e.,

increasing lipophilicity). It is worthy to note that GI membranes are covered by a mucus layer, which is thought to maintain an unstirred water layer (UWL) in contact with the epithelium. The aqueous content of the gut, mucus and UWL are considered to be significant barriers to passive diffusion of lipid-soluble compounds (Pang, 2003). Non-specific protein and lipoprotein binding, coupled with lipid partitioning, also serve to reduce flux of such compounds by virtually trapping them in cell membranes (Liu et al., 2011). Protein binding of a series of organic compounds has been shown to increase with their increasing lipophilicity, due to nonspecific hydrophobic bonding (Helmer et al., 1968; Laznicek and Laznickova, 1995). Wils et al. (1994) described a parabolic relationship between lipid solubility of drugs and their intestinal epithelial flux. Permeability of Caco-2 and HT29-18-C cells progressively increased with increasing lipophilicity to a point, beyond which higher log P values resulted in diminishing permeability. DLM and most other pyrethroids satisfy two of Lipinski et al.'s (2001) criteria for poor membrane permeation, namely Log P > 5 and molecular mass > 500.

Our analysis of some 16 serial blood samples from individual animals, taken over a 96-h period post dosing, yielded comprehensive plasma DLM concentration versus time profiles. The profiles resembled those of Anandon et al. (1996) and Kim et al. (2008) in some respects, but these researchers did not capture the absorption or full elimination phases. In the current study plasma DLM levels progressively increased, reaching maxima in 5 to 7 hours in the corn oil vehicle groups. DLM concentrations exhibited broad shoulders or plateaus lasting 2-3 hours (Fig. 2 & 3 Inserts) rather than actual peaks. This pattern is indicative of prolonged, rate-limited absorption of a highly lipophilic chemical in the aqueous gut environment. Elimination of DLM from the plasma was very slow. Although DLM is extensively metabolized to glucuronide conjugates and other water-soluble metabolites, several factors prolong the residence of the

parent compound. Sethi et al. (2014) recently reported that approximately 90% of toxicologically-relevant concentrations of DLM were bound to plasma proteins and lipoproteins. Kim et al. (2008) observed that DLM is deposited in large amounts and slowly cleared from adipose tissue of rats. Although Kim and coworkers were able to monitor fat DLM concentrations for 48 hours post gavage, their HPLC analytical method lacked the sensitivity to quantify DLM in other tissues or in plasma longer than 6 and 24 h at dosages of 0.4 and 2.0 mg/kg, respectively. With the present GC-MS method, it was possible to accurately measure DLM in plasma for up to 48 h for 0.05 and 0.1 mg/kg dosages and 72-96 h for 0.5, 1.0 and 5.0 mg/kg. It is essential to have this analytical capability to obtain valid measures of half-life and AUC. Plasma AUCs are frequently-used indices of the internal dose of toxicants.

The oral dosage vehicle and its volume slightly affected the TK of DLM as anticipated. Plasma levels rose more rapidly to a somewhat higher level in rats receiving the chemical in GF. Corn oil resulted in some delay in the absorption of DLM, as reflected by lower Cmax and longer half-life values. The oil apparently acted as a reservoir in the gut to retard systemic absorption. Time was required for its lipids to be emulsified, hydrolyzed and absorbed, releasing the lipophilic chemical. Kim et al. (1990 a, b) reported a similar phenomenon with carbon tetrachloride (CCl_4), a lipophilic hepatotoxin for which cytotoxicity appeared dependent primarily upon the Cmax. Oral administration of CCl_4 as an aqueous emulsion resulted in a much higher Cmax and hepatotoxic potency than when the agent was given in corn oil. In the present study, bioavailability and AUC_0^{∞} values are not largely dependent on the vehicle and its volume. Laher et al. (1984) reported that a 10-fold increase in the volume of olive oil did not significantly alter the oral bioavailability of benzo(a)pyrene in rats. Manifestations of acute DLM neurotoxicity such as salivation and hyperexcitability typically occur for several hours following

peak plasma levels (Rickard and Brodie, 1985). Thus, the AUC for this time period may be the most appropriate internal dosimeter for the insecticide. Judging from the fact that the TK parameters do not differ significantly among three groups, especially the findings of comparable AUCs in the corn oil and GF groups, it might be anticipated they would be equally toxic. Crofton et al. (1995) found this to be the case for inhibition of motor function in rats given DLM in the two vehicles. Results from oral toxicology studies employing either GF or a vegetable oil dosage vehicle should be relevant to risk assessments of ingested DLM and likely to other pyrethroids. Many diets include an oil or other fatty material.

The TK of DLM was linear, for the most part, over the 100-fold range of oral doses in corn oil in the present study. Cmax and AUC increased in direct proportion to dose from 0.05 to 1.0 mg/kg. Increases in Cmax and AUC from 1.0 to 5.0 mg/kg were somewhat less pronounced (i.e., ~4- and 3-fold, respectively). This less than proportional increase might be attributable to diffusion-limited absorption of the highest DLM dosage. Kim et al. (2008) did see a proportional increase in AUC_0^{∞} between 2 and 10 mg /kg DLM and no significant difference in t ½ . Terminal t ½ values did not differ significantly over the lower dosage range used in the present investigation. The lowest dose (i.e., 0.05 mg/kg) we could monitor was just ~50-fold higher than EPA (2014) estimates of total daily exposure of children to DLM. It would be anticipated that DLM will also exhibit linear kinetics at this lower dosage range such that estimates of internal dose (i.e., Cmax and AUC) and associated risks can be projected at "real life" exposure levels.

The serial sacrifice studies are the first studies, to our knowledge, that have examined the TK of a pyrethroid in a dosage range approaching the very low levels encountered in urban environments. The EPA (2015) estimates that total DLM exposure (food, water and residential) of members of the U.S. population is 0.6 μg/kg/day. Children 1 to 2 years old are estimated to

ingest a total of ~3 μg/kg/day, as they consume more food per unit body weight and engage in hand-to-mouth activities involving contaminated surfaces. Complete profiles for plasma and tissue were obtained in the current study for rats given 250 and 500 μg/kg. The lowest DLM dose administered was 100 μg/kg. It was possible to obtain plasma DLM concentration time-course profiles for all three age-groups of animals at this ingestion level, but our analytical method's LOQ of 1.5 and 3 ng/g precluded delineation of brain and liver concentration profiles for the adult animals. One hundred microgram/kilogram was 35- and 167-fold higher than the EPA's (2015) upper bound exposure estimates for children 1 to 2 years old and members of the general U.S. population, respectively.

Data from the previous study demonstrate that the TK of DLM was linear in this lower dosage range and that age-dependent differences in measures of internal dose tended to diminish with decrease in administered dose. PND 15 plasma AUC values were 4.6-, 2.4- and 1.5-fold higher than corresponding adult values at DLM doses of 0.5, 0.25 and 0.1 mg/kg. PND 15 plasma Cmax values were 5.6-, 3.5- and 1.4-times higher. This pattern of diminishing age differences in brain and liver dosimetry with decreasing exposure level was also generally exhibited by PND 15 and 21 pups, although DLM concentrations in adult tissues were below the LOQ. (Figures 4-6). A significant age difference in DLM internal dosimetry might be anticipated at contemporary exposure levels.

Several investigators have evaluated relationships between plasma or brain pyrethroid levels in neurotoxicity, in order to identify the most appropriate internal dosimeter, or dose metric. Dose metrics are important in risk assessments, as they describe the quantitative relationship between exposure (administered dose), the internal dose, and the magnitude of adverse effect. Rickard and Brodie (1985) reported finding a clear correlation between blood and

brain DLM concentrations and the onset of signs of neurotoxicity in rats given high single doses of the insecticide. Salivation was manifest initially in response to blood DLM levels of 750-1,200 ng/ml. Tremors and choreoathetosis subsequently occurred in turn with stepwise increases of about 50% of blood levels. Brain DLM concentrations and symptoms exhibited a similar pattern. The symptoms persisted as long as their respective blood or brain threshold was exceeded. It should be recognized that the blood and brain DLM concentrations in Rickard and Brodie's symptomatic animals were substantially (50- to 100-fold) greater than concentrations measured in the current investigation. Relatively modest differences (e.g., 50%) in the magnitude of internal exposure to DLM can have a pronounced effect on the incidence and severity of neurotoxic responses when external exposures are high. Rickard and Brodie (1985) observed that peak DLM levels in blood and brain did not correlate well with the onset of symptoms. There was generally a lag time after dosing, followed by response of increasing and decreasing magnitude over a period of hours. These observations argue for use of AUC during the period of effect, rather than Cmax or AUC_0^{∞} as a TK dose metric. Kim et al. (2010) reported relatively higher correlation between brain AUC_0^{∞} than plasma AUC_0^{∞} tremors/salivation in DLM-exposed rats. Scollen et al. (2011) concluded that the brain concentration of bifenthrin, a Type I pyrethroid, was a better predictor of decreased motor activity in rats than in blood concentration.

There are extensive toxicology databases for DLM and a number of other commonly used pyrethroids. DLM has been the subject of acute, subacute, chronic/carcinogenicity, reproductive, immunotoxicity and developmental neurotoxicity studies (EPA, 2015). Chronic studies have failed to reveal a qualitative or quantitative increase in toxicity with increase in duration of exposure. Therefore, the EPA has based its current risk assessment of DLM on the results of an acute study by Wolansky et al. (2006). These researchers determined that a single

oral dose of 2.5 mg /kg DLM produced a 30% decrease in spontaneous motor activity in rats. Both Type I and II pyrethroids reduce motor activity, weaken neuromuscular strength and impair motor coordination. A single dose of DLM as low as 1-2 mg/kg decreases acoustic startle response (ASR) amplitude in rats (Sheets et al., 1994). Type II pyrethroids decrease ASR, while Type I compounds increase ASR. It is important to note that thresholds for alteration of ASR and motor activity are well below doses that produced the aforementioned manifestations of T or CS syndrome (Wolansky and Harrill, 2008). The EPA (2015) calculated its margin of exposures (MOEs) for DLM by dividing 1.49 mg/kg/day, the BMDL of Wolansky et al. (2006), by its total exposure estimates. The resulting MOEs for the general U.S. population and children 1-2 years of age were 2,500 and 520, respectively. Thus, there is a large margin of safety between current DLM exposure levels and the dose required to affect the most sensitive neurotoxicity endpoint. Therefore, the modest differences we observed in DLM internal dosimetry between immature and mature animals at 100 μg/kg should be of negligible consequence toxicologically in real life. Timchalk and his colleagues described this phenomenon low-dose age equivalency for chlorpyrifos (CPF) (Timchalk et al., 2007). More pronounced inhibition of plasma and brain acetylcholinesterase (AChE) activities in pups than in adult rats at high chlorpyrifos (CPF) dosage levels was not manifest at low doses (Marty et al., 2012). A physiologically-based pharmacokinetic and pharmacodynamic PBPK/PD model for CPF did not predict differences between 3-year-old children and adults in brain or erythrocyte AChE inhibition (Hinderliter et al., 2011).

CHAPTER 5

SUMMARY

Pyrethroid insecticides are used extensively in the United States and Europe. Low-level exposure occurs there in most people. Empirical toxicokinetic (TK) data to use in risk assessments are quite limited, particularly for low doses approaching "real life" exposure levels.

The overall objective of the study was to characterize the plasma and tissue TK of a series of oral doses of deltamethrin (DLM), a type II pyrethroid, in age-dependent rats. Additional aims were to: describe the TK of an intravenous (i.v.) dose of DLM, in order to determine its bioavailability; and to assess the influence of vehicles and vehicle volume on the gastrointestinal (GI) absorption and disposition of DLM.

Serial blood samples were taken periodically via an arterial cannula from freely moving animals for up to 96 h following oral/i.v. administration of bolus doses ranging from 0.05-5.0 mg/kg in corn oil (CO) or glycerol formal (GF). Serial sacrifice was performed on group of adult, postnatal day (PND) 21, and PND 15 rats, after oral administration of bolus doses ranging from 0.1-0.5 mg/kg in CO. Plasma and tissue (brain, liver, and muscle) samples were processed and analyzed for DLM content by gas chromatography-negative chemical ionization-mass spectrometry. The resulting time-course data were analyzed to estimate standard TK parameters.

Absorption of DLM from the G.I. tract was protracted and apparently incomplete. The dosage vehicle and the vehicle volume influenced DLM's plasma TK profile. Systemic absorption of DLM was delayed when the lipophilic chemical was given orally in CO. The plasma levels subsequently diminished at about the same rate in the 1 ml/kg GF and CO groups.

As a result, AUCs were not significantly different with the different vehicles. Plasma time-courses for different dosage groups paralleled one another, typically exhibiting a progressive increase in concentration for 4-6 hours, a plateau, and a slow terminal elimination phase. The TK of DLM was, for the most part, linear over the 100-fold dosage range evaluated. Bioavailability was quite low (8-12%). A slow terminal elimination phase was noted at all dose levels, which is most likely due to prolonged DLM release from body fat.

Younger rats showed greater extent of systemic exposure compared to adults given the same dosage indicated by higher AUC. Brain, liver, and muscle concentrations were parallel to plasma concentrations. Brain concentrations were significantly lower than plasma levels, which was unexpected since DLM is a highly lipophilic compound. In both adults and 21-day-old pups, liver concentrations were lower than plasma, while higher liver concentrations exhibited in 15-day-old pups. Although TK parameters like Cmax and AUC in young rats at this dose range were generally proportional to dose, non-linearity was observed in some cases, showing evidence of saturation of systemic or brain uptake. These discoveries indicate age-dependency of DLM kinetics, which may be important in risk assessments in this dosage range.

CHAPTER 6

ABBREVIATIONS

AUC	area under the concentration versus time curve
Cl	clearance
CO	corn oil
Cmax	maximum plasma/tissue concentration
CNS	central nervous system
DLM	deltamethrin
EPA	Environmental Protection Agency
F	bioavailability
GC	Gas Chromatography
GF	glyceral formal
GI	gastrointestinal
HL	half-life
HPLC	High Performance Liquid Chromatography
i.v.	intravenous
Ka	absorption rate constant
LOD	Limit of Detection
LOQ	Limit of Quantitation
MS	Mass Spectrometry
NCI	Negative Chemical Ionization

PND	postnatal day
p.o.	per os
S-D	Sprague-Dawley
SD	standard deviation
T1/2	half-life
TK	toxicokinetics
Tmax	time to the maximum concentration
U.S.	United States
Vd	volume of distribution

REFERENCES

Anand, S.S., Kim, K.-B., Padilla, S., Muralidhara, S., Kim, H.J., Fisher, J.W., Bruckner, J.V., 2006. Ontogeny of the hepatic and plasma metabolism of deltamethrin in vitro: Role in age-dependent acute neurotoxicity. *Drug Metab. Dispos.* **34**, 389-397.

Anandon, A., Martinez-Larranaga, M.R., Diaz, M.J., Bringas, P. 1991. Toxicokinetics of permethrin in the rat. *Toxicol. Appl. Pharmacol.* **110**, 1-8.

Anandon, A., Martinez-Larranaga, M.R., Fernandez-Cruz, M.L., Diaz, M.J., Fernandez, M.C., Martinez, M.A., 1996. Toxicokinetics of deltamethrin and its 4'-HO-metabolite in the rat. *Toxicol. Appl. Pharmacol.* **141**, 8-16.

Anandon, A., Martinez, M., Martinez, M.A., Diaz, M.J., Martinez-Larranaga, M.R. 2006. Toxicokinetics of lamba-cyhalothrin in rats. *Toxicol. Lett.* **165**, 47-56.

Barr, D.B., Olsson, A.O., Wong, L.-Y., Udunka, S., Baker, S.E., Whitehead, R.D., Jr, Magsumbol, M.S., Williams, B.L., Needham, L.L., 2010. Urinary concentrations of metabolites of pyrethroid insecticides in the general U.S. population: National Health and Nutrition Examination Survey 1999-2002. *Environ. Health Perspect*. **118**, 742-748.

Coffin, J.C., Ge, R., Yang, S., Kramer, P.M., Tao, L., Pereira, M.A., 2000. Effect of trihalomethanes on cell proliferation and DNA methylation in female B6C3F1 mouse liver. *Toxicol. Sci.* **58**, 243 - 252.

Crofton, K.M., Kehn, L.S., Gilbert, M.E., 1995. Vehicle in route dependent effects of a pyrethroid insecticide, deltamethrin, on motor function in the rat. *Neurotoxicol. Teratol.* **17**, 489-495.

EPA (Environmental Protection Agency), 2011. Pyrethroid Cumulative Risk Assessment. Office of Pesticide Programs, Washington, DC.

EPA (Environmental Protection Agency), 2014. Deltamethrin: Human Health Risk Assessment, DP No. D413048. Office of Chemical Safety and Pollution Prevention, Washington, DC.

EPA (Environmental Protection Agency), 2015. Deltamethrin: A Human Health Risk Assessment for the Proposed Use of Deltamethrin as a Mosquito Adulticide Over Agricultural Crops, DP No. D417556. Office of Chemical Safety and Pollution Prevention. Washingto, DC.

Fortin, M.-C., Bouchard, M., Carrier, G., Dumas, P., 2008. Biological monitoring of exposure to pyrethrins and pyrethroids in a metropolitan population of the Province of Qu ébec, Canada. *Environ Res.* **107**, 343-350.

Foth, H., Kahl, R., Kahl, G.R., 1988. Pharmacokinetics of low doses of benzo(a)pyrene in the rat. *Food Cosmet. Toxicol.* **26**, 45-51.

Gray, A., Rickard, J., 1982. The Toxicokinetics of deltamethrin in rats after intravenous administration of a toxic dose. *Pest. Biochem. Physiol.* **18**, 205-215.

Gullick, D., Popovici, A, Young, H.C., Bruckner, J.V., Cummings, B.S., Bartlett, M.G., 2014. Determination of deltamethrin in rat plasma and brain using gas chromatography-negative chemical ionization mass spectrometry. *J. Chrom, B.* **960**, 158-165.

Helmer, F., Kiehls, K., Hansch, C., 1968. The linear free-energy relationship between partition coefficients and conformational perturbation of macromolecules by small organic compounds. *Biochemistry* **7**, 2858-2863.

Heudorf, U., Angerer, J., Drexler, H. 2004. Current internal exposure to pesticides in children and adolescents in Germany: Urinary levels of metabolites of pyrethroid and organophosphorus insecticides. *Int. Arch. Occup. Environ. Health* **77**, 67-72.

Hinderliter, P.M., Price, P.S., Bartels, M.J., Timchalk, C., Poet, T.S. 2011. Development of a source-to-outcome model for dietary exposure to insecticide residues: An example using chlorpyrifos. *Regul. Toxicol.. Pharmacol.* **61**, 82-92.

Jandacek, R.J., Rider, T., Yang, Q., Woollett, L.A., Tso, P., 2009. Lymphatic and portal vein absorption of organochlorine compounds in rats. Am. *J. Physiol. Gastrointest. Liver Physiol.* **296**, G226 - G234.

Kim, H.J., Odend'hal, S., Bruckner, J.V., 1990a. Effect of oral dosing vehicles on the acute hepatotoxicity of carbon tetrachloride. *Toxicol. Appl. Pharmacol.* **102**, 34-49.

Kim, H.J., Bruckner, J.V., Dallas, C.E., Gallo, J.M., 1990b. Effect of dosing vehicles on the pharmacokinetics of orally administered carbon tetrachloride. *Toxicol. Appl. Pharmacol.* **102**, 50-60.

Kim, K.B., Anand, S., Kim, H.J., White, C.A., Bruckner, J.V. 2008. Toxicokinetics and tissue distribution of deltamethrin in adult Sprague-Dawley rats. *Toxicol. Sci.* **101**, 197-205.

Kim, K.B., Anand, S.S., Kim, H.J., White, C.A., Fisher, J.W., Tomero-Velez, R., Bruckner, J.V., 2010. Age, dose, and time-dependency of plasma and tissue distribution of deltamethrin in immature rats. *Toxicol. Sci.* **115**:354-368

Laher, J.M., Rigler, M.W., Vetter, R.D., Barrowman, J.A., Patton, J.S., 1984. Similar bioavailability and lymphatic transport of benzo(a)pyrene when administered to rats in different amounts of dietary fat. *J. Lipid Res.* **25**, 1337 - 1342.

Laznicek, M., Laznickova, A., 1995. The effect of lipophilicity on the protein binding and blood cell uptake of some acidic drugs. *J. Pharm. Biomed. Anal.* **13**, 823-828.

Lawrence L, Casida J, 1982. Pyrethroid toxicology: mouse intracerebral structure-toxicity relationships. *Pestic Biochem Physiol* **18**:9-14

Lipinski, C.A., Lombardo, F., Dominy, B.W., Feeney, F.J., 2001. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Del. Rev.* **46**, 3-26.

Liu, X., Testa, B., Fahr, A., 2011. Lipophilicity and its relationship with passive drug permeation. *Pharm. Res.* **28**, 962-977.

Lu, C., Barr, D.B., Pearson, M., Bartell, R., Bravo, R., 2006. A longitudinal approach to assessing urban and suburban children's exposure to pyrethroid pesticides. *Environ. Health Perspect.* **114**, 1419-1423.

Lu, C.S., Schenck, F.J., Pearson, M.A., Wang, J.W., 2010. Assessing children's dietary pesticide exposure: Direct measurement of pesticide residue use and 24-hour duplicate food samples. *Environ. Health Perspect.* **118**, 1625-1630.

Marty, M.S., Andrus, A.K., Bell, M.P., Passage, J.K., Perela, A.W., Brzak, K.A., Bartels, M.J., Beck, M.J., Juberg, D.R., 2012. Cholinesterase inhibition and toxicokinetics in immature and adult rats after acute or repeated exposures to chlorpyrifos and chlorpyrifos-oxon. *Regul. Toxicol. Pharmacol.* **63**, 209-224

Narahashi T, 1996. Neuronal ion channels as the target sites of insecticides. *Pharmacol Toxicol.* **79**, 1-14

Palin, K.J., Wilson, C.G., Davis, S.S., Phillips, A.J., 1982. The effects of oils on the lymaphatic absorption of DDT. *J. Pharm. Pharmacol.* **34**, 707 - 710.

Pang, K.S., 2003. Modeling of intestinal drug absorption: Roles of transporters and metabolic enzymes. *Drug Metab. Dispos.* **31**, 1507-1519.

Power, L.E., Sudakin, D.L., 2007. Pyrethrin and pyrethroid exposures in the United States: A longitudinal analysis of incidents reported to poison centers. J. Med. Toxicol. 3, 94-99.

Ray D.E., Forshaw P.J., 2000. Pyrethroid insecticides: poisoning syndromes, synergies, and therapy. *J Toxicol Clin Toxicol* **38**:95-101.

Rickard, J., Brodie, M.E., 1985. Correlation of blood and brain levels of the neurotoxicant pyrethroid deltamethrin with the onset of symptoms in rats. *Pest. Biochem. Physiol.* **23**, 143-156.

Ross, M.K., Berazjani, A., Edwards, C.C., Potter, P.M., 2006. Hydrolytic metabolism of pyrethroids by human and other mammalian carboxylesterases. *Biochem. Pharmacol.* **71**, 657-669.

Scollon, E.J., Starr, J.M., Crofton, K.M., Wolansky, M.J., DeVito, M.J., Hughes, M.F. (2011). Correlation of tissue concentrations of the pyrethroid bifenthrin with neurotoxicity in the rat. *Toxicology* **290**, 1-6.

Scollon, E.J., Starr, J.M., Godin, S.J., DeVito, M.J., Hughes, M.F., 2009. In vitro metabolism of pyrethroid pesticides by rat and human hepatic microsomes and cytochrome P450 isoforms. *Drug Metab. Dispos.* **37**, 221-228.

Sethi, P.K., Muralidhara, S., Bruckner, J.V., White, C.A., 2014. Measurement of plasma protein and lipoprotein binding of pyrethroids. *J. Pharmacol. Toxicol. Methods* **70**, 106-111.

Sheets, L.P., Doherty, J.D., Law, M.W., Reiter, L.W., Crofton, K.M. 1994. Age-dependent difference in the susceptibility of rats to deltamethrin. *Toxicol. Appl. Pharmacol.* **126**, 186-190.

Sieber, S.M., 1976. The lymphatic absorption p, p-DDT and some structurally-related compounds in the rat. *Pharmacology* **14**, 443 - 454.

Tabarean I.V., Narahashi T, 1998. Potent modulation of tetrodotoxin-sensitive and tetrodotoxin-resistant sodium channels by the type II pyrethroid deltamethrin. *J Pharmacol Exp Ther* **284**:958-965

Tanabe, S., Nakagawa, Y., Tatsukawa, R., 1981. Absorption efficiency and biological half-life of individual chlorobiphenyls in rats treated with Kanechlor products. *Agric. Biol. Chem.* **45**, 717-726.

Timchalk, C., Kousba, A.A., and Poet, T.S. (2007). An age-dependent physiologically based pharmacokinetic/pharmacodynamic model for the organophosphate insecticide chlorpyzifos in the preweanling rat. *Toxicol. Sci.* **98**, 348-365.

Wang, X., Santostefano, M.J., Evans, M.V., Richardson, V.M., Diliberto, J.J., Birnbaum, L.S., 1997. Determination of parameters responsible for pharmacokinetic behavior of TCDD in male Sprague-Dawley rats. *Toxicol. Appl. Pharmacol.* **147**, 151-168.

Williams, M.K., Rundle, A., Holmes, D., 2008. Changes in pest infestation levels, self-reported pesticide use, and permethrin exposure during pregnancy after 2000-2001 U.S. Environmental Protection Agency restriction on organophosphates. *Environ. Health Perspect.* **116**, 1681-1688.

Wils, P., Warnery, A., Phung-Ba, V., Legrain, S., Scherman, D., 1994. High lipophilicity decreases drug transport across intestinal epithelial cells. *J. Pharmacol. Exp. Therap.* **269**, 654-658.

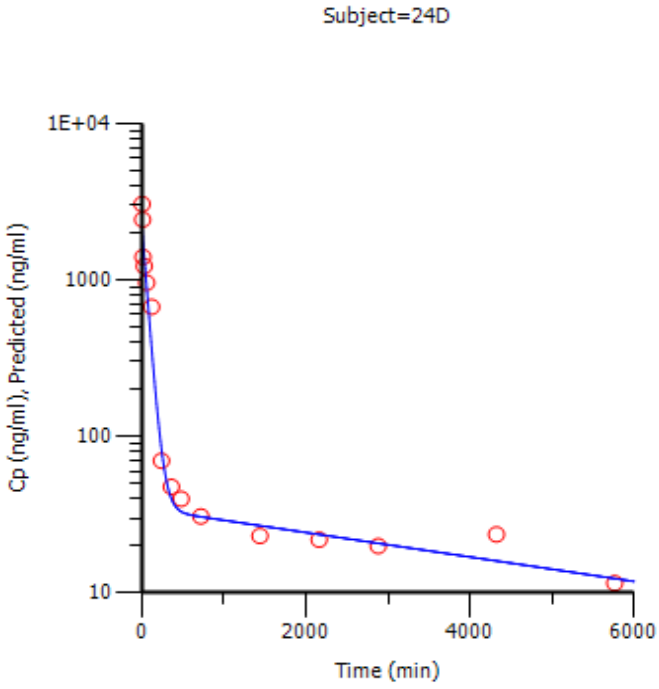
Wolansky, M.J., Gennings, C., Crofton, K. (2006). Relative potencies for acute effects of pyrethroids on motor function in rats. *Toxicol. Sci.* **89**, 271-277.

Wolansky, M.J., Harrill, J.A. (2008). Neurobehavioral toxicology of pyrethroid insecticides in adult animals. *Neurotoxicol. Teratol.* **30**, 55-78.

APPENDIX

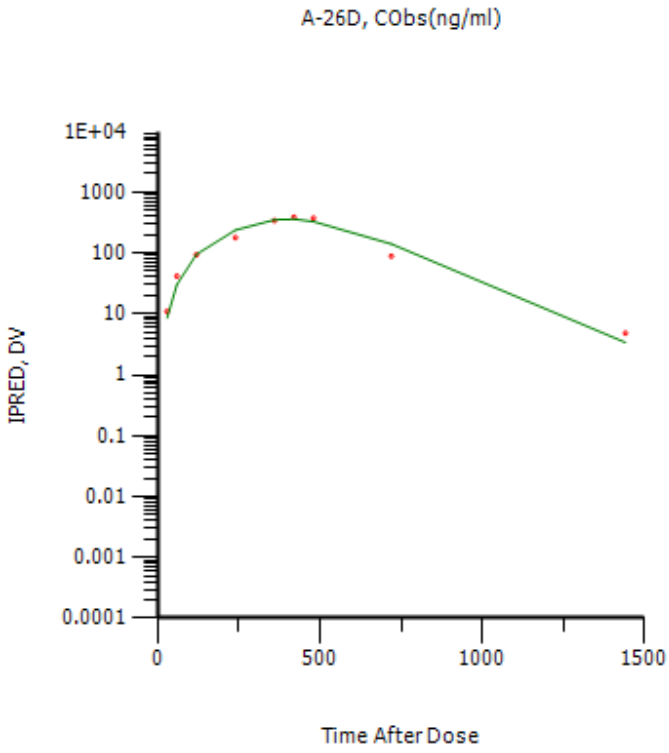
Actual data vs fitted line of one rat from each dosage group.

1. WinNonlin 6.4 i.v. 2-compartment model:

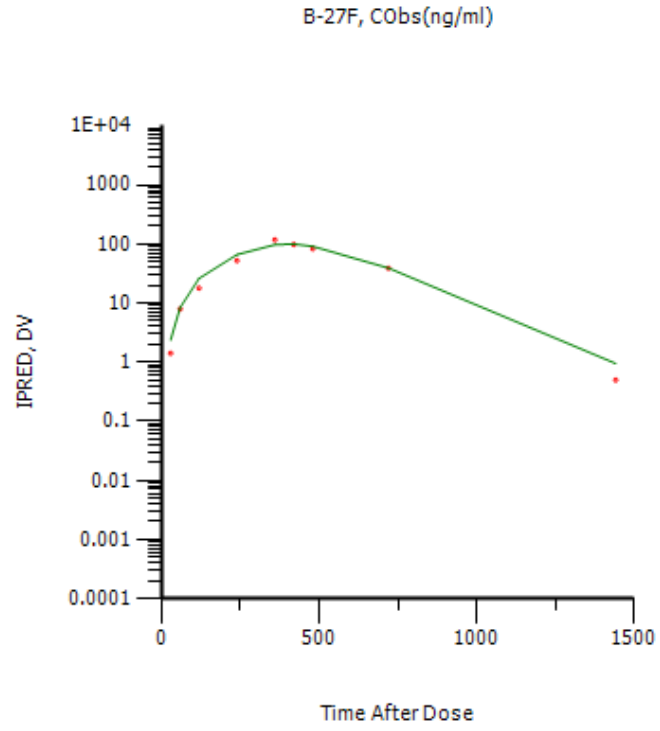


Rat 24D, 0.5 mg/kg DLM, 0.5 ml/kg GF i.v. administration.

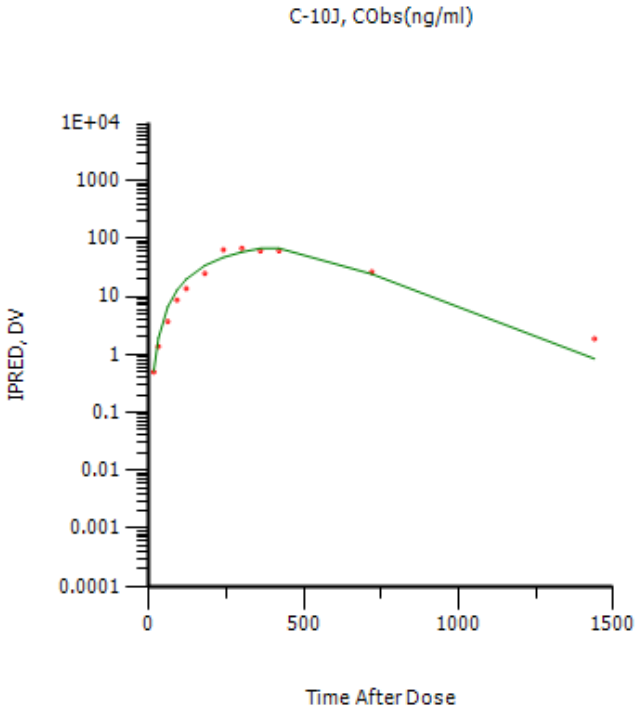
2. Pharsight 6.4 oral 1-compartment model:



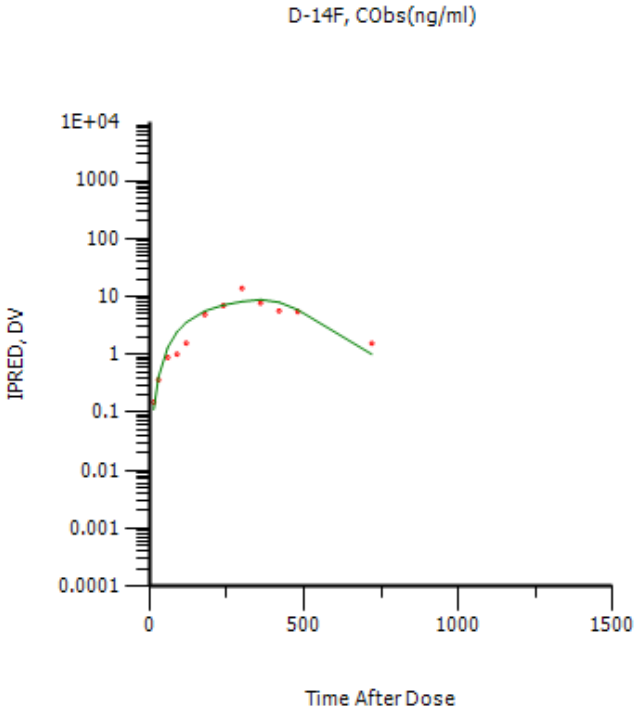
Rat 26D, 5 mg/kg DLM, 5 m/kg CO oral administration.



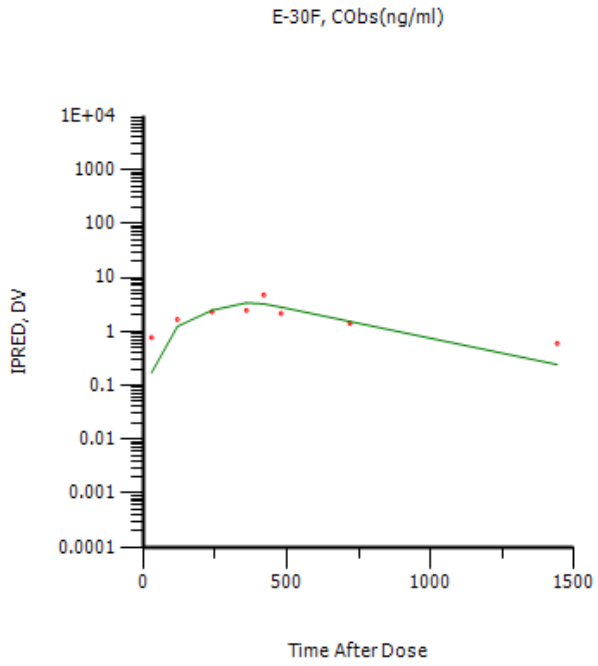
Rat 27F, 1 mg/kg DLM, 5 m/kg CO oral administration.



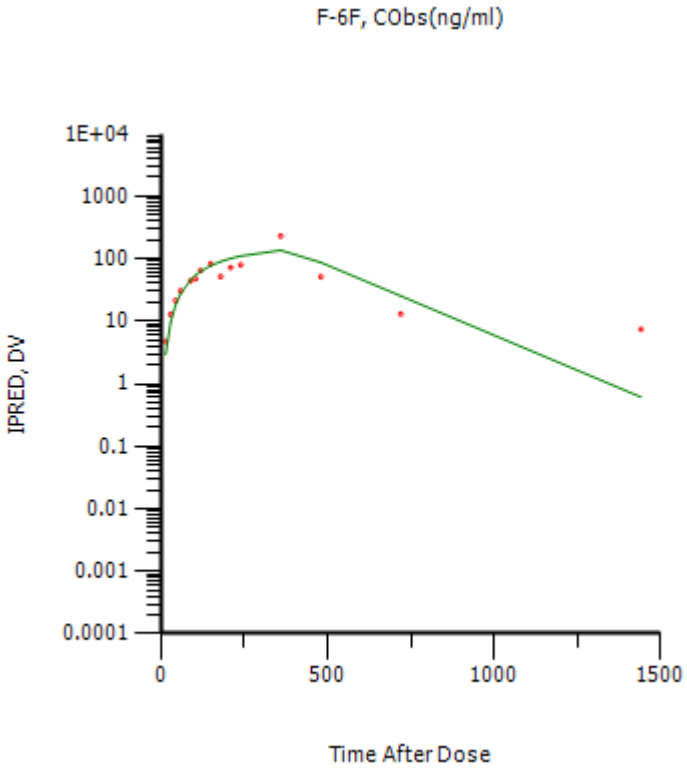
Rat 10J, 0.5 mg/kg DLM, 5 m/kg CO oral administration.



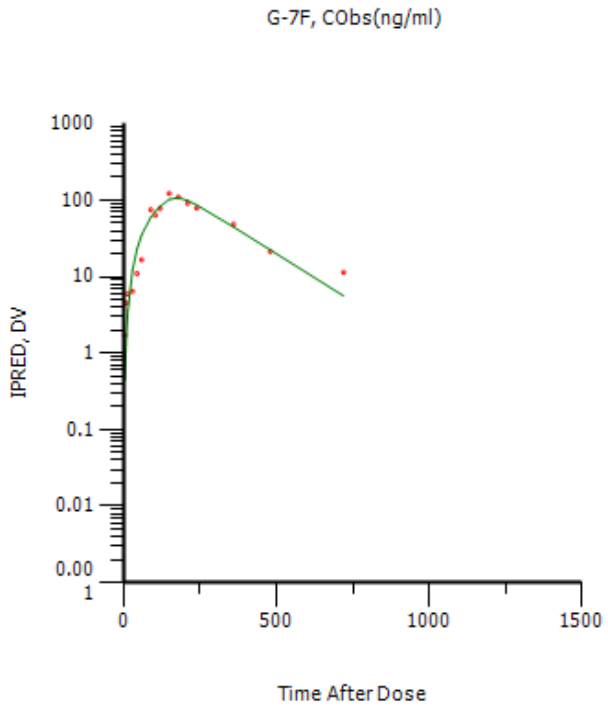
Rat 14F, 0.1 mg/kg DLM, 5 m/kg CO oral administration.



Rat 30F, 0.05 mg/kg DLM, 5 m/kg CO oral administration.



Rat 6F, 1 mg/kg DLM, 1 m/kg CO oral administration.



Rat 7F, 1 mg/kg DLM, 1 m/kg GF oral administration.