DELTAMETHRIN TOXICOKINETICS IN RATS: AGE AND DOSE DEPENDENCY

by

KYU-BONG KIM

(Under the Direction of JAMES V. BRUCKNER)

ABSTRACT

Deltamethrin (DLM) is a widely-used Type II pyrethroid insecticide and a relatively potent neurotoxicant. Previous studies have shown that immature rats are more susceptible to acute DLM neurotoxicity than adults. The main objectives of this study were: (1) to characterize the absorption, systemic/tissue distribution and elimination of DLM over a range of doses in adult rats; (2) to characterize the age- and dose-dependency of DLM toxicokinetics (TK) and tissue distribution in developing rats. New analytical method for DLM quantification in plasma and various tissues was developed and validated prior to TK studies. The limits of detection (LOD) and quantification (LOQ) were 0.01 and 0.05 μg DLM/ml or g, respectively. Selection of proper vehicles for DLM administration was an issue. To assess the influence of two common vehicles, Alkamuls® (AL, formerly Emulphor®) and glycerol formal (GF), on the bioavailability and toxicokinetics (TK) of DLM, adult Sprague-Dawley (S-D) male rats were administered DLM iv or po, either by dissolving it in GF or in AL. Bioavailability and target organ (brain) levels were significantly higher when the insecticide was given orally to rats in GF rather than as an unstable aqueous suspension in AL. To characterize the TK of DLM, adult male S-D rats were dosed orally with 0.4, 2 or 10 mg DLM/kg dissolved in GF. Another group received 2 mg DLM/kg iv. Oral bioavailability was low, and very small proportions of systemically-absorbed doses reached the brain, the target organ of DLM. Fat, skin and muscle accumulated relatively

large amounts of the highly lipophilic chemical and served as slow-release depots during elimination. Tissue deposition was dose-dependent, though not directly proportional to dose. Elimination kinetics were linear in this dosage range. To determine whether the susceptibility of immature rats to acute DLM neurotoxicity was due to age-dependent TK, DLM was administered via gavage at 0.4, 2 or 10 mg DLM/kg to male S-D rats of postnatal days (PND) 10, 21, and 40. Plasma and target organ (brain) DLM levels, like the magnitude of acute neurotoxicity, were inversely related to age. Inadequate metabolic detoxification capacity in the youngest pups was an important contributor to their susceptibility to DLM poisoning. These TK plasma and tissue time-course data will be invaluable in developing physiological models to use in predicting children's target organ dosimetry and risks for different exposure scenarios.

INDEX WORDS: Deltamethrin, Pyrethroid Insecticide, Neurotoxicity, Internal dosimetry, Toxicokinetics, Bioavailability, Tissue Distribution, HPLC, Adult and Developing Rats,

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DEDICATION TO MY FAMILY,

ESPECIALLY MY FATHER, WEE-JIN, AND NIECE, DASOM

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ABBREVIATIONS

AL	Alkamuls®
AUC	Area under the DLM concentration versus time curve
BBB	Blood brain barrier
Cl	Clearance
Cmax	The maximum plasma or tissue concentration
CNS	Central nervious system
DLM	Deltamethrin
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
GF	Glycerol formal
GI	Gastrointestinal
HDL	High density lipoprotein
HPLC	High-performance liquid chromatography
ICH	International Conference on Harmonization of Technical Requirements for Registarion of Pharmaceuticals for Human Use
Ka	Absorption rate constant
LDL	Low density lipoprotein
LOD	Limit of detection
LOQ	Limit of quantitation
OP	Organophosphate
PAH	Polyaromatic hydrocarbon
PBT(P)K	Physiologically-based toxicokinetic (pharmacokinetic)
PC	Partition coefficient
PCB	Polychlorinate biphenol
P-gp	P-glycoprotein
PND	Postnatal day
T1/2	Elimination half-life (h)
R.S.D	Relative standard deviation
S-D	Sprague-Dawley
Tmax	The time to maximum concentration
Vd	Volume of distribution

INTRODUCTION/LITERATURE REVIEW

Over a decade after the well-known apple crisis involving Alar® in 1989, overall use of pesticides has continuously increased. There is a great deal of concern that exposure of infants and children may result in relatively serious acute poisoning and in possible neurological dysfunction in adulthood. Many pesticides are generally believed to be more harmful to children than adults (Pogoda and Preston-Martin, 1997; Guillette et al., 1998; Bruckner, 2000). It is likely that the exposures of infants and children to pesticides are frequently greater than in adults due to their rapid breath rate per body weight (NRC, 1993), crawling and mouth activity, and greater consumption of fruits and vegetables contaminated with insecticides. Moreover, the nervous system rapidly grows and develops after birth. Most pesticides are designed to impair the nervous system and seem to be much more injurious to the developing nervous system (Casida and Quistad, 1998; Shafer et al., 2005). Once damaged by chemicals, children's developing nervous system changes are hard to reverse. The dysfunction may elicit the loss of intelligence and alteration of normal behavior in the future. A study of Mexican children exposed to a mixture of agricultural chemicals showed impacts on motor skills, memory, attention, and learning (Guillette et al., 1998). Researches clearly show that immature rats are more sensitive than adults to acute and subacute neurotoxicity of organophosphates mediated by cholinesterase inhibition (Atterberry et al., 1997; Moser and Padilla, 1998). An experiment by Sheets et al. (1994) revealed the same phenomenon for high doses of deltamethrin (DLM), a synthetic pyrethroid insceticide.

Exposure to pyrethroids has been widely documented in pregnant women, infants and children (Schettgen et al., 2002; Whyatt et al., 2002; Berkowitz et al., 2003; Heudorf et al., 2004). Heudorf et al. (2004) recently reported that pyrethroid metabolites were found in 75% of

an urban German population without occupational exposure to the insecticide. Levels in children were comparable to those in adults. Currently, 16 pyrethroids are registered for use in the U.S. in a variety of agricultural and consumer products (Bryant and Bite, 2003). Deltamethrin (DLM) is the active ingredient in 27 products marketed in the U.S., including products used by professional applicators and the general public (CALEPA, 2001). Registered uses of DLM include structural pest control (e.g., termites), turf (e.g., fire ants), indoor pests (e.g., ants and roaches), and pet collars. When applied indoors as an aqueous liquid, DLM has been shown to persist at measurable levels for at least 2 years (Berger-Preib et al., 1997). Levels in settled dust and indoor air soon after standard application were ~50 mg/kg and 3 $\mu g/m^3$, respectively. After 1 year, concentrations stabilized at ~5 mg/kg in settled dust and ~2 ng/m^3 in door air. These data indicate that relatively high-level acute exposures and lower-level chronic exposures of infants and children can occur in residential environments.

Pyrethroid insecticides are widely used in agriculture to protect crops, in the household to control pests, and in public health to control diseases caused by vectors or intermediate hosts (IPCS, 1990; Soderlund et al., 2002). Pyrethroids accounted for about 25% of the worldwide insecticide market in 1998 (Casida and Quistad, 1998). That percentage share has increased substantially over the last few years in the U.S. (Lee et al., 2002), as a result of the U.S. Environmental Protection Agency's (EPA's) restrictions on household and agricultural use of organophosphates. Pyrethroids' popularity also stems from their insecticidal potency, slow development of pest resistance, and relatively low toxicity of most congeners in mammals.

Traditionally, pyrethroids are divided into two classes, based upon their chemical structure and clinical manifestations of acute exposure. Type I compounds do not contain a cyano moiety, while Type II do. Hyperexcitation, tremors and skin parathesias are the most

common signs of acute poisoning by Type I compounds. Type II pyrethroids' "hallmark" effects are salivation, tremors and choreoathetosis (Lawrence and Casida, 1982; Ray and Forshaw, 2000). Voltage-sensitive sodium channels in central nervous system neurons appear to be their principal site of action (Narahashi, 1996). Deltamethrin (DLM), [*(S)*-α-cyano-d-phenoxybenzyl-(1*R*,3*R*)-e-(2,2-dibromovinyl)-2,2-dimethylcyclo-propane-1-carboxylate] is one of the most neurotoxic pyrethroids. It acts by delaying closure of sodium channels, resulting in a tail current that is characterized by a slow influx of sodium during the end of neuronal depolarization (Chinn and Narahashi, 1986; Tabarean and Narahashi, 1998).

It has been recognized for almost 30 years that biotransformation of pyrethroids occurs primarily via two pathways: hydrolysis of the molecules' ester linkage by esterases; and aromatic hydroxylation by cytochrome P450s, with subsequent conjugation (Ruzo et al., 1978; Rickard and Brodie, 1985). Anand et al. (2006a) recently found that carboxylesterases (CaEs) in rat liver and plasma, as well as rat liver cytochrome P450s 1A1, 1A2 and to a lesser degree 2C11, are primarily responsible for DLM biotransformation *in vitro*. Intrinsic clearance by plasma CaEs is far less important quantitatively than metabolism in the liver. Anand et al. (2006b) also found that limited capacity of these enzymes contributes significantly to increased systemic exposure and neurotoxic effects of the toxic parent compound in immature rats.

DLM, the compound chosen for the current study, is one of the most potent pyrethroids *in vitro* (Choi and Soderlund, 2006) and *in vivo* (Wolansky et al., 2006a). Rickard and Brodie (1985) find that the onset and severity of signs of DLM poisoning in mice are proportional to brain levels of DLM. Additional evidence that the parent pyrethroid is the primary toxic moiety is furnished by Lawrence and Casida (1982). They report that intracerebral injection of DLM produces signs of poisoning in mice within a min or less. It is also reported that inhibitors of

enzymes that metabolize pyrethroids exacerbate their toxicity (Soderlund and Casida, 1977; Casida et al., 1983).

DLM is a colorless, crystalline powder with a melting point at 98-101°C. It is odorless, and non-corrosive (IPCS, 1990). Its molecular weight is 505.2 g/mol, and it has very low solubility of 2 µg/l in water at 20°C. DLM is soluble in most aromatic solvents, acetone, ethanol, and dioxane. DLM is a very stable compound and does not degrade at 40°C for two years. It is resistant to air and photo-oxidation. The common formulations of DLM are emulisifiable concentrates, wettable powder, dusts, granules, and flowable powder. DLM is apparently absorbed to a limited extent from the GI tract. It is also readily absorbed by inhalation of spray mist. Dermal absorption is incomplete in mammals (Perger and Szadkowski, 1994), including rats (Kavlock et al., 1979). There is inadequate evidence for the carcinogenicity of DLM in animals. DLM is not classifiable as to its carcinogenicity to humans (Group 3) (IARC, 1991). It is non-phytotoxic (Tomlin, 1994) and non-genotoxic (IPCS, 1990). No evidence of teratogenic activity was observed in mice, rats or rabbits even at doses sufficient to produce clinical signs of toxicity in pregnant dams (IPCS, 1990). DLM was slightly embryotoxic in a 3-generation rat study, but did not adversely affect reproduction (IPCS, 1990). Acceptable daily intakes for DLM have been set to 0.01 mg/kg by the Joint FAO/WHO Meeting on Pesticide Residue (IPCS, 1990).

Several studies have demonstrated that immature rats are more susceptible to acute DLM neurotoxicity than adults. The exposed pups may exhibit residual effects later in life. Sheets et al. (1994) determined oral LD_{50}s for 11-, 21- and 72-day-old rats to be 5, 11 and 81 mg DLM/kg, respectively. There have been studies of altered behavior and learning associated with changes in levels/binding of neurotransmitters in the offspring of rats dosed with DLM (0.08 mg/kg) during pregnancy (Aziz et al., 2001; Lazarini et al., 2001). Eriksson and Fredriksson (1991) were among

the first to demonstrate residual neurological effects following neonatal pyrethroid exposure. Ten-day-old male NMRI mice, given orally 0.7 mg DLM or bioallethrin/kg daily for 7 days, exhibited increased spontaneous motor activity and decreased density of muscarinic cholinergic receptors in the cerebral cortex at the adult age of 4 months. Oral administration of 7 mg DLM/kg to weanling rats on days 22-37 postpartum produced significant neurochemical and behavioral deficits (Husain et al., 1994).

Oral dosage vehicles can have a marked effect on the acute toxicity of pyrethroids. Published acute LD_{50} values for most pyrethroids given orally to rats in corn or other vegetable oils range from 50 to 500 mg/kg (Soderlund et al., 2002). Oral LD_{50} values listed for DLM ranged from 87 mg/kg (in corn oil) to > 5,000 mg/kg (in a 1% methylcellulose aqueous suspension). Pham et al. (1984) reported that oral DLM was 100 times less toxic to rats when suspended in gum Arabic solution than when dissolved in glycerol formal (GF). Crofton et al. (1995) assessed the ability of DLM to depress motor activity of rats gavaged with DLM in four different vehicles. The chemical was equipotent in corn oil and GF. Its potency was substantially lower when administered in Emulphor®, and even lower when given as an aqueous suspension in methylcellulose. Crofton and his co-workers (1995) emphasized the need for toxicokinetic (TK) studies to clarify the reason(s) for the vehicle-dependent differences they observed in acute DLM neurotoxicity.

The EPA has been given responsibility in the U.S. for assessing pesticides and other chemicals that may pose risk to the health of children and adults. The degree and duration of toxicity are largely dependent upon the concentration of toxicant in the target tissue and how long it remains there. Physiologically-based pharmacokinetic (PBPK) models are a useful tool in risk assessment, in that they can be used to predict concentrations of toxic forms of chemicals in

target organs (e.g., brain) over time following a variety of exposure scenarios (Beck and Clewell, 2001; Andersen, 2003). Construction of a PBPK model for a toxic chemical requires data from toxicology and PK studies in animals. Mature and immature rodents are commonly used as surrogates for adults and children. Inherent limitations of such studies include, among others, small biological sample size and limitation of the dose of chemical that can be given, due to toxicity. TK data for DLM and most other pyrethroids are very limited and in some cases contradictory. Anadon and his co-workers have reported some of the most comprehensive TK studies of permethrin (1991), DLM (1996) and lambda-cyanohalothrin (2006). These investigators delineated the time-courses of the parent compounds in the plasma, multiple regions of the brain and one or two other tissues after administration of one toxic dosage level to rats. Pyrethroid levels in most brain areas were found to be substantially higher than plasma levels. This observation was in direct contrast to that of Gray and Rickard (1982) and Rickard and Brodie (1985), who measured markedly higher ^{14}C-DLM concentrations in rat plasma than in brain. Marei et al. (1982) calculated the DLM half-life in brain of orally-dosed rats to be similar to that reported by Anadon et al. (1996) for plasma, though the former research group found the half-life to be 5 days for fat. Common deficiencies in studies from which the current DLM TK database (ATSDR, 2003) was obtained include: use of frankly toxic doses; administration of a single dosage, sometimes by an irrelevant exposure route; lack of data for key organs and tissues; and failure to sample long enough post dosing to delineate elimination profiles and TK indices. Hence, it is important to clarify the comprehensive TK and brain distribution of DLM across age and different doses for the future children's PBPK modeling and risk assessment.

The specific aims of the current project are: (1) to develop a more rapid and sensitive HPLC technique to quantify DLM in the large numbers of low-volume blood and tissue samples generated in PK studies in small animals; (2) to investigate the influence of two common vehicles/diluents on the bioavailability, TK and acute neurotoxicity of orally-administered DLM; (3) to conduct a comprehensive investigation of the TK of DLM over a range of oral doses in the adult rat; (4) to determine whether the susceptibility of immature rats to acute DLM neurotoxicity was due to age-dependent toxicokinetics of DLM.

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CHAPTER 1

RAPID DETERMINATION OF THE SYNTHETIC PYRETHROID INSECTICIDE, DELTAMETHRIN, IN RAT PLASMA AND TISSUES BY HPLC[1]

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[1]Kyu-Bong Kim, Michael G. Bartlett, Sathanandam S. Anand, James V. Bruckner, and Hyo Jung Kim (2006) *Journal of Chromatography B* 834: 141-148

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ABSTRACT

Deltamethrin (DLM), [*(S)*-α-cyano-d-phenoxybenzyl-(1*R*,3*R*)-e-(2,2-dibromovinyl)-2,2-dimethylcyclo-propane-1-carboxylate], is a pyrethroid insecticide widely used in agriculture and households. There are several methods for analysis of DLM in biological fluids and tissues, but these methods are time consuming. They generally involve the extraction of DLM with lipid-soluble solvents such as *n*-pentane, *n*-hexane, diethylether or acetone, and subsequent evaporation of the solvent. A more rapid and sensitive high-performance liquid chromatography (HPLC) method to analyze DLM in plasma and tissues (liver, kidney, and brain) was developed and validated according to U.S. Food and Drug Administration and International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines. The limit of detection (S/N of 3/1) for DLM was 0.01 µg/ml for plasma, liver, kidney and brain. The method performances were shown to be selective for DLM and linear over the concentration range 0.01-20.0 µg/ml. For five replications of samples at 0.05, 0.1, 0.2, 1.5 and 4.0 µg/ml, intraday precision and accuracy values were in the range of 0.7 to 13.1% relative standard deviation (%R.S.D.) and 1.8 to 14.1 %Error, respectively. Interday (n = 15) precision and accuracy values at 0.05, 0.1, 0.2, 1.5, and 4.0 µg/ml were in the range of 3.2 to 15.2% (%R.S.D.) and 3.7 to 14.8 %Error, respectively. The absolute recoveries of DLM ranged from 93 to 103% for plasma, 95 to 114% for liver, 97 to 108% for kidney, and 95 to 108% for brain. This method can be quite useful for DLM pharmacokinetic and tissue distribution studies, for which multiple plasma and tissue samples have to be analyzed quickly with high reproducibility.

INTRODUCTION

Pyrethroid insecticides are widely used in agriculture to protect crops, in the household to control pests, and in public health to control diseases caused by vectors or intermediate hosts (IPCS, 1990; Soderlund et al., 2002). Pyrethroids accounted for about 25% of the worldwide insecticide market in 1998 (Casida and Quisad, 1998). That percentage share has increased substantially over the last few years in the U.S. (Lee et al., 2002), as a result of the U.S. Environmental Protection Agency's (EPA's) restrictions on household and agricultural use of organophosphates. Pyrethroids' popularity also stems from their insecticidal potency, slow development of pest resistance, and relatively low toxicity of most congeners in mammals.

There have been a variety of approaches developed since the 1970s to quantify pyrethroids in biological samples. These approaches can be categorized as biological, immunological and chemical. Biological assays such as the LC_{50} (i.e., concentration required to kill 50% of an insect population) were carried out by entomologists (Sommer et al., 2001; Vale et al., 2004). Immunoassays were developed to rapidly detect trace levels of pyrethroids in environmental and food samples (Lee et al., 1998; Lee et al., 2003). Chemical assays have included gas chromatography (Valverde et al., 2001; Ramesh and Ravi, 2004), thin-layer chromatography (Cole et al., 1982; Akhtar et al., 1986), and high-performance liquid chromatography (HPLC) with ultraviolet detection. HPLC methods have been utilized to determine concentrations of the pyrethroid deltamethrin (DLM) in milk, foods, environmental specimens, blood and various tissues (Mourot et al., 1979; Yao et al., 1992; Anadon et al., 1996; Bissacot and Vassilieff, 1997; Ding et al., 2004a). Isolation and concentration of DLM in samples has involved a combination of liquid-liquid and solid phase extraction.

Deltamethrin (DLM), [*(S)*-α-cyano-d-phenoxybenzyl-(1*R*,3*R*)-e-(2,2-dibromovinyl)-2,2-dimethylcyclo-propane-1-carboxylate] (Fig. 1-1), is one of the most neurotoxic pyrethroids. It acts by delaying closure of sodium channels, resulting in a tail current that is characterized by a slow influx of sodium during the end of neuronal depolarization (Chinn and Narahashi, 1986; Tabarean and Narahashi, 1998). Typical signs and symptoms of acute poisoning of laboratory animals and humans by DLM and other Type II pyrethroids include salivation, hyperexcitability and choreoathetosis. Immature rats are much more susceptible to acute DLM neurotoxicity than adults (Sheets et al., 1994; Sheets, 2000), due in large measure to inefficient metabolic detoxification of the parent compound (Anand et al., 2006). DLM is hydrolyzed by esterases and hydroxyated by cytochrome P450s (Ruzo et al., 1978; Villarini et al., 1995; Anand et al., 2005). At present there is concern that DLM and possibly other pyrethroids, like certain organophosphates, may exhibit potential to be developmental neurotoxicants in infants and children (Eriksson and Fredriksson, 1991; Shafer et al., 2005).

The EPA has been given responsibility in the U.S. for assessing pesticides and other chemicals that may pose risk to the health of children and adults. The degree and duration of toxicity are largely dependent upon the concentration of toxicant in the target tissue and how long it remains there. Physiologically-based pharmacokinetic (PBPK) models are a useful tool in risk assessment, in that they can be used to predict concentrations of toxic forms of chemicals in target organs (e.g., brain) over time following a variety of exposure scenarios (Beck and Clewell, 2001; Andersen, 2003). Construction of a PBPK model for a toxic chemical requires data from toxicology and PK studies in animals. Mature and immature rodents are commonly used as surrogates for adults and children. Inherent limitations of such studies include, among others, small biological sample size and limitation of the dose of chemical that can be given, due to

toxicity. Few PK data for DLM are therefore available. Anadon et al. (1996) did conduct an experiment with adult rats, but had to administer a neurotoxic dose [i.e., 26 mg DLM/kg body weight (bw)] in order to characterize a relatively complete time-course of DLM in the blood and brain. Immature rats succumb to this and lower doses. Therefore, analytical sensitivity is a key consideration in development of an analytical technique suitable for DLM kinetics time-course studies in small animals and their offspring. Detection limits as low as 0.001 and 0.005 µg/ml of sample have been reported (Bissacot and Vassilieff, 1997; Anadon et al., 1996), but these HPLC procedures were not adequately validated, were time consuming and required large sample volumes. Serial blood sampling and sacrifices of groups of animals in PK time-course experiments produce a myriad of samples to be analyzed. To our knowledge, only Ding et al. (2004a, b) reported a fully-validated HPLC analytical method for DLM. Its limit of quantitation was 0.1 µg/ml plasma. The method utilized protein precipitation with acetonitrile rather than liquid-liquid or solid-phase extraction for sample preparation. It was not applied to liver, kidney or brain and was somewhat time consuming, in that it required solvent extraction and evaporation to dryness.

The objective of the current effort was to develop a more rapid and sensitive HPLC technique to quantify DLM in the large numbers of low-volume blood and tissue samples generated in PK studies in small animals. An important aim was to validate the procedure by U.S. FDA (2001) and ICH (1994) guidelines for analytical method validation including accuracy, precision, linearity, limit of detection, limit of quantitation, recovery and stability.

METHODS

Chemicals and animals. Standard DLM (purity, 98.8%) was kindly provided by Bayer CropScience AG (Monheim, Germany). Acetonitrile (HPLC-grade) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol, sulfuric acid and deionized water (HPLC-grade) were obtained from J.T. Baker (Phillipsburg, NJ, USA)

Male Sprague-Dawley (SD) rats (Charles River Laboratories, Raleigh, NC, USA) were used to provide blank biological matrices and for PK studies with DLM. The protocol for this study was approved by the University of Georgia Animal Care and Use Committee. Each rat was housed in a cage with a 12-h light/dark cycle at ambient temperature (22°C) and relative humidity (55 ± 5%). Food (5001 Rodent Diet, PMI Nutrition International LLC, Brentwood, MO, USA) and tap water were provided *ad libitum*.

Preparation of stock, standard solutions, and calibration standards. A DLM stock solution was prepared in acetonitrile at a final concentration of 1.0 mg/ml. The stock solution was stored in a freezer at -20°C, though DLM is generally believed to be stable at least 6 months at room temperature (IPCS, 1990). Working standard solutions with concentrations of 0.05, 0.1, 0.5, 1.25, 1.5, 2.5, 3.75, 5.0, 10.0, 15.0, 25.0, and 100 µg/ml were prepared by appropriate dilution of the 1.0 mg/ml stock solution with acetonitrile. Plasma, liver, kidney and brain specimens were collected from SD rats for blank matrices. Mixtures of appropriate volume (30 µl) of a working solution and blank matrix (120 µl) were prepared for calibration standards. The final calibration standards were 0.01, 0.02, 0.1, 0.25, 0.3, 0.5, 0.75, 1.0, 2.0, 3.0, 5.0, 20.0 µg DLM/ml.

Analysis system. The analysis was conducted with a HPLC system consisting of a Shimadzu HPLC (Shimadzu, Canby, OR, USA) equipped with a pump (LC-10AT), degasser (DGU-14A), auto-sampler (SIL-HT), detector (SPD-10AV) and computer with an EZStart 7.2 SP1 Rev B.

The analytical column was an Ultracarb 5 ODS (20) column (250 x 4.6 mm; 5 µm particle) (Phenomenex, Torrance, CA, USA), and the guard column was a Phenomenex fusion RP 4 mm x 3 mm (Torrance, CA). The mobile phase was 80% acetonitrile and 20% sulfuric acid (1%, v/v) (v/v). The flow rate was set at 1.0 ml/min. The eluate was monitored at 230 nm. Under these chromatographic conditions, DLM eluted at approximately 14.5 min.

Extraction procedure

Plasma. To generate plasma, blood samples were collected in heparinized tubes were centrifuged for 5 min at 13,000 rpm using a microcentrifuge (Microfuge 22R Centrifuge, Beckman Coulter, Fullerton, CA, USA). Sixty-five µl of plasma were added to microcentrifuge tubes containing 130 µl of acetonitrile. These were vigorously agitated on a vortex mixer (Mini Vortexer, VWR, West Chester, PA, USA) for 30 s. The tubes were then centrifuged for 5 min at 13,000 rpm in the microcentrifuge, and 50 µl of the clear supernatant injected onto the column.

Liver, kidney, and brain. Liver, kidney and brain were isolated from the rats and homogenized in 4 volumes of 50% (v/v) acetonitrile in distilled water with a Tissumizer (Tekmar, Cincinnati, OH, USA). Sixty-five µl of the tissue homogenates were added to microcentrifuge tubes containing 130 µl of acetonitrile. These tubes were vigorously mixed with a vortex mixer for 30 s and centrifuged for 5 min at 13,000 rpm in the microcentrifuge. Fifty µl of the clear supernatant were injected onto the column.

Method validation

Preparation of samples. Thirty µl of DLM were added to 120 µl of blank plasma or tissue homogenate in a 1.5-ml microcentrifuge tube. The final concentrations of samples were 0.05, 0.1, 0.2, 1.5, and 4.0 µg/ml, respectively. This mixture was vortexed for 10 s and then used.

Specificity. Blank samples were analyzed as described, and the chromatograms were visually evaluated for occurrence of substances that might interfere with the DLM peak.

Absolute recovery, accuracy and precision. Absolute recovery was calculated from the peak areas of DLM in plasma, liver, kidney and brain compared with those of standard solutions. For intraday accuracy and precision, the samples (n = 5) spiked at concentrations of 0.05, 0.1, 0.2, 1.5, and 4.0 µg/ml were analyzed. The accuracy was expressed as the absolute error percentage and calculated from (|mean of measured concentration – added concentration|/added concentration) x100. The precision was expressed as the relative standard deviation (%R.S.D.) and calculated from the standard deviation divided by the mean of the detected concentration. Interday accuracy and precision were determined in 5 replicates of the biological sample spiked at concentrations of 0.05, 0.1, 0.2, 1.5, and 4.0 µg/ml and performed on 3 different days.

Limit of detection and limit of quantification. The limit of detection (LOD) was defined as the lowest concentration of DLM (S/N = 3), and the limit of quantitation (LOQ) was set at the lowest validation point.

Linearity of calibration curve. The calibration curves were obtained by peak area vs. DLM concentration.

Application to pharmacokinetic studies. Pharmacokinetic tissue distribution experiments with adult (~ 90-day-old) and immature (10-day-old) male SD rats were conducted. Blood was serially collected from the same adult animals to decrease intersubject variability in the pharmacokinetic profiles. Each adult rat was anesthetized by IM injection of 0.1 ml/100 g bw of a "cocktail" consisting of ketamine hydrochloride (100 mg/ml), acepromazine maleate (10 mg/ml), and xylazine hydrochloride (20 mg/ml) (3:2:1, v:v:v). A cannula (PE50 polyethylene tubing) was surgically inserted into the right carotid artery and securely ligated. The cannula

was passed under the skin and exteriorized at the nape of the neck, so the animals could move about freely following their recovery. Water was provided but food was withheld during the 24-h post-surgical recovery period before dosing. The cannulated adult rats were given a single oral dosage of 10 mg DLM/kg bw (in 2 ml glycerol formal/kg bw). Arterial blood samples of 150 µl were collected from the indwelling cannula from 4 animals (0.25, 0.5, 1, 2, 4, 6, 9, 12, and 24 h post dosing). Plasma samples were processed by the procedure previously described. In order to study DLM deposition in brain, adult rats (n=5) per time-point were given 10 mg DLM/kg bw orally (in 2 ml glycerol formal/kg bw) and euthanized by CO_2 asphyxiation 0.5, 1, 2, 6, 12 and 24 h after dosing. Ten-day-old immature rats (n=3 per time-point) were given 2 mg DLM/kg bw orally (in 2 ml glycerol formal/kg bw) for determination of plasma, brain, liver and kidney disposition. They were decapitated 0.5, 1, 2, 6, 12 and 24 h after dosing. Tissue sample preparation was as described previously.

RESULTS AND DISCUSSION

Analytical method

The HPLC method proved to be rapid and sensitive for quantitation of DLM in blood and tissues. Other published HPLC methods (Mourot et al., 1979; Yao et al., 1992; Anadon et al., 1996; Bissacot and Vassilieff, 1997; Ding et al., 2004a) are laborious, in that they require day-long evaporation to dryness and have longer column retention times. With the current method, DLM was extracted from plasma by vortexing it in twice its volume of acetonitrile for 30 s. Acetonitrile in water (50%, v/v) was initially used to homogenize tissues to achieve better extraction. DLM was extracted by adding this homogenate to twice the volume of acetonitrile.

An aliquot of supernatant was injected directly onto the column without taking the samples through an evaporation process. Eliminating acetonitrile volatilization enabled us to analyze many more samples in a day. This is essential for PK studies, as described in the Introduction. DLM is a highly lipophilic compound with an octanol-water partition coefficient (K*ow*) of 4.53 (Laskowski, 2002). This value means that DLM can be transferred much more efficiently into the lipid-soluble solvent, acetonitrile, than water. Thus, acetonitrile acts not only to precipitate proteins but to solubilize the highly lipophilic DLM. Evaporation of solvent and reconstitution are thereby avoided, and acetonitrile containing DLM can be injected directly into the HPLC.

The chemical structure and chromatographic separation of DLM are shown in Figs. 1-1 and 1-2, respectively. The retention time was 14.5 min when a C18 column was used. With a C8 column, the retention time was 9.1 min. Baseline resolution was achieved using the chromatographic conditions described in the Experimental section. DLM was completely separated, as no interfering or co-eluting peaks with similar retention times were found in the chromatograms of blank biological samples (Fig. 1-2).

Method validation for plasma, liver, kidney, and brain

The calibration curves for plasma, liver, kidney, and brain showed good linearity over the range from 0.01 to 20.0 µg/ml for DLM ($r^2 > 0.998$ in all cases). The LOD for DLM was determined by analysis of standard-spiked samples of gradually decreasing concentration. The LOD was defined as the concentration at which the signal/noise ratio was ~ 3. The LOD and LOQ were found to be approximately 0.01 and 0.05 µg/ml, respectively. This LOQ is lower than that reported by Ding et al. (Ding et al., 2004a), and the present method's LOD is 3-fold lower. Although other investigators have reported even lower LODs (0.001 µg/ml by Bissacot

and Vassilieff (1997) and 0.005 µg/ml by Anadon et al. (1996)), their methods' applications to pharmacokinetic studies have the following limitations: 1) Both methods utilized time-consuming solvent evaporation, and Bissacot and Vassilieff (1997) also used a complex extraction procedure; 2) Both methods required very large volumes of blood, which is a limiting factor if these methods are to be applied to mice, young rats or serial sampling from an adult rat or larger animal; and 3) Neither method was fully validated.

As shown in Table 1-1, absolute recoveries of DLM from spiked plasma, liver, kidney and brain were in the range of 93 – 114%. The present extraction efficiencies (93 – 103% recovery from rat plasma) were higher than those (91% and 83 – 89%) for HPLC methods previously developed by our laboratory (Ding et al., 2004a, b). This observation implies that the evaporation and reconstitution steps formerly used may be responsible for the lower recoveries of DLM. Recoveries of DLM from rat liver (95 – 114%), kidney (97 – 108%), and brain (95 – 108%) were also higher than from those achieved from placenta or fetal tissues (Ding et al., 2004b).

Intraday and interday accuracy and precision were determined to evaluate the reliability of the current analytical method. The intraday and interday accuracy and precision were evaluated using 0.05, 0.1, 0.2, 1.5 and 4.0 µg DLM/ml (Tables 1-2 and 1-3). These concentrations were similar to those employed previously by Ding et al. (2004a). Both intra- and interday accuracy and precision for DLM in plasma and tissues were between 1.8 and 14.8 (%Error), and 0.7 and 15.2 (% R.S.D.), respectively. The inter- and intraday accuracy and precision for DLM in plasma (4.7 – 11.2 %Error and 1.6 – 13.5% R.S.D.) and in brain (3.3 – 14.8 %Error and 0.9 – 14.8% R.S.D.) are in the range reported previously (Anadon et al., 1996; Ding et al., 2004a). Ding (2004b) found good intraday and interday accuracy and precision for

DLM analyses of amniotic fluid, placenta and fetal tissue. The currently observed inter- and intraday accuracy and precision for DLM in liver (1.8 – 11.7 %Error and 1.9 – 15.2 %R.S.D.) and in kidney (2.0 – 13.1 %Error and 0.7 – 10.5 %R.S.D.) were comparable to those tissues and acceptable according to the criteria of the U.S. FDA (2001). Stability tests were not performed, because DLM has been shown to be a very stable compound in plasma by Ding et al. (2004a).

Like Ding et al. (2004a), the present method can also measure 3-phenoxybenzoic acid (PBA), one of DLM's major hydrolytic metabolites. Its retention time is at 3.9 min (data not shown). We, however, have not validated PBA quantitation, because the parent compound is the primary moiety of interest in toxicokinetic studies, as it is the proximate neurotoxicant. The cytochrome P450-mediated oxidative metabolites are not commercially available. Synthesis of hydroxy metabolites is currently underway in our laboratory. A method for analysis of hydroxy and hydrolytic metabolites of DLM is planned for the future.

Application of DLM analysis to PK studies in rats

The most recent analytical method was applied to tissue disposition experiments with 10-day-old and adult SD rats. Due to DLM's greater toxicity in young rats, they were gavaged with just 2 mg DLM/kg bw. The adults received 10 mg/kg orally. Plasma and tissue samples were collected and analyzed for DLM as described in the Experimental section. Plasma and tissue DLM concentration versus time profiles for the mature and immature animals are shown in Figs. 1-3 and 1-4, respectively. All animals exhibited transient salivation. The maximum DLM concentrations (C_{max}s) of 0.95 µg/ml plasma and 0.21 µg/g brain in adult rats were observed 1 h and 2 h, respectively, after dosing (Fig. 1-3). The finding of lower DLM levels in brain than in plasma is in agreement with a report by Rickard and Brodie (1985). C_{max}s of 0.8 µg/ml plasma

and 0.1 µg/g brain were measured in the 10-day-old rats, although they received only 20% of the dose given the adults. This disproportionately high internal exposure to the neurotoxic parent compound is consistent with reports of the immature rats' limited detoxification capacity and increased susceptibility (Sheets et al., 1994; Sheets, 2000; Anand et al., 2005). DLM concentrations decreased slowly in plasma and tissues of immature and adult rats during the 24-h monitoring period (Fig. 1-4). Previously, this insecticide could be detected for just 8.3 h in the plasma of an adult SD rat dosed orally with 20 mg DLM/kg (Ding et al., 2004a). A preliminary elimination half-life of 5.17 h was calculated from this abbreviated dataset, though kineticists prefer that chemical concentrations be monitored for 4 to 5 half-lives to yield accurate parameters. The currently-described analytical method is sensitive enough to allow this with the small sample volumes that are available from immature rodents.

CONCLUSION

Although the acute toxicity of DLM is well characterized, there has been a lack of published simple and reliable analytical methods to support pharmacokinetic studies of this compound. Therefore, such a method for quantitation of DLM in plasma and tissues was developed and validated following U.S. FDA and ICH guidelines. The present procedure does not include the time-consuming solvent evaporation step used previously. Our procedure yielded high recoveries, showed good linearity, precision and accuracy within the range of 0.05 – 4.0 µg/ml. It should be quite useful for pharmacokinetic studies of low doses of toxic pyrethroids, where complete blood and tissue concentration time-profiles are required for accurate calculation

of key pharmacokinetic parameters. This method is currently being used in our laboratory to investigate the plasma and tissue disposition of DLM in developing and mature rats.

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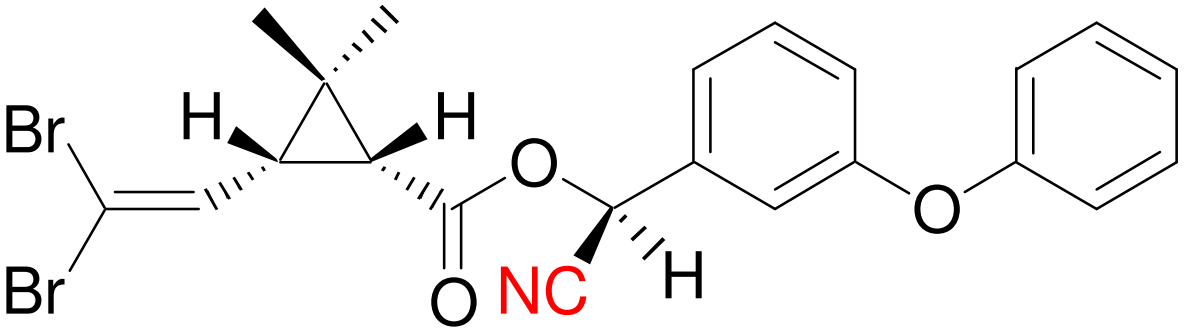

Deltamethrin

Figure 1-1. Chemical structure of deltamethrin.

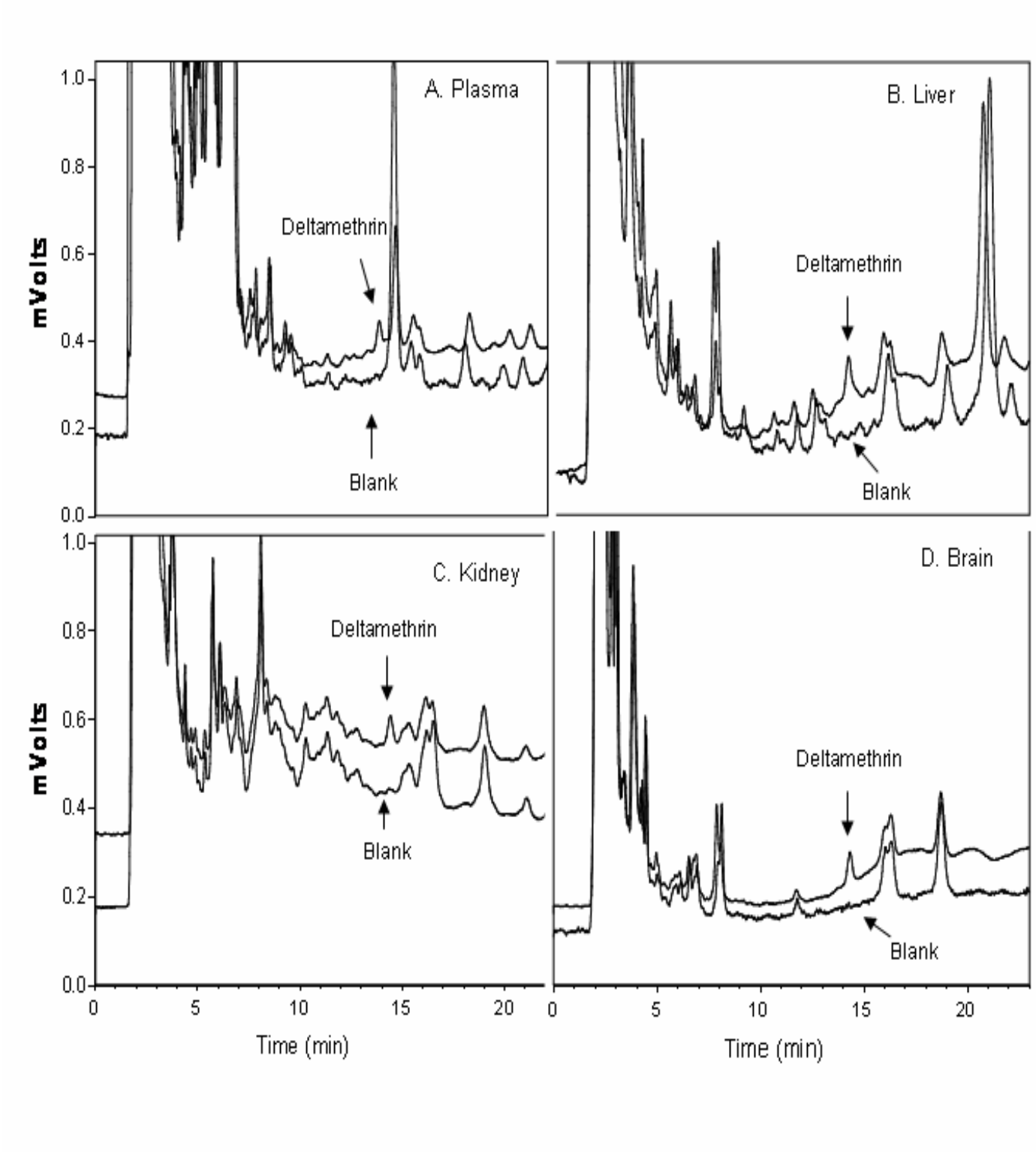

Figure 1-2. Chromatograms of blank and DLM (0.05 µg/ml)-spiked plasma (A), liver (B), kidney (C) and brain (D) on a C18 Ultracarb 5 ODS (20) (4.6 × 250 mm, 5 µm) analytical column.

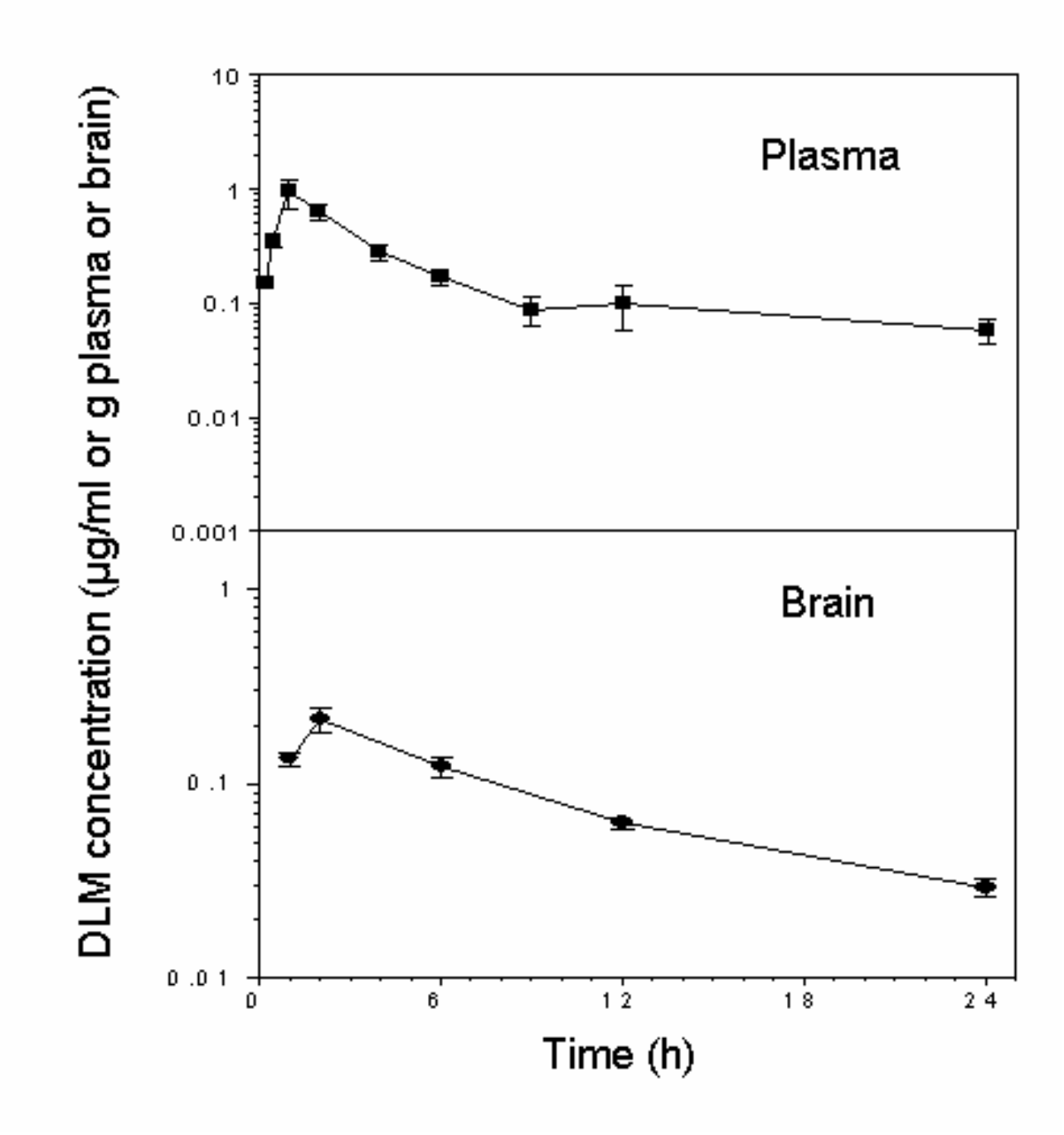

Figure 1-3. Plasma and brain concentration versus time profiles of deltamethrin (DLM) after administration of an oral dose of 10 mg DLM/kg to adult rats (n = 4 for plasma and n = 5 for brain DLM analyses at each time-point). Values represent mean ± SD.

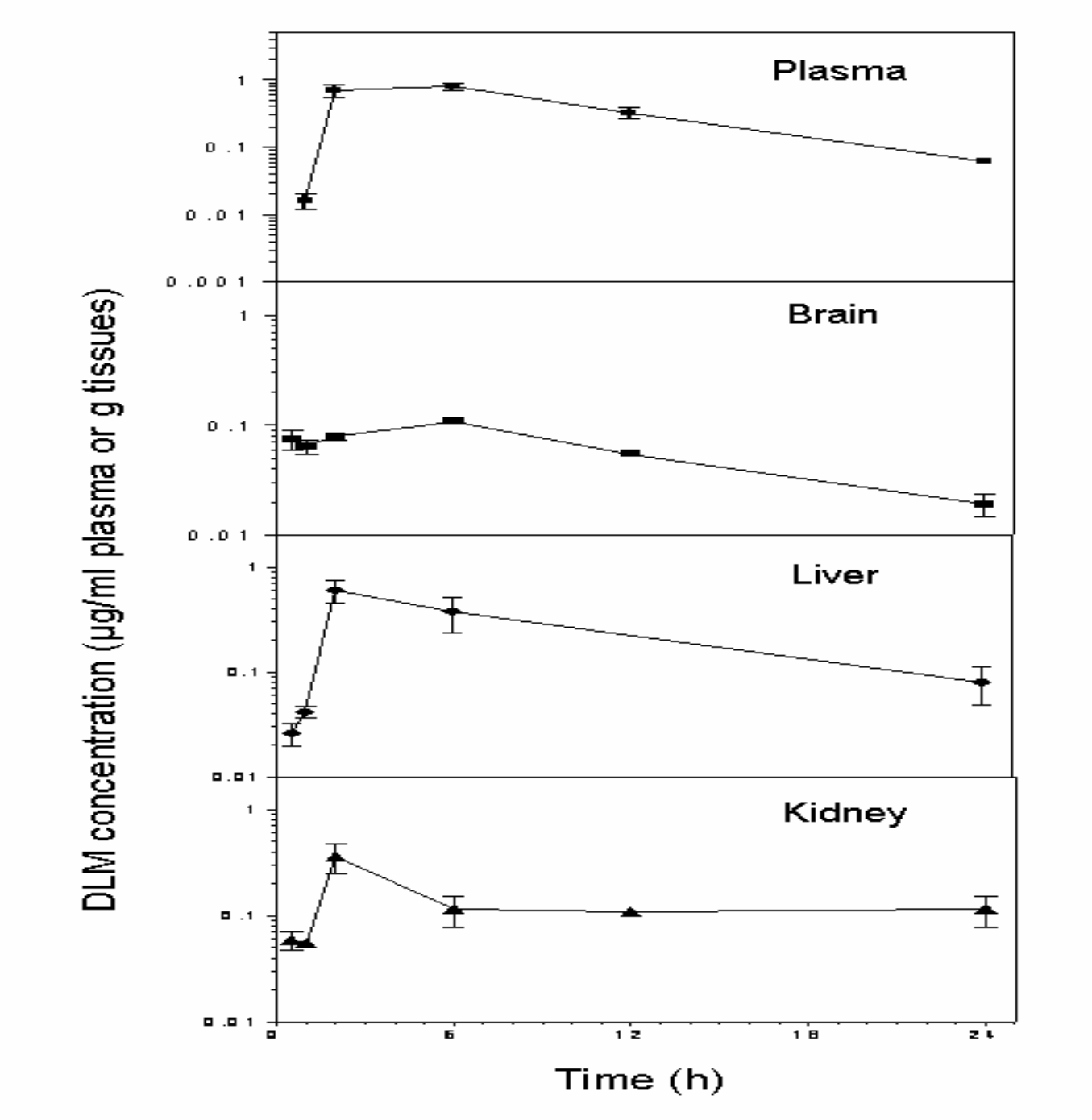

Figure 1-4. Plasma and tissues concentration versus time profiles of deltamethrin (DLM) after oral administration of 2 mg DLM/kg to 10-day-old rats (n = 3 at each time-point). Values represent mean ± SD.

Table 1-1. Absolute recovery (%) of DLM in rat plasma and tissues.

Sample	n	DLM concentration Added (μg/ml)	Absolute Recovery (%)[a]
Plasma	5	4.0	93± 7
		1.5	101 ± 7
		0.2	102 ± 2
		0.1	102 ± 5
		0.05	103 ± 6
Liver	5	4.0	103 ± 3
		1.5	95 ± 2
		0.2	107 ± 2
		0.1	114 ± 7
		0.05	100 ± 10
Kidney	5	4.0	104 ± 4
		1.5	97 ± 4
		0.2	97 ± 1
		0.1	102 ± 6
		0.05	108 ± 4
Brain	5	4.0	108 ± 5
		1.5	104 ± 4
		0.2	95 ± 3
		0.1	106 ± 1
		0.05	103 ± 9

[a] Absolute recovery was calculated from the peak areas of DLM in plasma, liver, kidney and brain compared with standard solutions.
All the values are expressed as mean ± SD.

Table 1-2. The intraday precision (% R.S.D.) and accuracy (%Error) of DLM analyses

Sample	n	DLM concentration Added (μg/ml)	DLM		
			Mean conc.[a] ± SD	Accuracy[b]	Precision[c]
Plasma	5	4.0	4.03 ± 0.29	4.7	7.2
		1.5	1.57 ± 0.10	5.0	6.4
		0.2	0.19 ± 0.00	4.8	1.6
		0.1	0.09 ± 0.01	7.4	5.5
		0.05	0.047 ± 0.004	9.1	8.2
Liver	5	4.0	4.07 ± 0.12	2.6	2.8
		1.5	1.51 ± 0.03	1.8	1.9
		0.2	0.21 ± 0.01	3.9	4.2
		0.1	0.09 ± 0.01	11.7	7.6
		0.05	0.047 ± 0.002	7.0	3.6
Kidney	5	4.0	4.02 ± 0.17	3.3	4.1
		1.5	1.52 ± 0.06	3.7	4.1
		0.2	0.20 ± 0.00	2.0	0.7
		0.1	0.09 ± 0.01	7.5	6.2
		0.05	0.056 ± 0.005	13.1	8.1
Brain	5	4.0	4.17± 0.18	4.7	4.3
		1.5	1.64 ± 0.06	9.1	3.5
		0.2	0.21 ± 0.01	3.3	3.2
		0.1	0.11 ± 0.00	4.8	0.9
		0.05	0.057 ± 0.007	14.1	13.1

[a] Mean conc., mean concentration (μg/ml) was determined from calibration curve.
[b] The absolute error percentage was calculated from the formula of (|mean of found concentration – added concentration|/added concentration)×100 (%).
[c] The precision was evaluated as the %R.S.D.

Table 1-3. The interday precision (%R.S.D.) and accuracy (%Error) of DLM

Sample	n	DLM concentration Added (μg/ml)	DLM		
			Mean conc.[a] ± SD	Accuracy[b]	Precision[c]
Plasma	15	4.0	3.80 ± 0.23	5.7	6.0
		1.5	1.58 ± 0.14	8.1	8.9
		0.2	0.20 ± 0.02	5.4	7.8
		0.1	0.10 ± 0.01	6.6	7.3
		0.05	0.047 ± 0.006	11.2	13.5
Liver	15	4.0	3.96 ± 0.25	5.1	6.3
		1.5	1.48 ± 0.06	3.7	4.4
		0.2	0.20 ± 0.01	4.2	5.2
		0.1	0.10 ± 0.01	6.4	6.4
		0.05	0.047 ± 0.007	10.1	15.2
Kidney	15	4.0	4.08 ± 0.20	4.5	4.9
		1.5	1.53 ± 0.08	4.5	5.1
		0.2	0.19 ± 0.01	4.6	4.2
		0.1	0.10 ± 0.01	7.4	8.1
		0.05	0.054 ± 0.006	9.9	10.5
Brain	15	4.0	4.11± 0.26	6.1	6.4
		1.5	1.54 ± 0.11	6.7	7.3
		0.2	0.21 ± 0.01	6.7	3.2
		0.1	0.10 ± 0.01	4.7	4.6
		0.05	0.056 ± 0.008	14.8	14.8

[a] Mean conc., mean concentration (μg/ml) was determined from calibration curve.
[b] The absolute error percentage was calculated from the formula of (|mean of found concentration – added concentration|/added concentration)×100 (%).
[c] The precision was evaluated as the %R.S.D.

CHAPTER 2

FORMULATION-DEPENDENT TOXICOKINETICS EXPLAINS DIFFERENCES IN THE GI ABSORPTION, BIOAVAILABILITY AND ACUTE NEUROTOXICITY OF DELTAMETHRIN IN RATS[1]

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[1]Kyu-Bong Kim, Sathanandam S. Anand, Srinivasa Muralidhara, Hyo J. Kim, and James V. Bruckner (2007) *Toxicology* 234: 194-202

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ABSTRACT

The acute neurotoxicity of pyrethroid insecticides varies markedly with the dosage vehicle employed. The objective of the present study was to assess the influence of two common vehicles on the bioavailability and toxicokinetics (TK) of a representative pyrethroid insecticide, deltamethrin (DLM), to determine whether the vehicles influence toxic potency by modifying the chemical's TK. Adult, male Sprague-Dawley rats were administered DLM iv or po, either by dissolving it in glycerol formal (GF) or by suspending it in Alkamuls® (AL). Groups of rats received 10 mg DLM/kg by gavage in each vehicle, as well as 2 mg/kg in GF or 10 mg/kg in AL by iv injection. Serial blood samples were collected over 96 h and analyzed for their DLM content by HPLC. In a second experiment, plasma, brain, fat, liver and lung DLM concentrations were measured 2 h after giving 10 mg DLM/kg orally in GF or AL. In a third experiment rats received 2 or 10 mg DLM/kg iv in AL or 2 mg DLM/kg iv in GF. Lung DLM content was determined 15 min post injection. DLM particle size in both formulations was measured under a phase contrast microscope. DLM appeared to be completely dissolved in GF, while particle size ranged from < 5 to > 50 µm in AL. The bioavailability of DLM in the aqueous AL suspension was ~ 9-fold lower than in GF (1.7% vs. 15%). Blood C_{max} (0.95 ± 0.27 vs. 0.09 ± 0.01 µg/ml) and AUC_0^{48h} (5.49 ± 0.22 vs. 0.61 ± 0.14 µg•h/ml) were markedly higher in the GF gavage group. Tissue DLM levels were also significantly higher in the GF animals at 2 h. The 10 mg/kg po and 2 mg/kg iv doses of DLM in GF produced moderate salivation and slight tremors. Rats receiving the insecticide in AL were asymptomatic. IV injection of the AL suspension resulted in trapping of much of the dose in the pulmonary capillaries. As anticipated, the injected suspension had a longer half-life and slower clearance than did the GF formulation. In summary, limited dissolution of the highly lipophilic DLM particles in the AL suspension

severely limited DLM's GI absorption, bioavailability, target organ deposition and acute neurotoxic potency.

INTRODUCTION

Pyrethroids have been used for more than 30 years for control of insects in agriculture, public health and the home (ATSDR, 2003). By the mid 1990s, pyrethroid use had grown to represent 23% of the U.S. dollar value of the worldwide insecticide market (Casida and Quistad, 1998). That percentage share and the number of human incidents have continued to increase substantially in the U.S. during the last several years with the declining use of organophosphates (Sudakin, 2006). It was estimated by the ATSDR (2003) that 1 million pounds of permethrin, the most popular pyrethroid, were applied in the U.S. in 2001. Occupational (Vijverberg and van dan Bercken, 1990; Soderlund et al., 2002) and nonoccupational (Berkowitz et al., 2003; Heudorf et al., 2004; Lu et al., 2006) exposures of wide segments of the general population to pyrethroids have been well documented. Nevertheless, knowledge of their mechanism(s) of action and long-term toxicity is limited. Information on their toxicokinetics (TK) is even more limited.

Traditionally, pyrethroids are divided into two classes, based upon their chemical structure and clinical manifestations of acute exposure. Type I compounds do not contain a cyano moiety, while Type II do. Hyperexcitation, tremors and skin parathesias are the most common signs of acute poisoning by Type I compounds. Type II pyrethroids' "hallmark" effects are salivation, tremors and choreoathetosis (Lawrence and Casida, 1982; Ray and Forshaw, 2000). Voltage-sensitive sodium channels in central nervous system neurons appear to be their principal site of action (Narahashi, 1996). Deltamethrin (DLM), the compound chosen for the current study, is one of the most potent pyrethroids *in vitro* (Choi and Soderlund, 2006) and *in vivo* (Wolansky et al., 2006). Rickard and Brodie (1985) find that the onset and severity of signs

of DLM poisoning in mice are proportional to brain levels of DLM. Additional evidence that the parent pyrethroid is the primary toxic moiety is furnished by Lawrence and Casida (1982). They report that intracerebral injection of DLM produces signs of poisoning in mice within a min or less. It is also reported that inhibitors of enzymes that metabolize pyrethroids exacerbate their toxicity (Soderlund and Casida, 1977; Casida et al., 1983).

It has been recognized for almost 30 years that biotransformation of pyrethroids occurs primarily via two pathways: hydrolysis of the molecules' ester linkage by esterases; and aromatic hydroxylation by cytochrome P450s, with subsequent conjugation (Ruzo et al., 1978; Rickard and Brodie, 1985). Anand et al. (2006a) recently found that carboxylesterases (CaEs) in rat liver and plasma, as well as rat liver cytochrome P450s 1A1, 1A2 and to a lesser degree 2C11, are primarily responsible for DLM biotransformation *in vitro*. Intrinsic clearance by plasma CaEs is far less important quantitatively than metabolism in the liver. Anand et al. (2006b) also found that limited capacity of these enzymes contributes significantly to increased systemic exposure and neurotoxic effects in immature rats.

The number of comprehensive TK studies of orally-administered pyrethroids in animals is very limited. Our laboratory (Kim et al., 2007) has delineated the time-course of DLM in the blood and a variety of tissues of adult rats gavaged with DLM in glycerol formal (GF). Mirfazaelian et al. (2006) utilized these data to develop a physiologically-based pharmacokinetic model for DLM. Anadon and his colleagues used GF as an iv injection vehicle, but a vegetable oil for oral administration of permethrin (1991), DLM (1996) and lambda-cyhalothrin (2006) in their TK studies.

Oral dosage vehicles can have a marked effect on the acute toxicity of pyrethroids. Published acute LD_{50} values for most pyrethroids given orally to rats in corn or other vegetable

oils range from 50 to 500 mg/kg (Soderlund et al., 2002). These authors cited LD_{50} values for lambda-cyhalothrin of 79 and 56 mg/kg when given in corn oil, versus 299 and 433 mg/kg when given as an aqueous suspension. Oral LD_{50} values listed for DLM ranged from 87 mg/kg (in corn oil) to > 5,000 mg/kg (in a 1% methylcellulose aqueous suspension). Pham et al. (1984) reported that oral DLM was 100 times less toxic to rats when suspended in gum Arabic solution than when dissolved in GF. Crofton et al. (1995) assessed the ability of DLM to depress motor activity of rats gavaged with DLM in four different vehicles. The chemical was equipotent in corn oil and GF. Its potency was substantially lower when administered in Emulphor®, and even lower when given as an aqueous suspension in methylcellulose. Crofton and his co-workers (1995) emphasized the need for TK studies to clarify the reason(s) for the vehicle-dependent differences they observed in acute DLM neurotoxicity.

The overall objective of the current study was to investigate the influence of two common vehicles/diluents on the bioavailability, TK and acute neurotoxicity of orally-administered DLM. An aim of our investigation was to test the hypothesis that DLM dissolved in GF is more acutely neurotoxic to rats than DLM in Alkamuls®, because solubilized DLM is better absorbed from the GI tract and reaches the target organ (brain) in larger quantities.

MATERIALS AND METHODS

Chemicals. Deltamethrin (DLM) [(S)-α-cyano-3-phenoxybenzyl-(1R, *cis*)-2,2-dimethyl-3-(2,2-dibromovinyl)-cyclopropanecarboxylate] (purity, 98.8%) was kindly provided by Bayer CropScience AG (Monheim, Germany). Acetonitrile (HPLC grade) and glycerol formal (GF) were purchased from Sigma-Aldrich (St. Louis, MO). Alkamuls El-620® (formerly Emulphor®)

(AL) was a gift from Rhodia (Cranbury, NJ). Methanol, sulfuric acid and deionized water (HPLC grade) were obtained from J.T. Baker (Phillipsberg, NJ). All other chemicals used were of the highest grade commercially available.

GF, a binary solvent, is used to solubilize a wide variety of hydrophobic and hydrophilic chemicals and pharmaceuticals (Sanderson, 1959). It is a condensation product of glycerol and formaldehyde. It is a 60:40 mixture of two chemicals: 4-hydroxymethyl-1,3-dioxolane and 5-hydroxy-1,3-dioxane. GF produces no apparent toxic effects or macroscopic pathology when rats are dosed orally with up to 4,000 mg/kg. The only effect manifest at the highest doses is narcosis (Sanderson, 1959). Emulphor® (now Alkamuls®), a polyethoxylated vegetable oil, is widely used to prepare stable aqueous emulsions of aliphatic and aromatic hydrocarbons of low to moderate molecular weight.

Animal Maintenance and Preparation. Male, adult Sprague-Dawley (S-D) rats (~ 90 days old) were obtained from Charles River Laboratories (Raleigh, NC). The rats were acclimated (2 rats/cage) for at least 10 days in an AAALAC-approved animal care facility maintained at 72 ± 2° F and 50 ± 10% humidity with a 12-h light/dark cycle (light 0600 – 1800 h). These animals were housed in polycarbonate cages lined with sterilized recycle paper bedding (Tek-Fresh®, Harlan TEKLAD, Madison, WI). Food (5001 Rodent Diet®, PMI Nutrition Internat., St. Louis, MO) and tap water were provided *ad libitum*. The experimental protocol was reviewed and approved by the University of Georgia Animal Care and Use Committee.

Groups of rats were cannulated so that serial blood samples could be taken to characterize the time-course of DLM given in GF and in AL. Briefly, each rat was anesthetized by an injection of 0.1 ml/100 g bw of a "cocktail" consisting of ketamine hydrochloride (100 mg/ml), acepromazine maleate (20 mg/ml), and xylazine hydrochloride (10 mg/ml) (3:2:1, v:v:v). A

cannula (PE-50 polyethylene tubing) was surgically inserted into the left carotid artery until its tip rested just above the aortic arch. The cannula was then securely ligated to the artery, passed sc and exteriorized at the nape of the neck. Thus, the animals could not disturb the cannula, but could move about freely once they awakened. Water was provided *ad libitum*, but food was withheld during the 24-h recovery period before dosing. Food was provided 3 h after dosing.

DLM treatments and sampling. We were limited in the range of DLM doses that could be evaluated by the insecticide's acute toxicity and by its analytical limit of quantitation. Oral dosages ≥ 20 mg/kg in GF produced salivation, marked tremors and choreoathetosis. DLM was even more neurotoxic when injected iv in GF. Oral and iv doses in GF had to be ≥ 2 mg/kg, in order to obtain complete time-courses (i.e., uptake and elimination profiles) of the parent chemical in plasma.

In the time-course experiment DLM was administered iv and po either as a solution in GF or as an aqueous suspension in 5% AL. The oral dose of 10 mg DLM/kg was given by gavage in each vehicle in a total volume of 2 ml/kg. The iv doses of 2 and 10 mg DLM/kg in GF and AL, respectively, were injected in a total volume of 0.2 ml/kg into a lateral tail vein. Each animal was dosed between 0900 and 1000 h. Their average body weight at the time of dosing was ~ 360 g. Serial arterial blood samples of 150 µl were drawn from the left carotid artery cannula and collected in heparinized tubes at the following time-points: 0.02, 0.08, 0.25, 0.5, 1, 2, 4, 6, 9, 12, 24, 36, 48, 60, 72 and 96 h after iv dosing, and 0.25, 0.5, 1, 2, 4, 6, 9, 12, 24, 36, 48, 60, 72 and 96 h after oral dosing. An equivalent volume of heparinized saline was injected ia via the cannula after each blood withdrawal. Plasma was separated by centrifugation within 30 min of blood collection. DLM concentrations were analyzed immediately as described below by the method of Kim et al. (2006).

A second experiment was performed to delineate the influence of the dosing vehicles on the deposition of DLM in selected tissues. Groups of 3 – 4 rats were fasted overnight before being gavaged between 0900 and 1000 h with 10 mg DLM/kg in GF or AL. Two h post dosing the rats were euthanized by CO_2 asphyxiation. Samples of blood, whole brain, liver, kidney, lung and perirenal fat were collected and processed for DLM analysis as described below.

In a third experiment groups of 3 – 4 rats received an iv injection of one of the following: 2 mg DLM/kg in GF; 2 mg DLM/kg in AL; or 10 mg DLM/kg in AL. Each solution was injected in a total volume of 0.2 ml/kg into a lateral tail vein. All of the animals were euthanized by CO_2 asphyxiation after 15 min and the lungs removed for analysis of their DLM content by the method of Kim et al. (2006). The purpose of this experiment was to compare the pulmonary deposition of DLM given in the two vehicles.

Neurotoxicity Assessment. Rats in the DLM time-course study were observed during the 96-h blood sampling period for toxic signs. Complex measures of behavioral effects (e.g., functional observational batteries, motor activity in mazes) have been utilized by some investigators to assess some pyrethroids (McDaniel and Moser, 1993; Wolansky et al. 2007). No quantitative biochemical measures of acute pyrethroid neurotoxicity, however, are apparently available. Therefore, we chose to monitor the animals for the primary clinical signs of acute Type II poisoning: salivation, tremors, choreoathetosis; and death, as observed in previous studies of DLM (Anadon et al., 1996; Anand et al., 2006b). The severities of these signs were not scored subjectively, but merely observed and recorded.

Analysis of DLM. DLM in plasma and tissues was quantified by a high performance liquid chromatography (HPLC) method with UV absorbance detection at 230 nm (Kim et al., 2006). Briefly, DLM was separated on a reverse phase Ultracarb 5 ODS column (250 x 4.6 mm; 5-µm

particle) (Phenomenex, Torrance, CA), protected by a Security Guard® Fusion-RP guard column cartridge (Phenomenex, Torrance, CA) on a Shimadzu HPLC system (LC-10AT pump, DGU-14A degasser, SIL-HT autosampler, SPD-10AV detector) (Shimadzu, Canby, OR). The mobile phase was 80% acetonitrile and 20% sulfuric acid (1%). The flow rate was set at 1.0 ml/min. Most of the DLM in blood was present in the plasma (data not shown). Therefore, plasma was separated from blood and 65 µl of plasma were added to microcentrifuge tubes containing 130 µl of acetonitrile for extraction. These tubes were vortexed for 15 sec and centrifuged for 10 min at 2500 x g (Beckman Coulter®, Atlanta, GA). Various tissues were homogenized for 10 sec in 4 volumes of a mixture of deionized water and acetonitrile (1:1 ratio). Sixty-five µl of each homogenized tissue were mixed with 130 µl of acetonitrile and centrifuged. Fifty µl of supernatant were injected onto the guard column. The limits of detection and quantitation for plasma and tissues were 0.01 and 0.05 µg/ml, respectively.

Particle size measurement. Both the GF and AL formulations were viewed soon after preparation at 40X magnification under a phase-contrast microscope (Bausch & Lomb, Rochester, NY) fitted with a calibrated ocular micrometer. No DLM particles were visible in the GF solution. The microscope's stage was moved in a coordinated manner until a total of 1,000 DLM particles were measured in the AL formulation. The average and ranges of particle size were then calculated. The particles were photographed using a Pixera Pro600ES camera (Pixera Corporation, Los Gatos, CA) attached to the microscope.

Data analyses. The maximum plasma concentration (C_{max}) and time to maximum concentration (T_{max}) values were determined by visual inspection of the plasma DLM concentration versus time profile data for each formulation. Other pharmacokinetic parameters were calculated using Winnonlin (ver. 4.1) noncompartmental model analysis (Scientific Consulting, Inc., Cary, NC).

Data are reported as means ± SE. The statistical significance ($p < 0.05$) of apparent differences in pharmacokinetic parameters between the two formulations was assessed by Student's t test using Prism (ver. 3.03) (GraphPad Software, Inc., San Diego, CA). A one-way analysis of variance (ANOVA), followed by the Bonferroni Test (Prism 3.03, San Diego, CA) was performed to determine whether DLM concentrations in plasma and different tissues were significantly different ($p < 0.05$) from one another for each formulation. Student's t test was used to assess the statistical significance of vehicle dependent differences in DLM levels in each tissue 2 h after oral administration of 10 mg/kg.

RESULTS

Toxicokinetics of iv DLM in GF vs. AL

Plasma elimination curves for rats given 2 and 10 mg DLM/kg iv in GF and AL, respectively, are shown in Fig. 2-1. Differences in DLM elimination in the two vehicle groups during the initial 2 h post injection can be seen more easily in the inset. As the plasma DLM concentrations were well below its K_m (Anand et al., 2006a), the TK of DLM should be linear in this dosage range. The mean arterial plasma DLM concentration in the GF group 1-min after injection was 26-fold higher than that in the AL group, despite the 5-fold higher dose in the latter group. Plasma DLM levels decreased very rapidly in both vehicle groups for the first 15 min post injection (Fig. 2-1 Inset). Kinetic analysis confirmed that plasma elimination was biexponential in both vehicle groups. The initial rapid drop was followed by a slow, prolonged decline for the remainder of the 96-h monitoring period in the AL rats. Sequestered DLM particles in their pulmonary capillaries, as described below, apparently acted as a sustained

release. The terminal elimination half-life ($t_{½}$) was twice as long in this group as in the GF group (Table 2-1). DLM levels in the AL vehicle group exceeded levels in the GF group at all sampling times after 2 h (Fig. 2-1). As a result, the AL area under the plasma concentration versus time curve (AUC) was not significantly different from that for the GF group (Table 2-1). The AL volume of distribution (Vd) was markedly higher, due to trapping of DLM particles in the pulmonary microcirculation. This was confirmed by measurement of DLM levels in the lungs 15 min after iv injection of 2 mg DLM/kg in AL and in GF. Concentrations of the insecticide were 10-fold higher in the lungs of the animals receiving the compound in AL (Fig. 2-2A). Another group given 10 mg DLM/kg iv in AL exhibited an 86-fold higher DLM level than the 2 mg/kg GF group (Fig. 2-2B).

Plasma uptake and elimination curves for rats gavaged with 10 mg DLM/kg in GF and AL are pictured in Fig. 2-3. DLM is detectable for 48 h in both vehicle groups. It is obvious that DLM is absorbed from the GI tract to a greater extent when the chemical is administered in GF. The peak plasma level (C_{max}) for this vehicle group is 10.6-fold higher, while the AUC is 9-fold greater (Table 2-2) than in the AL group. Although bioavailability (F) is 8.8-fold greater, it is still only 15% in the GF animals. As DLM particles in AL were trapped in the pulmonary capillaries, it was necessary to utilize the GF iv data to estimate a F value for the AL group. Interestingly, the shape of the GF and AL uptake and elimination profiles are similar (Fig. 2-3). Accordingly, several of the TK parameter estimates [e.g., absorption rate constant (ka), time to maximum blood concentration, $t_{½}$, Vd and clearance (Cl)] (Table 2-2) are comparable for the two vehicle groups.

Tissue and plasma DLM concentrations 2 h after oral administration of 10 mg DLM/kg in GF and AL are presented in Fig. 2-4. DLM concentrations are significantly higher in each

biological specimen when the insecticide is given in GF. DLM levels in plasma are substantially higher than in tissues of the GF group 2 h post dosing. Surprisingly, DLM levels are significantly lower in the brain than in plasma and the other tissues in both vehicle groups. Levels in fat are little different from concentrations in the other tissues in either vehicle group at this particular time-point. Comprehensive time-course studies in our laboratory demonstrate that DLM levels in adipose tissue far exceed levels in plasma and other tissues for a prolonged period at later time-points (Mirfazaelian et al., 2006; Kim et al., 2007).

DLM particle size was contrasted in the two vehicles (Fig. 2-5). The lipophilic chemical appeared to be completely dissolved in GF. In contrast, particles of varying size were visible under a phase-contrast microscope in the AL formulation. The mean particle size was determined to be 10 µm. The size distribution was as follows: < 5 µm = 22.2%; > 5 – 12.5 µm = 48.9%; > 12.5 – 25 µm = 20.8%; and > 25 – 50 µm = 8.1%.

Manifestations of acute neurotoxicity were consistent with the aforementioned TK findings. Moderate salivation and slight tremors were evident in rats administered 2 or 10 mg DLM/kg iv and po, respectively, in GF. These signs lasted for 2 to 3 h. No acute neurotoxicity was evident at either dosage level in animals given DLM in AL by either route.

DISCUSSION

This investigation demonstrates that oral DLM's greater lethality (Soderlund et al., 2002) and motor depressant activity (Crofton et al., 1995) are due largely to increased GI absorption and bioavailability upon the chemical's dissolution. The systemic, or internal dose of DLM, as reflected by AUC_0^{48} values, was 9-fold higher when the chemical was administered orally in GF

rather than AL. The GF group's observed C_{max} was 10.6-fold higher. The concentration of DLM in the whole brain of GF rats was significantly higher than in the brain of the AL rats 2 h post dosing. The animals that received 10 mg DLM/kg in GF exhibited transient salivation and tremors, whereas their AL counterparts were asymptomatic. Thus the oral dosage vehicle, or diluent can have a toxicologically-significant impact on DLM and very likely on other ingested pyrethroids.

Particle size is an important determinant of systemic absorption of hydrophobic drugs and other chemicals from the GI lumen (Jinno et al., 2006; Liversidge and Cundy, 1995). Reducing the size of particulates increases their surface area, enhancing a compound's opportunity for dissolution and diffusion across GI epithelial membranes (Gibaldi, 1984). Jinno et al. (2006) were able to produce microparticles of cilostazol, a hydrophobic drug, that ranged in size from 0.1 – 100 µm. Bioavailability of the smallest (~ 0.1 – 0.3 µm) NanoCrystals® was about 5-fold higher than NanoCrystals® of ~ 0.5 – 10 µm, when the two formulations were given orally to dogs as an aqueous suspension. The researchers demonstrated that miniaturization of the particles of the highly lipophilic drug significantly increased their solubility in water, and hence their systemic absorption. Damge et al. (1996) measured the uptake of solid microspheres instilled into the ileal lumen of adult rats. Only ~ 0.11% of the 5- to 10- µm microspheres were taken up versus ~ 12.7% of these 1 – 5 µm. These researchers observed the microspheres to cross the intestinal mucosa by phagocytotic uptake by specialized membranous cells in the Peyer's patches, lymphoid nodules abundant in the ileum. Nevertheless, it appears that solid nanoparticles of pyrethroids would be taken up in very limited amounts by this mechanism.

Bioavailability and C_{max} in the current study were significantly lower when DLM was given as an aqueous AL suspension than when dissolved in GF. AL has routinely been utilized

in our laboratory to prepare stable aqueous emulsions of a variety of volatile organic chemicals that were liquids at room temperature. DLM, however, is a solid (powder). Our AL formulation proved to be an unstable aqueous suspension, in that the DLM particles settled soon after vigorous shaking. Some 22% of the DLM particles in the suspension were < 5 µm, but the size distribution of even smaller particles could not be determined. Judging from the aforementioned studies, nanoparticles 0.1 – 1 µm would best undergo dissolution (and diffusion through GI membranes). Although systemic uptake from our AL suspension was quite limited, T_{max} and ka, two indices of the rate of absorption, were not significantly different between the AL and GF groups. This, it seems reasonable to assume that much less DLM was available for systemic uptake in the AL animals, but that compound which was available (i.e., dissolved) was absorbed from the gut at a comparable rate as that dissolved in GF. Masuh et al. (2000) demonstrated that a substantial reduction of particle size in an aqueous suspension of *cis*-permethrin, a relatively non-toxic pyrethroid, resulted in that formulation having greater insecticidal activity than a commercial suspension of DLM.

DLM and other pyrethroids are marketed in a variety of formulations for application as insecticides. One of the most common DLM preparations is an emulsifiable concentrate (Pawlisz et al., 1998). Some emulsifiable concentrates incorporate the pyrethroid into a petroleum solvent (Mueller-Beilschmidt, 1990). This should dissolve the pyrethroid and result in a true emulsion. Absorption of the dissolved pyrethroid should be more rapid and extensive from the solvent-based product than from aqueous suspensions. Most petroleum solvents themselves quickly diffuse across membranes. The other most common commercial formulation is the flowable suspension concentrate (Pawlisz et al., 1998). It is a dispersion of the powdered insecticide in water. A dispersing agent is included to keep the particles in a deflocculated state.

Additional products include a wettable powder, a dustable powder, an aerosol and water-dispersible granules (Tomlin, 1997). The results of the current investigation and of other studies discussed suggest that the oral bioavailability and toxic risks posed to humans by formulations of DLM particulates will be relatively modest, unless the particle size is very small (i.e., < 1µm).

The processes, or mechanisms by which DLM and other pyrethroids are absorbed across the GI epithelium and enter the arterial circulation are not clear. DLM dissolved in GF may be absorbed by passive diffusion into the portal venous blood and be subject to extensive first-pass metabolism by hepatic cytochrome P450s and CaEs (Anand et al., 2006a; Mirfazaelian et al., 2006). This process may account for the low oral bioavailability we found, though it has not been established how efficiently DLM molecules are absorbed. Systemic uptake of DLM from a vegetable oil vehicle would be anticipated to be relatively slow, as the oil should serve as a reservoir in the gut until the lipids are digested. Crofton et al. (1995), however, found DLM given orally in corn oil and GF to have the same time of onset and to be equipotent in reducing motor activity in rats. The oral bioavailability of 14.4% for DLM in sesame oil in rats (Anadon et al., 1996) is virtually the same as our value of 15% for GF. Long-chain fatty acid micelles from digested triglycerides may carry DLM with them into enterocytes, where they are incorporated into chylomicrons and enter the mesenteric lacteals. Chylomicrons are known to serve as GI carriers for other highly lipophilic, polycyclic hydrocarbons such as DDT (Palin et al., 1982), TCDD (Lakshmanan et al., 1986) and hexachlorobenzene (Roth et al., 1993). DLM absorbed in this manner from a vegetable oil will bypass first-pass hepatic metabolism, possibly accounting for the comparable bioavailability and bioactivity observed in the aforementioned vegetable oil and GF vehicle groups.

The vehicle-dependent differences we observed in plasma DLM levels were also manifest in organ levels 2 h following oral dosing. In each instance, tissues of rats receiving DLM in GF had significantly higher DLM concentrations than tissues of rats given DLM in AL. It is worthy of note that concentrations of the insecticide in target organ (i.e., brain) were substantially lower than in plasma or the other tissues. The reason(s) for this phenomenon is(are) not clear, though it has been observed with pyrene (Withey et al., 1991) and TCDD (Dilberto et al., 1996) in rats, as well as with 11 chlorinated pesticides and 14 polychlorinated biphenyl congeners in human tissue lipids measured at autopsy in Greenland natives (Dewailly et al., 1999). Although fat DLM levels were not significantly different from liver and lung levels in our rats 2 h after dosing, DLM levels in fat markedly exceeded levels in plasma and in other tissues at later sampling times (Kim et al., 2007; Mirfazaelian et al., 2006). The relatively high DLM concentrations in the lungs 2 h after oral administration can be attributed to the organs' high blood content.

The lungs' high DLM content following iv injection of the compound in AL was due to trapping of DLM particulates in the pulmonary microcirculation. Some 88% of the DLM particulates in AL ranged from > 5 to > 50 μm. The diameter of pulmonary capillaries varies from 7 – 10 μm. First-pass pulmonary sequestration of a number of highly lipophilic drugs has been reported following their iv injection. Dutta and Ebling (1998), for example, found peak lung concentrations of propofol, a lipophilic narcotic, to be 300-fold higher when infused iv in a lipid-free medium rather than in a solubilized form.

In summary, the vehicle in which DLM is administered orally has a pronounced influence on the compound's particle size, dissolution, absorption and bioavailability, as well as target and storage tissue deposition. Bioavailability and target organ (brain) levels were significantly

higher when the insecticide was given orally to rats in GF rather than as an unstable suspension in AL. Animals receiving 10 mg DLM/kg po in GF exhibited transient salivation and tremors, whereas AL animals were asymptomatic. Persons who ingest solid formulations of DLM are likely to be at a substantially lower risk of neurotoxicity than those who consume the agent in a solubilized aqueous or oil-based formulation. Further toxicity studies will be needed to substantiate this conclusion.

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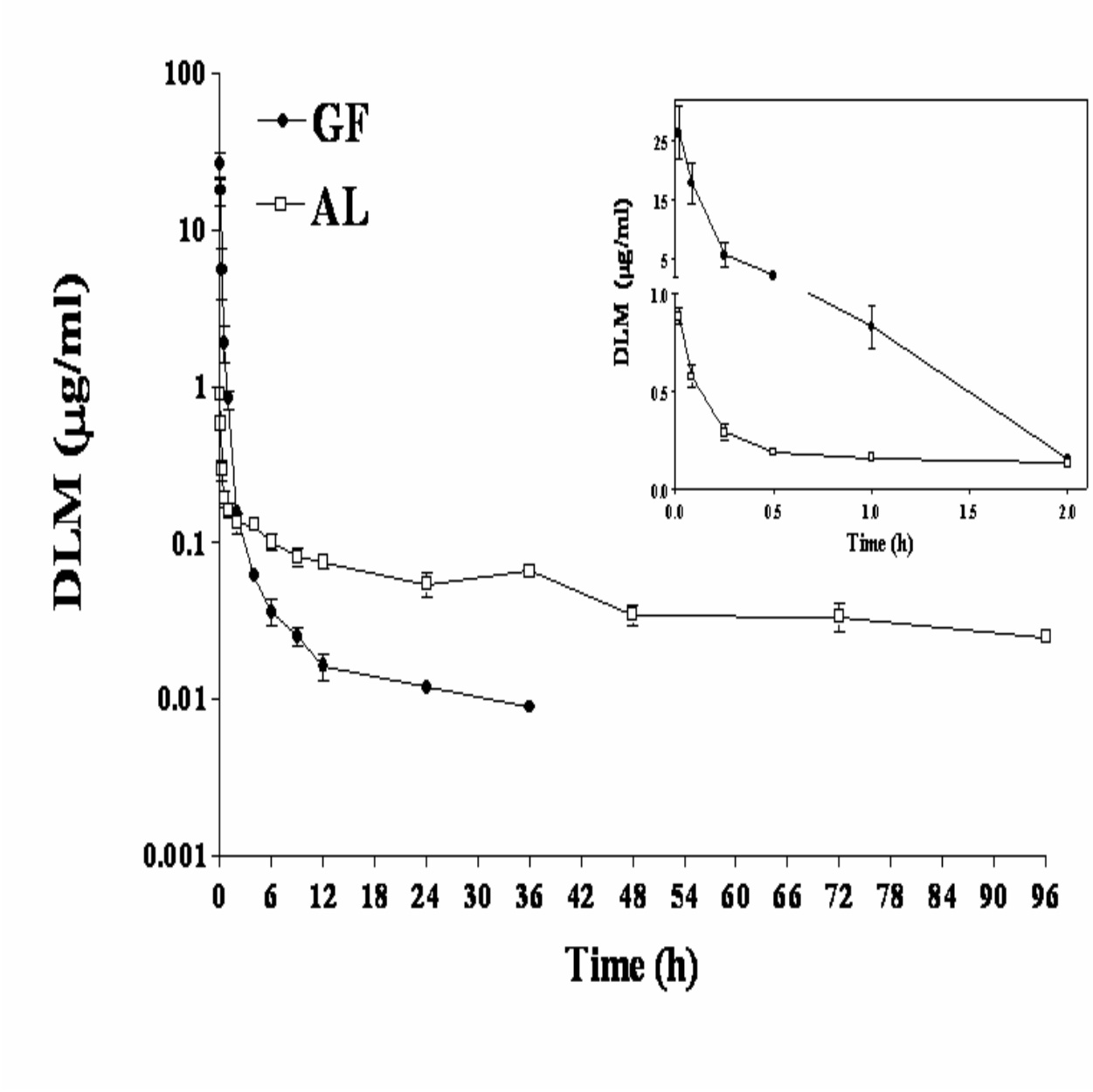

Figure 2-1. Plasma concentration-time profiles following iv injection of DLM in GF and AL. Rats received 2 mg DLM/kg in GF or 10 mg DLM/kg iv in AL. Plasma DLM concentrations were measured serially in blood samples taken from a carotid artery cannula from 0.02 – 96 h post injection. Data points represent means ± SE of groups of 3 or 4 rats. DLM levels were undetectable beyond 36 h in the GF animals. Inset shows DLM levels in the two vehicle groups during the first 2 h. Note the linear scale of the inset's Y axis.

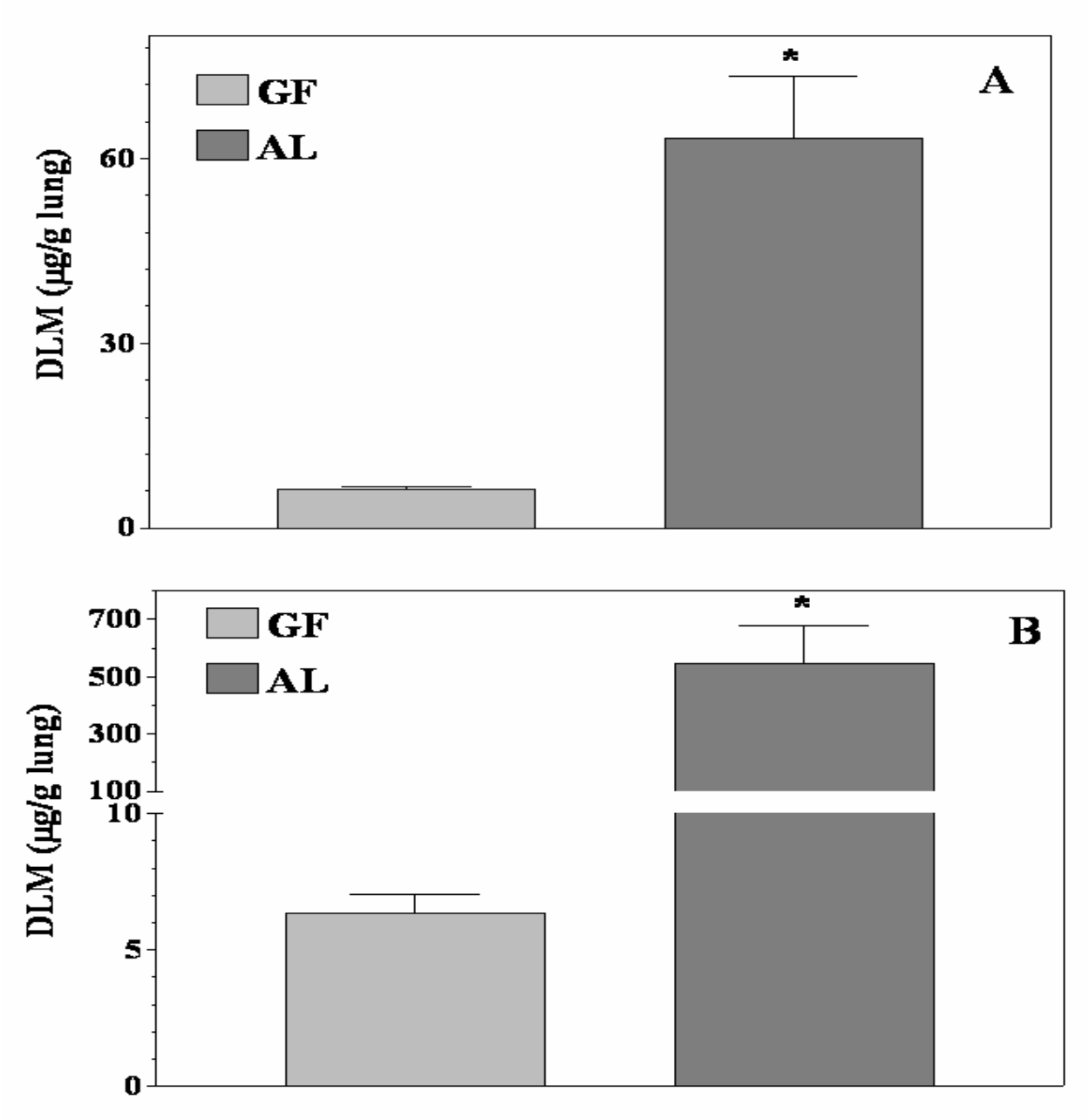

Figure 2-2. DLM concentrations in lungs of rats 15 min after iv injection of: [A] 2 mg DLM/kg in glycerol formal (GF) or Alkamuls® (AL); [B] 2 mg DLM/kg in GF and 10 mg DLM/kg in AL. Bar heights represent mean ± SE for groups of 3 – 4 rats. *Denotes statistically significant difference from 2 mg/kg GF vehicle group.

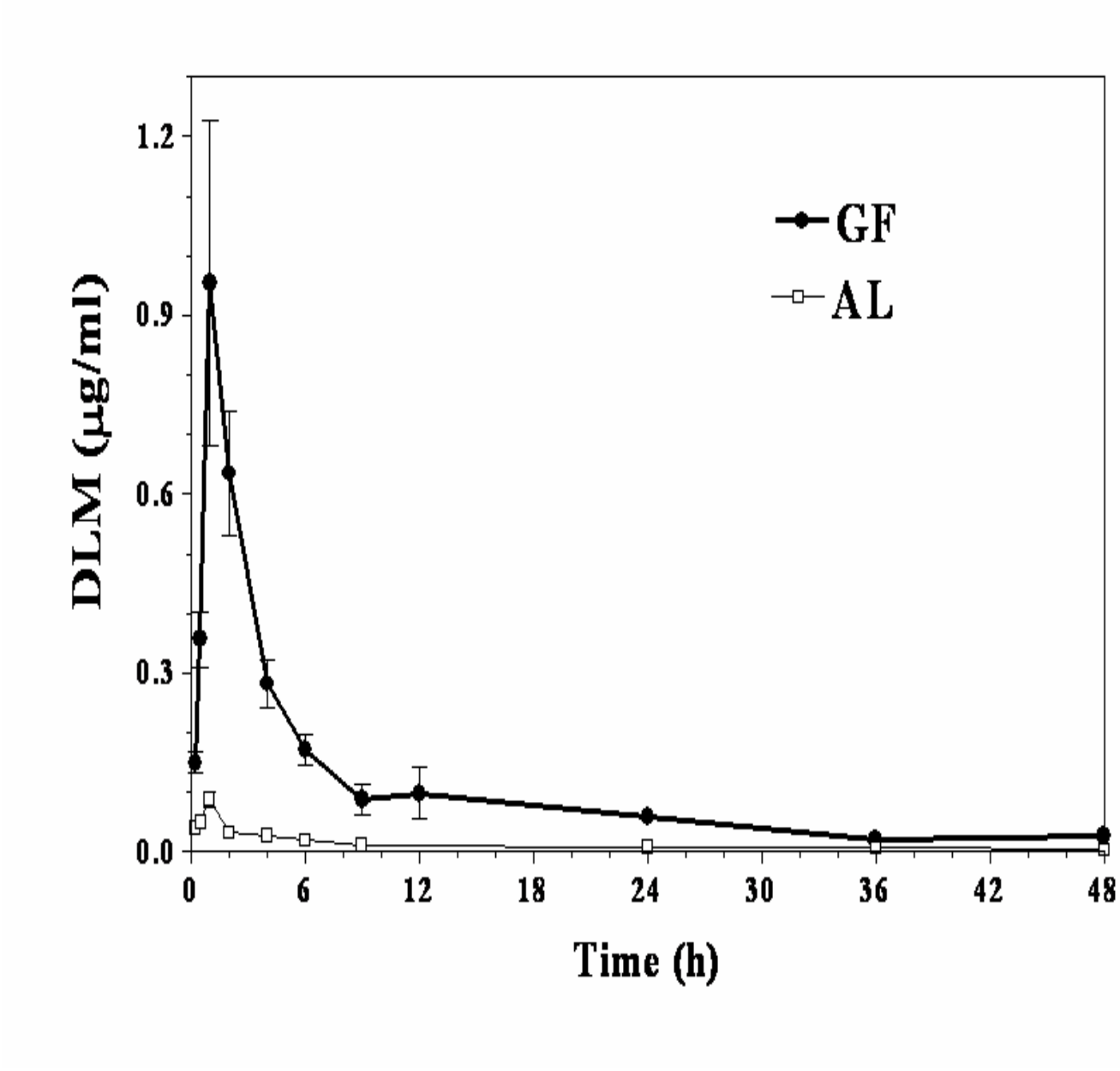

Figure 2-3. Plasma concentration-time profiles on linear scales following oral administration of 10 mg DLM/kg in GF or AL. Serial plasma concentrations of DLM were measured over a period of 0.25 – 96 h. Data points and brackets represent means ± SE of 4 or 5 rats. DLM was undetectable beyond 48 h.

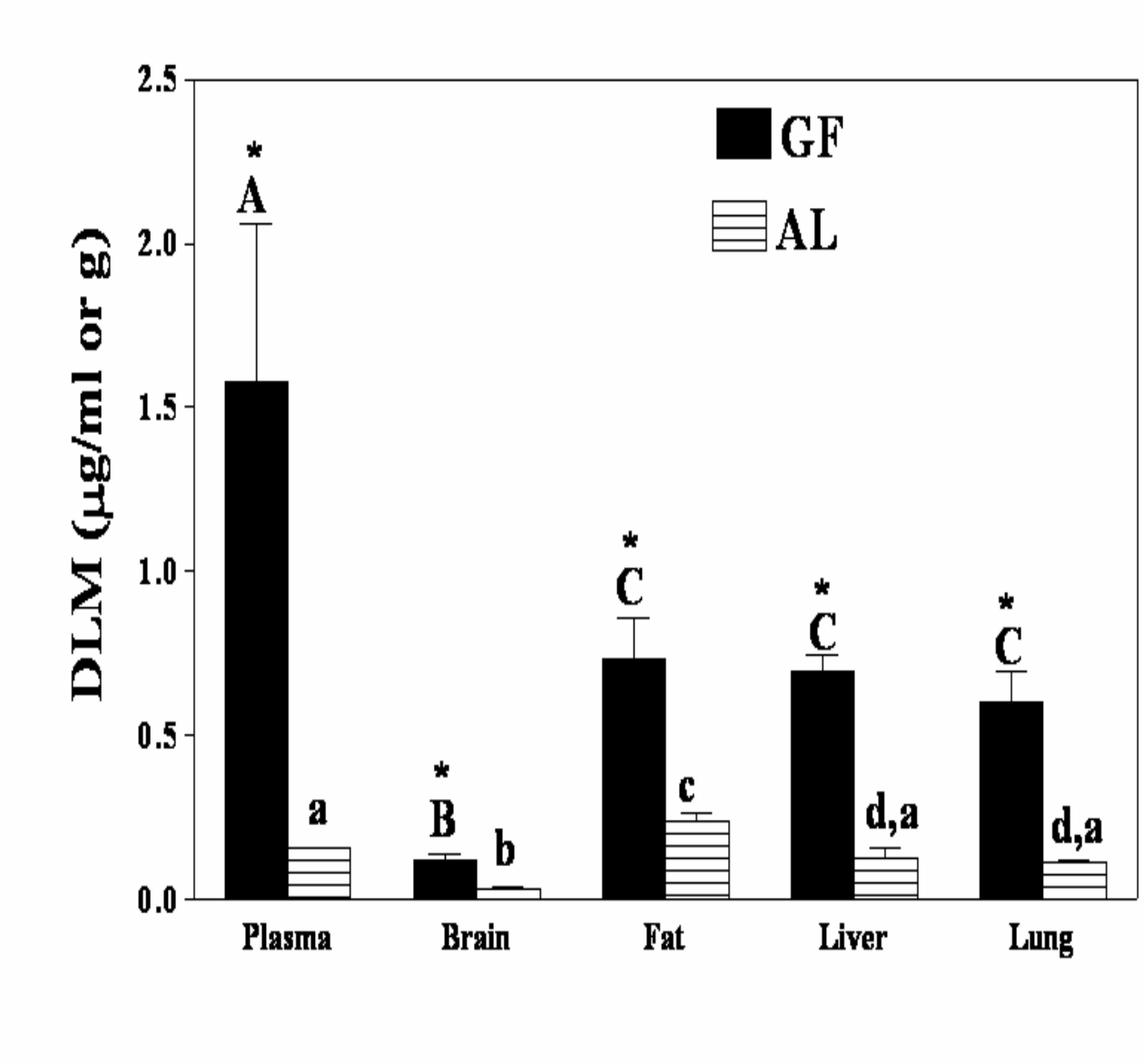

Figure 2-4. Plasma and tissue concentrations of DLM 2 h after an oral dose of 10 mg DLM/kg dissolved in GF or suspended in 5% AL. Bars and brackets represent means ± SE for 3 or 4 rats. *Denotes DLM concentrations in plasma and each tissue that were significantly higher when the insecticide was given in GF. Different capital and lower case letters designate statistically significant difference within the GF and AL groups, respectively.

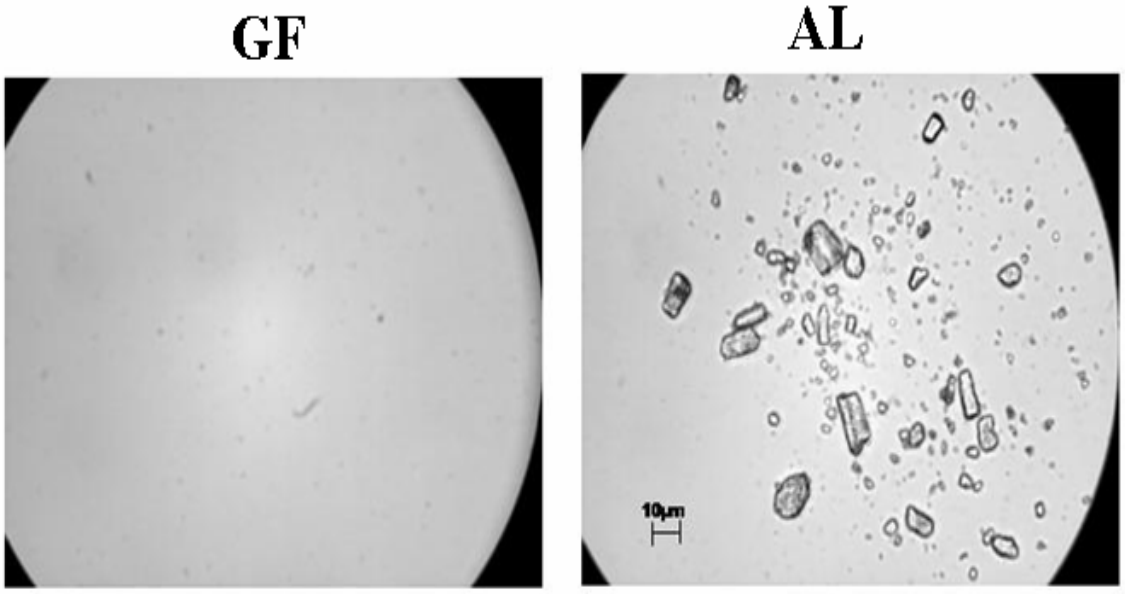

Figure 2-5. DLM GF and AL formulations as seen under a phase-contrast microscope (original magnification X 40). Particle size in the AL formulation was measured using a calibrated ocular micrometer attached to the microscope. A 10 µM scale is included to aid in judgement of particle size in AL.

Table 2-1. Toxicokinetic parameter estimates following iv injection of DLM in GF or AL

Toxicokinetic parameters	**Glycerol Formal (GF) 2 mg DLM/kg**	**Alkamuls (AL) 10 mg DLM/kg**
AUC (µg•h/ml)	7.19 ± 1.77	5.21 ± 1.64
$t_{1/2}$ (h)	15.03 ± 1.31*	30.52 ± 10.60
Vd (l/kg)	7.36 ± 2.70*	85.30 ± 22.45
Cl (l/h)	0.12 ± 0.04*	0.85 ± 0.27

Values represent means±SE of groups of 3 or 4 rats. * Indicates significant difference ($p<0.05$) between the two formulations.

Table 2-2. Toxicokinetic parameter estimates following oral administration of 10 mg DLM/kg in GF or in AL

Toxicokinetic parameters	**Glycerol Formal (GF)**	**Alkamuls (AL)**
ka (h^{-1})	1.38 ± 0.66	1.60 ± 1.01
Cmax (µg/ml)	0.95 ± 0.27*	0.09 ± 0.01
Tmax (hr)	1.50 ± 0.58	1.0 ± 0.00
AUC (µg•h/ml)	5.49 ± 0.22*	0.61 ± 0.14
F	0.15 ± 0.02*	0.017 ± 0.004
$t_{1/2}$ (h)	20.15 ± 3.22	24.82 ± 5.10
Vd (l/kg)	7.99 ± 0.48	8.48 ± 1.17
Cl (l/h)	0.11 ± 0.00	0.10 ± 0.01

Values represent means±SE for groups of 4 or 5 rats. * Indicates significant difference ($p<0.05$) between the two formulations. Bioavailability of DLM in AL was calculated using GF iv data, because of substantial pulmonary trapping of DLM given iv in AL.

CHAPTER 3

TOXICOKINETIC AND TISSUE DISTRIBUTION STUDY OF DELTAMETHRIN IN ADULT SPRAGUE-DAWLEY RATS[1]

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[1]Kyu-Bong Kim, Sathanandam S. Anand, Hyo Jung Kim, Catherine A. White, Jeffrey W. Fisher and James V. Bruckner. To be submitted to *Drug Metab. Dispos.*

ABSTRACT

The primary objective of this study was to characterize the absorption**,** systemic/tissue distribution and elimination of deltamethrin (DLM) over a range of doses in Sprague-Dawley (S-D) rats. DLM is one of the more neurotoxic members of a relatively new and commonly-used class of insecticides, the pyrethroids. Despite widespread exposure of the general population to pyrethroids, there is little basic toxicokinetic (TK) data to use in development of physiologically-based pharmacokinetic models or in health risk assessments. Male S-D rats of ~ 388 g were dosed orally with 0.4, 2 or 10 mg DLM/kg dissolved in glycerol formal. Another group received 2 mg/kg iv. Serial blood and tissue samples were taken and analyzed by HPLC for their DLM content, in order to obtain comprehensive time-course data sets for TK analyses. GI absorption of DLM was rapid but incomplete. Bioavailability was low. Some 83% of DLM in blood was present in the plasma. Very small proportions of systemically- absorbed doses reached the brain, the target organ of the bioactive parent compound. Fat, skin and muscle accumulated large amounts of the highly lipophilic chemical and served as slow-release depots. Tissue deposition was dose-dependent, though generally not proportional to dose. Elimination kinetics was linear in this dosage range. Much remains to be learned about key physiological/biochemical processes and barriers that govern the TK of DLM and other pyrethroids in laboratory animals and humans.

INTRODUCTION

Pyrethroids, synthetic derivatives of pyrethrins, have enjoyed increasing use as wide-spectrum insecticides. Casida and Quistad reported pyrethroids accounted for 25% of the worldwide insecticide market in 1998. Pyrethroids popularity stems from their insecticidal potency, slow development of pest resistance, relatively low acute toxicity of most congeners in mammals, and lack of persistence in the environment (Soderlund et al., 2002). Their use in the U.S. has increased substantially since 1998, due to the EPA's concern about possible adverse effects of organophosphates (OPs) on neurodevelopment in children. The number of human OP exposure incidents in the U.S. has decreased significantly since 2001, while pyrethroid incidents have increased (Sudakin, 2006). The widespread use of pyrethroids in forestry, agriculture and the home has resulted in their frequent detection in individuals who apply them and in large populations without apparent exposure (Berkowitz et al., 2003; Heudorf et al., 2004; Whyatt et al., 2002).

Traditionally, pyrethroids are divided into two classes (Types I and II) based on their structure and toxic effects. Type I compounds do not contain a cyano group, but Type IIs do. Type I compounds can cause tremors and skin parathesias, and are generally less potent neurotoxins than Type IIs. Major signs of acute poisoning by Type II compounds include salivation, hyperexcitability, tremors and choreoathetosis (Soderlund et al., 2002). Deltamethrin (DLM), a commonly-used Type II pyrethroid, was selected for the current investigation. Unlike most other commercial pyrethroids, it is available as a single isomer. DLM is one of the most potent neurotoxicants of this class of chemicals (Choi and Soderlund, 2006; Wolansky et al., 2006). The parent compound is believed to be the proximate toxicant, as demonstrated by: very

rapid induction of neurotoxicity upon intracerebral injection in mice (Lawrence and Casida, 1982); and correlation of brain DLM levels with the magnitude of clinical signs in rats dosed by ip injection (Rickard and Brodie, 1985). DLM is believed to act primarily by binding to voltage-dependent sodium channels in CNS neurons, thereby prolonging their opening (Wang et al., 2001; Choi and Soderlund, 2006).

The U.S. EPA and other regulatory agencies are currently planning how to evaluate health risks of pyrethroids and are collecting pertinent data on DLM and other frequently-used congeners. Toxicokinetic (TK) studies are playing an increasingly important role in reducing uncertainties inherent in risk assessments. Chemical toxicity is a dynamic process, in which the degree and duration of adverse effect in a target tissue or cell are dependent on the amount of toxic moiety reaching the target and the length of time it remains there. The target tissue dose and effects are dependent upon the net effect of a number of concurrent TK and toxicodynamic processes including systemic absorption, tissue deposition, metabolism, interaction with cellular components, elimination and recovery/repair. Gaining an understanding of these processes and learning how they differ with exposure route, dose and species will greatly reduce the number of unsubstantiated assumptions that must be made in risk assessments of pyrethroid exposures of humans.

TK data for DLM and most other pyrethroids are very limited and in some cases contradictory. Anadon and his co-workers have reported some of the most comprehensive TK studies of permethrin (1991), DLM (1996) and lambda-cyanohalothrin (2006). These investigators delineated the time-courses of the parent compounds in the plasma, multiple regions of the brain and one or two other tissues after administration of one toxic dosage level to rats. Pyrethroid levels in most brain areas were found to be substantially higher than plasma

levels. This observation was in direct contrast to that of Gray and Rickard (1982) and Rickard and Brodie (1985), who measured markedly higher ^{14}C-DLM concentrations in rat plasma than in brain. Marei et al. (1982) calculated the DLM half-life in brain of orally-dosed rats to be similar to that reported by Anadon et al. (1996) for plasma, though the former research group found the half-life to be 5 days for fat. Common deficiencies in studies from which the current DLM TK database (ATSDR, 2003) was obtained include: use of frankly toxic doses; administration of a single dosage, sometimes by an irrelevant exposure route; lack of data for key organs and tissues; and failure to sample long enough post dosing to delineate elimination profiles and TK indices.

The primary objective of this study was to conduct a comprehensive investigation of the TK of DLM over a range of oral doses in the adult rat. This study was part of an ongoing research project intended to characterize the TK of selected pyrethroids in immature and adult animals, and to utilize the data to develop and validate physiologically-based toxicokinetic (PBTK) models for use in risk assessments for different age groups. In light of DLM's high lipophilicity, experiments were designed to test the following hypotheses about the parent compound: gastrointestinal (GI) absorption is rapid and complete; brain, skin and fat accumulate and retain relatively high levels for a prolonged period; DLM partitions into erythrocyte membranes for transport in the blood; and modest levels in blood and lean tissues (skeletal muscle, liver and kidney) will be eliminated relatively quickly.

MATERIALS AND METHODS

Chemicals. [(*S*)-α-cyano-3-phenoxybenzyl-(1*R, cis*)-2,2-dimethyl-3-(2,2-dibromovinyl)-cyclopropane-1-carboxylate] (DLM) was kindly provided by Bayer CropScience AG (Monheim, Germany). DLM's chemical structure is shown in Fig. 3-1. Acetonitrile (HPLC grade) and glycerol formal (GF) were purchased from Sigma-Aldrich (St. Louis, MO). Methanol, sulfuric acid and deionized water were obtained from J.T. Baker (Phillipsberg, NJ). All other chemicals used were of the highest grade commercially available.

Animals. Male adult (~ 90 days old) Sprague-Dawley (S-D) rats were obtained from Charles River Breeding Laboratories (Raleigh, NC). The protocol for this study was approved by the University of Georgia Animal Care and Use Committee. The animals were housed in pairs in polycarbonate cages in an Association for Assessment and Accreditation of Laboratory Animal Care-approved animal facility with a 12-h light/dark cycle (light 0600 – 1800 h) at 22° C and 55 ± 5% relative humidity. Food (5001 Rodent Diet®, PMI Nutrition International) (Brentwood, MO) and tap water were provided *ad libitum* during an acclimation period of at least 10 days.

Plasma TK Experiments. Rats of 330 ± 19 g (x ± S.D.) were cannulated, in order that serial blood samples could be taken to delineate DLM's uptake and elimination profiles. Each animal was anesthetized by im injection of 0.1 ml/100 g bw of a "cocktail" consisting of ketamine hydrochloride (100 mg/ml), acepromazine maleate (10 mg/ml) and xylazine hydrochloride (20 mg/ml) (3:2:1, vol:vol:vol). A cannula (PE-50 polyethylene tubing) was surgically placed into the right carotid artery. The cannula was inserted toward the heart, until its tip rested just above the aortic arch. It was then securely ligated to the artery, passed under the skin and exteriorized at the nape of the neck, so the animal could move about freely upon recovery. Water was

provided, but food was withheld during a 24-h post-surgical recovery period before dosing. DLM was dissolved in GF prior to dosing. One group of 4 cannulated rats received 2 mg DLM/kg (0.2 ml/kg) iv via a caudal tail vein. Another group of 4 rats was administered 10 mg DLM/kg po in a total volume of 2 ml/kg by gavage. The animals were provided food 3 h after dosing, which was performed between 0900 and 1000 h. Serial arterial blood samples of 150 µl were withdrawn and collected in heparinized tubes at the following times following oral dosing: 0.25, 0.5, 1, 2, 4, 6, 9, 12, 24, 36, 48, 60, 72 and 96 h. Blood samples were similarly taken 0.02, 0.08, 0.25, 0.5, 1, 2, 4, 6, 9, 12, 24, 36, 48, 60, 72 and 96 h post iv injection. An equivalent volume of heparinized saline was injected via the arterial cannula following each blood withdrawal.

Tissue Disposition Experiment. The aim of this experiment was to delineate plasma and tissue uptake and elimination time-courses for a series of DLM doses. Three oral dosages were selected: 0.4, 2.0 and 10.0 mg DLM/kg. It was not possible to utilize a lower dosage due to the limit of quantitation of our assay. It was not advisable to exceed 10 mg/kg, as this dosage produced transient salivation and tremors. Rats of 388 ± 56 g (x ± S.D.) were provided water *ad libitum* but fasted for 12 h prior to treatment, in order to avoid intersubject variability in GI absorption resulting from varying food intake. Uncannulated rats were gavaged with each of the three dosages in glycerol formal (total volume = 2 ml/kg). Access to food was provided 3 h after dosing. Groups of 5 rats were euthanized with CO_2 after 0.5, 1, 2, 6, 12, 24, 48, 72 and 96 h, as well as 2 and 3 weeks post dosing. Blood samples were drawn from the inferior vena cava and collected in heparinized tubes. The whole brain, left kidney, ventral skin (hair removed), a part of the liver's median lobe, and portions of perirenal fat and thigh muscle were then excised and stored at -80° C until analysis.

DLM Extraction and Analysis. The DLM content of blood and tissue samples was quantified by the procedure of Kim et al. (2006). Plasma was obtained by centrifuging the 150-µl blood samples at 3,000 rpm for 5 min at 4° C in a microcentrifuge (Microfuge® 22R, Beckman Coulter) (Fullerton, CA). Sixty-five µl of plasma were transferred to microcentrifuge tubes containing 130 µl of acetonitrile. The tubes were vortexed (Mini Vortexer®, VWR) (West Chester, PA) for 30 sec, and then centrifuged for 5 min in the microcentrifuge at 13,000 rpm. The clear supernatant was subsequently injected into a high performance liquid chromatograph (HPLC) as described below. Each tissue was homogenized in 4 volumes of 50% acetonitrile in distilled water (vol:vol) with a Tekmar Tissumizer® (Cincinnati, OH). Sixty-five µl of the tissue hemogenates were transferred to microcentrifuge tubes containing 130 µl of acetonitrile. The tubes were vigorously agitated on the vortex mixer for 30 sec, and subsequently centrifuged for 5 min at 13,000 rpm. An aliquot of the clear supernatant was then injected into a HPLC.

The HPLC unit was a Shimadzu (Canby, OR) equipped with a LC-10AT pump, a DGU-14A degasser, a SIL-HT autosampler, a SPD-10AV detector and an EZStart 7.2 SP1 Rev B computer. The analytical column was an Ultrasorb 5 ODS 20 (250 mm X 4.6 mm, 5 µm particle) (Phenomenex, Torrance, CA), and the guard column was a Phenomenex Fusion RP (4 mm X 3 mm). The mobile phase was 80% acetonitrile and 20% sulfuric acid (1%, v:v) (v:v). The flow rate was 1 ml/min. The eluate was monitored at 230 nm. DLM eluted at ~ 14.5 min under these conditions. A series of standards was prepared and run each day biological samples were analyzed. The limits of detection (LOD) and quantitation (LOQ) for the method were 0.01 and 0.05 µg DLM/ml, respectively.

TK Data and Statistical Analyses. Means and S.E.s were calculated with Microsoft Excel 2003 (Microsoft Co., Redmond, WA). TK parameters, including area under the DLM

concentration versus time curve (AUC^{∞}_{o}), time of peak blood concentration after dosing (T_{max}), volume of distribution (Vd), clearance (Cl) and terminal elimination half-life ($t_{½}$), were calculated using Winnonlin (ver. 4.1) noncompartmental model analysis by Scientific Consulting, Inc. (Cary, NC). The maximum blood concentrations (C_{max}) were observed values. Student's t test (prism 3.03, San Diego, CA) was used to assess whether differences in TK parameters for the 10 mg/kg po and 2 mg/kg iv groups were significantly different ($p < 0.05$) and one-way analysis of variance (ANOVA), followed by the Newman-Keuls Multiple Comparison Test was performed to determine whether C_{max} values for blood and tissues time-courses were significantly different ($p < 0.05$) from values for adjacent time-points.

RESULTS

Plasma TK.

Plasma DLM time-courses in rats administered a single oral dose of 10 mg DLM/kg or injected iv with 2 mg/kg are pictured in Fig. 3-2. Both doses elicited salivation and tremors. These signs disappeared within 3 h in the oral group, but persisted for several more hours in the iv animals. It can be seen in Fig. 3-2 that DLM is very rapidly distributed systemically following iv injection. In contrast, its systemic elimination is quite slow, as reflected by a terminal elimination half-life of 15 h (Table 3-1). DLM administered orally in GF was rapidly absorbed ($ka = 1.38\ h^{-1}$; $T_{max} = 1 – 2$ h), distributed, and excreted at a rate that appeared to be comparable following iv injection. Despite its rapid GI absorption and distribution, the bioavailability of DLM was only 15%. Volume of distribution, $t_{½}$ and clearance did not vary significantly between the po and iv groups (Table 3-1).

Blood:Plasma Ratio.

In vivo experiments revealed that DLM is carried in the bloodstream largely in the plasma. Plasma, blood and red blood cell (rbc) DLM concentrations are contrasted 2, 6 and 12 h after adult S-D rats received 10 mg DLM/kg po (Fig. 3-3). It is evident that plasma levels far exceed rbc levels. Blood levels are lower than plasma levels as a result of blood's larger volume. The plasma/blood ratios do not change substantially over time. Mean plasma/blood ratios were also calculated by averaging the plasma/blood ratios for each sampling time from 1 to 48 h for each animal given the 2 and the 10 mg/kg po doses (data not shown). The mean plasma/blood ratios for the 2 and 10 mg/kg group were 0.83 ± 0.06 and 0.82 ± 0.5, respectively (x ± S.E, n = 30). Thus ~ 83% of DLM in blood was present in the plasma.

Dose-Dependence of Tissue Disposition.

Plasma and tissue DLM concentration-versus-time curves in orally-dosed rats are shown in Figs. 3-4 and 3-5. GI absorption and deposition of DLM in well-perfused organs were quite rapid, as manifest by T_{max} values of 1 or 2 h for all three dosage-levels. Peak levels were not reached until much later in the poorly perfused, lipoidal tissues (i.e., 6 and 12 h in the adipose tissue and skin, respectively) (Table 3-2). Concentrations in skeletal muscle were maximal from 2 – 6 h in the 10 mg/kg animals. DLM was measurable for up to 6, 24 and 48 h in the plasma and brain of the 0.4, 2 and 10 mg/kg groups, respectively. Unfortunately, the parent compound was not detectable after 6 h in any of the biological samples from rats given 0.4 mg/kg. Therefore it was not feasible to estimate accurate TK parameters for this lowest dose. Visual inspection revealed that the plasma and liver profiles resembled one another, (Figs. 3-4A and 3-5A), but that brain concentrations (Fig. 3-4B) were substantially lower at each time-point. Fat

concentrations (Fig. 3-4D), in contrast, were initially lower than plasma or blood levels, but subsequently exceeded them and remained elevated for a much longer time. The time-course of DLM in skin (Fig. 3-5B) resembled that in fat, although skin levels were initially lower. Unexpectedly, elimination from skeletal muscle (Fig. 3-5C) was also relatively slow, but DLM concentrations were much lower during the uptake and elimination phases.

It was possible to assess the dose- and tissue-dependency of a number of DLM TK indices for the 2 and 10 mg/kg groups (Table 3-2). The magnitude of the plasma and tissue profiles was clearly dose-dependent (Figs. 3-4 and 3-5). In most instances the 1- and 2-h DLM concentrations differed little from one another in both the 2 and 10 mg/kg groups. The poorly perfused, lipoidal tissues (i.e., fat and skin) exhibited the longest T_{max}s (i.e., 6 and 12 h, respectively) (Table 3-2). The T_{max} did not vary with dose, with the exception of the 6-h value for muscle in the 10 mg/kg group. Careful inspection of Fig. 3-5C revealed that the 2-, 6- and 12-h muscle levels differed little from one another, as was the case for the 6-, 12- and 24-h fat and skin levels (Figs. 3-4D and 3-5B Insets). C_{max}s were dose-dependent, but did not increase in direct proportion to dose. The increases in C_{max} from 2 to 10 mg/kg were only ~ 2-fold, with the exception of the brain, fat and kidney. Increases in AUCs in this dosage range were more nearly proportional to dose in some instances (e.g., plasma, fat, liver and kidney) (Table 3-2). The fat AUC values far exceeded those of plasma and other tissues. DLM content in the skin over time was relatively high, followed by muscle. AUCs are presented as bar graphs for ease of comparison in Fig. 3-6.

Relative Tissue DLM Content.

Total DLM content of the blood, plasma and each tissue, analyzed 2 and 12 h after oral administration of 0.4, 2 and 10 mg DLM/kg, is listed in Table 3-3. Brain DLM burdens, even at the T_{max} (2 h), are extremely low and account for only 0.1 – 0.3% of the body burden at 2 and 12 h, respectively. Conversely, the animals' adipose tissue and skin contain large amounts of the highly lipophilic insecticide. Concentrations are modest at 2 h, but increase significantly by 12 h, when concentrations in most other tissues have diminished substantially. The 2- and 12-h concentrations in skin are lower than those in fat (Table 3-3). This is counterbalanced by the skin's relatively large volume, resulting in comparable burdens and % body burdens in skin and fat 12 h after dosing (Table 3-4). Skeletal muscle's large volume and long $t_{½}$ are responsible for the tissue's sizable DLM content at 12 h, despite its modest DLM concentrations. Some 97% of the amount of DLM remaining in monitored tissues 12 h after administration of 2 and 10 mg/kg was present in the fat, muscle and skin. Tissue DLM burdens were dose-dependent, though the increases were not directly proportional to dose.

Partition Coefficients.

In vivo tissue:blood distribution ratios, or partition coefficients (PCs) are included in Table 3-2. A common way to determine these values is to contrast chemical concentrations in each media when steady-state is reached. In the present case, where comprehensive time-course data are available for compounds with long half-lives, AUC^{∞}_{o} values can be contrasted. Both tissue:plasma and tissue:blood PCs are presented. The tissue:blood values are larger, because blood DLM concentrations are lower than plasma concentrations (Fig. 3-3). PCs are essential input parameters for PBTK models. The PCs do not vary appreciably with DLM dose, with the

exception of ~ 2-fold higher muscle and skin values in the 10 than in the 2 mg/kg group. As expected, adipose tissue exhibits the highest PCs, followed in turn by skin and skeletal muscle. The PCs for brain are lowest of all the biological specimens.

Tissue and Systemic Elimination.

The terminal elimination half-lives varied substantially among the different organs and plasma/blood (Table 3-2). The brain, kidney and plasma exhibited the shortest $t_{½}$s. Plasma levels diminished somewhat more rapidly than whole brain levels, resulting in a progressive decline in plasma:brain AUC ratios (Fig. 3-7). The $t_{½}$ for liver was longer, though it is the major site of DLM metabolism (Anand et al., 2006). DLM was most slowly eliminated from fat and skin, the most lipophilic tissues evaluated. Unexpectedly, elimination from skeletal muscle was equally slow. The rate of decrease in DLM concentrations in plasma and most tissues did not appear to be dose-dependent. Half-life values were comparable in each instance, with the exception of the skin.

DISCUSSION

Relatively little is known about the TK of DLM or other pyrethroids in humans and other mammals, despite frequent exposures of the populace to this relatively new class of insecticides. The time-course data and TK parameter estimates presented here provide a relatively comprehensive overview of the systemic uptake, disposition and elimination of iv and oral DLM in rats. A key contribution of the work to toxicity risk assessment of DLM is information on the

relationships among administered dose, internal dose (i.e., blood/plasma levels) and target organ dose (i.e., brain C_{max} and AUC).

DLM appeared to be rapidly but incompletely absorbed from the GI tract of fasted adult rats. Peak blood levels were manifest in 1 – 2 h with each dosage level, but bioavailability was only 15%. We recently reported that DLM is completely dissolved and rapidly absorbed when given orally to rats in glycerol formal (Kim et al., 2007). Interestingly, Anadon et al. (1996) reported a T_{max} of 1.83 h and bioavailability of 14.4% in fasted rats gavaged with 26 mg DLM/kg in sesame oil. It might be anticipated that the oil would serve as a reservoir in the gut to delay the absorption of lipophilic chemicals (Kim et al., 1990). Digestible oils, however, have been shown to serve as carriers for highly lipophilic compounds such as dioxins and hexachlorobenzene into the lacteals (Lakshmanan et al., 1986; Roth et al., 1993), thereby bypassing first-pass hepatic metabolism. This may account for the comparable C_{max} and F values we and Anadon et al. (1996) observed for DLM given in different dosage vehicles.

DLM was apparently not well absorbed from the GI tract, contrary to what was hypothesized. A review of the literature revealed bioavailability was also low for a number of other highly lipid-soluble, slowly-metabolized chemicals including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Wang et al., 1997), benzo(*a*) pyrene (Foth et al., 1988) and other polyaromatic hydrocarbons (PAHs) (Roth et al., 1993). Tanabe et al. (1981) observed that the efficiency of oral absorption of PCB isomers diminished with increasing chlorine content (i.e., increasing lipophilicity). No logical explanations have been given for the limited GI absorption of PAHs. In recent experiments, we found ~ 40% of a 10 mg DLM/kg oral dosage to be eliminated in the feces of rats (data reported elsewhere). DLM was not detectable in the feces following its iv injection, suggesting that the chemical does not reenter the GI lumen by biliary excretion or by

passive diffusion from mesenteric blood. P-glycoprotein (P-gp) is located on the GI luminal brush border membrane (Zhang and Benet, 2001; Brady et al., 2003) and may diminish DLM absorption in rats by acting as an efflux transporter. There has been little evidence reported to date, however, that DLM or other pyrethroids are P-gp substrates. Permethrin and fluvalinate have been found to inhibit P-gp-mediated efflux of doxorubicin from mouse melanoma cells transfected with the human *MDR1* gene (Bain and LeBlanc, 1996). Efficient first-pass GI and liver metabolism could contribute to DLM's low bioavailability. The cytochrome P450s (CYPs) 3A4 and 1A1 and carboxylesterases (CaEs) located in human and rat enterocytes (Mendoza et al., 1971; Paine et al., 1999; Poet et al., 2003) may play some role in metabolism of ingested DLM, particularly at low dosage levels. Efficient first-pass hepatic metabolism would contribute significantly to the low oral bioavailability. DLM has been shown to be primarily metabolized in vitro by rat hepatic microsomal CYP1A1 and CYP1A2, as well as by hepatic and plasma CaEs (Anand et al., 2006). Intrinsic clearance was much higher by the liver than by plasma. Godin et al. (2006) recently reported that DLM was metabolized primarily by oxidation in rat liver microsomes, but that hydrolysis predominated in human liver microsomes. Ross et al. (2006) found that CaEs in hepatic microsomes from mice, rats and humans hydrolyzed *cis*- and *trans*-permethrin at similar rates in each species.

Relatively little DLM was found to be associated with erythrocytes, in contrast to one of the proposed hypotheses. Direct measurements revealed that ~ 83% of DLM was present in the plasma 2, 6 and 12 h after rats received 10 mg DLM/kg po. It has not been established whether DLM binds to plasma proteins. Halogenated biphenyls are not thought to bind to specific sites on plasma proteins, but to partition into and be associated with hydrophobic regions (Matthews et al., 1984). Very lipophilic chemicals are preferentially absorbed from the gut into the neutral

lipid core of chylomicrons, which may enter blood and/or lymphatic vessels (Roth et al., 1993). Low density lipoproteins (LDLs) and high density lipoproteins (HDLs) are the major plasma acceptors of PAHs and halogenated aliphatic hydrocarbons. A relatively low proportion of most PAHs in blood are associated with erythrocyte membranes, as proved to be the case for DLM.

Unexpectedly low concentrations of DLM were present in its target organ. We had theorized that substantial amounts of DLM would accumulate in the brain, due to its relatively high lipid content. Anadon et al. (1996) measured markedly higher DLM levels in 5 of 6 brain regions than in plasma of orally-dosed rats. Based upon the brain DLM C_{max} of 0.18 µg/g at the T_{max} (2 h) and an assumed brain weight of 2 g in a 388-g rat, it was calculated that only 0.29 % of the total body burden of rats given 10 mg/kg was present in the whole brain (Table 3-3). Gray and Rickard (1982) and Rickard and Brodie (1985) also found that brain DLM levels were much lower than blood levels. This unanticipated phenomenon remains to be explained. The brain's largely phospholipid composition may be a factor in limited partitioning of DLM. DLM readily exits the blood and enters the other organs and tissues studied, but may be limited by the blood-brain barrier. The substantial amounts of P-gp in the blood-brain (Brady et al., 2002) and cerebrospinal (Choudhuri et al., 2003) barriers may serve as effective efflux transporters. This supposition is supported by DLM's short $t_{½}$ in brain (Table 3-2), though as noted previously, the pyrethroid is yet to be shown to be a P-gp substrate. Rat brain is reported to slowly metabolize DLM to a limited extent (Rickard and Brodie, 1985). Relatively low levels of PAHs such as PCB 153, dioxin, pyrene and chlordane are also found in the CNS of orally-dosed rodents. Brain lipids of Inuit people from Greenland contain lower concentrations of 11 organochlorines and 14 PCB congeners than do lipid extracts from their liver, omental fat and subcutaneous fat (Dewailley et al., 1999).

Adipose tissue, skin and skeletal muscle were found to be the major depots for DLM. T_{max} values were relatively long for these poorly-perfused tissues. As hypothesized, perirenal fat exhibited substantially higher concentrations than other tissues/organs (Table 3-2, Fig. 3-6). DLM levels in skin were lower, but the skin's relatively large volume and similarly long *t½* resulted in comparable total DLM content 2 and 12 h post dosing (Table 3-3). Muscle contained modest DLM concentrations, but its mass (40.4% of total bw) and slow elimination rate resulted in it serving as the third major depot for the parent compound. It is not clear at the present time whether DLM binds to muscle, and if so whether this and slow perfusion account for the chemical's long half-life there. It is clear that fat, skin and muscle are important factors in DLM's potential for systemic accumulation and its delayed elimination and prolonged duration of action. Judging from comparable tissue *t½* values for the 2 and 10 mg/kg and dosages, the elimination kinetics of DLM in this dosage range is not dose-dependent. The $t_{½}$ values for the 10 mg/kg po and 2 mg/kg iv doses, as well as these group's AUC, Vd and CL values did not differ significantly (Tables 3-1 and 3-2). Unfortunately, acute neurotoxicity precluded administration of higher iv doses.

Blood and tissue time-course data are essential for development and validation of PBTK models. Partition coefficients (PCs), or distribution ratios are an essential model input parameter. *In vivo* PCs, calculated as the ratio of the tissue AUC to the blood and plasma AUC, are included in Table 3-2. Such tissue:plasma PCs were recently used in construction of a PBTK model for DLM in adult rats (Mirfazelian et al., 2006). The 2 and 10 mg/kg blood, plasma, brain and fat DLM concentration versus time profiles were utilized to assess the accuracy of the model's simulations. Such a validated model can be used with reasonable certainty to predict target organ (brain) doses of DLM for a variety of doses and exposure scenarios.

In summary, we have characterized the systemic absorption, distribution and elimination of a necessarily narrow range of oral doses of DLM in adult, male S-D rats. GI absorption of the insecticide is rapid but incomplete. Bioavailability is low. DLM in blood is largely present in plasma. Very small proportions of the absorbed doses reach the brain. Fat, skin and muscle ultimately accumulate large amounts of the highly lipophilic chemical and serve as slow-release depots. Other orally-administered pyrethroids may be found to exhibit similar disposition characteristics. Much remains to be learned about specific physiological processes and barriers that govern pyrethroids' systemic absorption, transport, metabolism, target organ deposition, binding and elimination.

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TABLE 3-1

Plasma DLM toxicokinetic parameter estimates for oral and iv administration

Male S-D rats were given 10 mg DLM/kg by gavage or 2 mg DLM/kg iv. Serial blood samples were taken for up to 48 h post dosing and analyzed for their DLM content. The T_{max} was observed. Other TK indices were calculated using Winnonlin noncompartmental analysis. Oral and iv $t_{1/2}$, Vd and CL values are not significantly different from one another at $p < 0.05$. Values are means ± S.E. (n = 3 - 4).

Toxicokinetic parameters	Dose	
	Oral *(10 mg/kg)*	iv *(2 mg/kg)*
ka (h^{-1})	1.38 ± 0.66	-
C_{max} *(µg/ml)*	0.95 ± 0.27	-
T_{max} *(h)*	1.50 ± 0.58	-
AUC *(µg●h/ml)*	5.49 ± 0.22	7.19 ± 1.77
F	0.15 ± 0.02	-
$t_{1/2}$ *(h)*	20.15 ± 3.22	15.03 ± 1.31
Vd *(l/kg)*	7.99 ± 0.48	7.36 ± 2.70
CL *(l/h)*	0.11 ± 0.00	0.12 ± 0.04

TABLE 3-2

DLM toxicokinetic parameter estimates for tissues following oral dosing

Male S-D rats were gavaged with 0.4, 2 or 10 mg DLM/kg. Serial sacrifices were performed for up to 3 weeks after dosing, and DLM levels were measured. T_{max} was observed. Other TK parameters were calculated using Winnonlin. Values are means ± S.E. for groups of 3 - 5 rats.

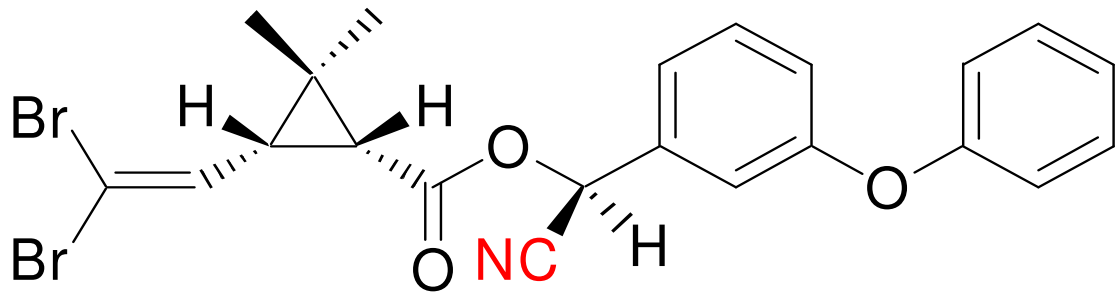
Dosage group *(mg/kg)*	Tissue	*Cmax (µg/ml or g)*	*Tmax (h)*	Half-life *(h)*	AUC $_0^\infty$ observed *(µg·h/ml)*	$\frac{AUC_{Tissue}}{AUC_{Plasma}}$	$\frac{AUC_{tissue}}{AUC_{blood}}$
0.4	Plasma	0.12 ± 0.01	1	-	-	-	-
	Blood	0.09 ± 0.03	1	-	-	-	-
	Brain	0.02 ± 0.00	2	-	-	-	-
	Fat	0.22 ± 0.03	2	-	-	-	-
	Liver	0.21 ± 0.06	1	-	-	-	-
	Kidney	0.08 ± 0.01	2	-	-	-	-
	Muscle	0.08 ± 0.03	2	-	-	-	-
	Skin	0.11 ± 0.00	2	-	-	-	-
2	Plasma	0.53 ± 0.14	1	15.8	2.7	1.0	1.6
	Blood	0.37 ± 0.10	1	16.3	1.7	0.6	1.0
	Brain	0.04 ± 0.01	1	12.5	0.8	0.3	0.1
	Fat	0.80 ± 0.34	6	168.2	143.0	53.0	84.1
	Liver	0.51 ± 0.12	1	31.0	3.5	1.3	2.1
	Kidney	0.19 ± 0.06	1	13.6	1.6	0.6	0.9
	Muscle	0.18 ± 0.01	2	191.4	13.8	5.1	8.1
	Skin	0.34 ± 0.11	12	204.2	48.1	17.8	28.3
10	Plasma	1.22 ± 0.36	2	14.6	11.2	1.0	1.7
	Blood	0.91 ± 0.27	2	10.7	6.5	0.6	1.0
	Brain	0.18 ± 0.02	2	19.5	2.2	0.2	0.2
	Fat	2.38 ± 0.99	6	200.2	514.2	45.9	79.1
	Liver	1.02 ± 0.37	1	32.4	10.0	0.9	1.5
	Kidney	0.65 ± 0.19	1	16.2	5.9	0.5	0.9
	Muscle	0.29 ± 0.06	6	190.5	27.2	2.4	4.2
	Skin	0.77 ± 0.53	12	145.2	104.9	9.4	16.1

TABLE 3-3

Disposition of DLM in blood and tissues 2 and 12 h after dosing

Male S-D rats were gavaged with 0.4, 2 or 10 mg DLM/kg, and DLM levels measued 2 and 12 h later. Tissue burdens were calculated by multiplying each tissue's % bw by the rats' mean bw (388 g) times the mean tissue concentration. The rat tissue volumes (% bw) were taken from Brown et al. (1997) and Schoeffner et al. (1999). Disposition is also expressed as % of the total body burden. DLM concentrations are mean ± S.E. (n = 3-5).

Dosage group *(mg/kg)*	Tissue	% Body Wt.	2 h			12 h		
			DLM Conc. *(µg/ml or g)*	Tissue Burden *(µg)*	% Body Burden	DLM Conc. *(µg/ml or g)*	Tissue Burden *(µg)*	% Body Burden
0.4	Plasma	3.80	0.11 ± 0.03	1.6	5.2	-	-	-
	Blood	7.40	0.07 ± 0.02	2.0	6.5	-	-	-
	Brain	0.54	0.02 ± 0.00	0.04	0.12	-	-	-
	Fat	6.48	0.22 ± 0.03	5.6	17.8	-	-	-
	Liver	3.44	0.19 ± 0.07	2.5	8.1	-	-	-
	Kidney	0.79	0.08 ± 0.01	0.2	0.8	-	-	-
	Muscle	40.40	0.08 ± 0.03	12.5	40.3	-	-	-
	Skin	19.30	0.11 ± 0.00	8.2	26.5	-	-	-
	Total	78.35	-	31.1	100.0	-	-	-
2	Plasma	3.80	0.38 ± 0.13	5.6	9.2	0.02 ± 0.00	0.3	0.6
	Blood	7.40	0.22 ± 0.06	6.3	10.4	0.02 ± 0.01	0.6	1.1
	Brain	0.54	0.04 ± 0.01	0.08	0.13	0.02 ± 0.01	0.04	0.7
	Fat	6.48	0.35 ± 0.06	8.8	14.5	0.78 ± 0.12	19.7	37.5
	Liver	3.44	0.21 ± 0.08	2.8	4.5	0.03 ± 0.01	0.4	0.8
	Kidney	0.79	0.17 ± 0.03	0.5	0.9	0.03 ± 0.01	0.1	0.2
	Muscle	40.40	0.18 ± 0.10	28.2	46.3	0.04 ± 0.01	6.3	11.9
	Skin	19.30	0.19 ± 0.06	14.2	23.3	0.34 ± 0.10	25.5	48.5
	Total	78.35	-	61.0	100.0	-	52.5	100.0
10	Plasma	3.80	1.22 ± 0.36	18.0	13.7	0.21 ± 0.14	3.1	2.1
	Blood	7.40	0.91 ± 0.27	26.1	20.0	0.11 ± 0.03	3.2	2.1
	Brain	0.54	0.18 ± 0.02	0.35	0.27	0.04 ± 0.01	0.08	0.05
	Fat	6.48	1.05 ± 0.24	26.5	20.2	2.04 ± 1.24	51.5	34.6
	Liver	3.44	0.67 ± 0.21	8.8	6.8	0.11 ± 0.04	1.5	1.0
	Kidney	0.79	0.64 ± 0.25	2.0	1.5	0.09 ± 0.03	0.3	0.2
	Muscle	40.40	0.28 ± 0.08	43.9	33.5	0.22 ± 0.13	34.5	23.2
	Skin	19.30	0.31 ± 0.09	23.2	17.7	0.77 ± 0.53	57.7	38.8
	Total	78.35	-	131.0	100.0	-	148.6	100.0



Deltamethrin

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

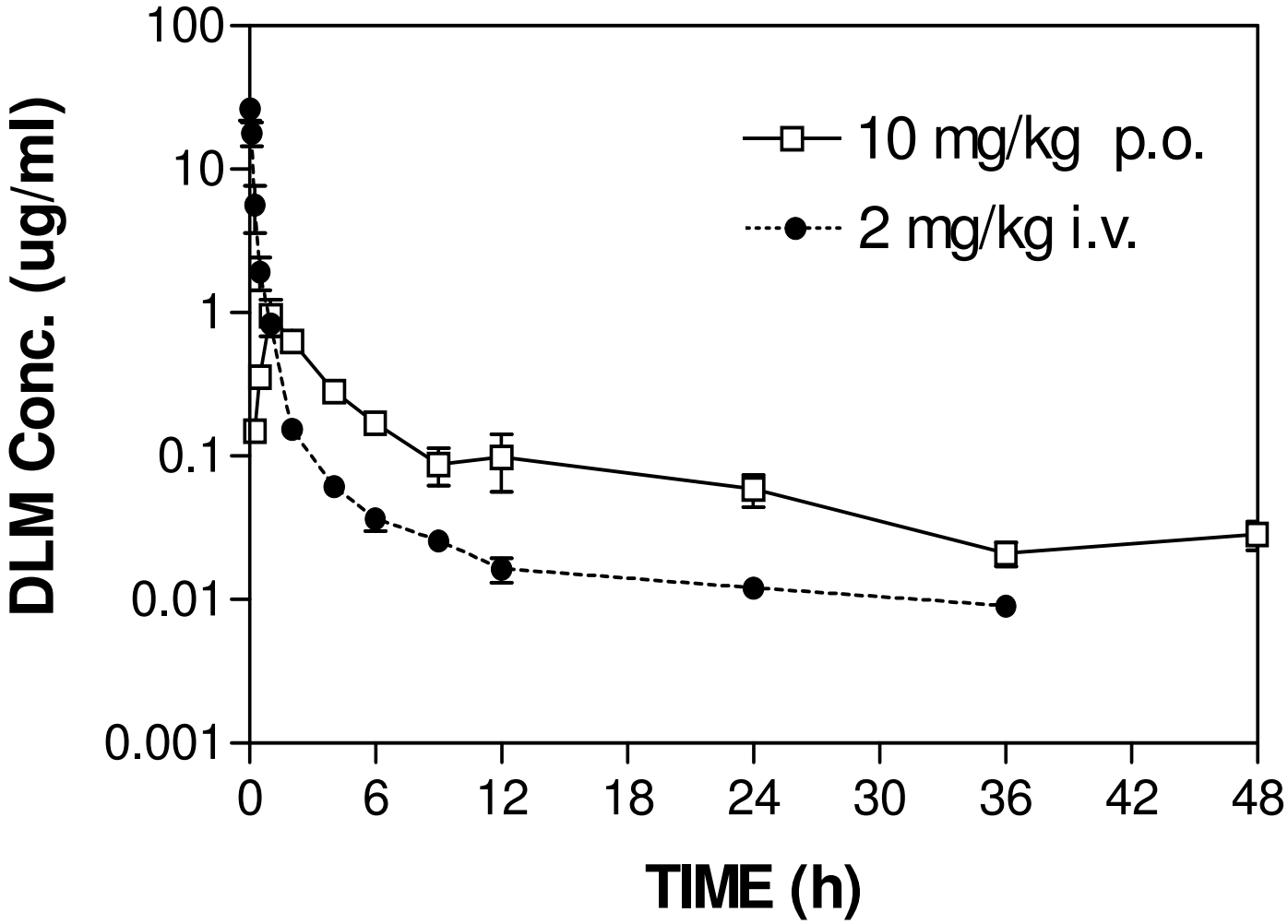


Figure 3-2. Plasma DLM concentration-versus-time profiles of male S-D rats given 10 mg DLM/kg po or 2 mg DLM/kg iv in glycerol formal. Serial plasma samples were analyzed for the parent compound by HPLC as described in the Materials and Methods. Symbols represent means ± S.E. for groups of 3 – 4 animals.

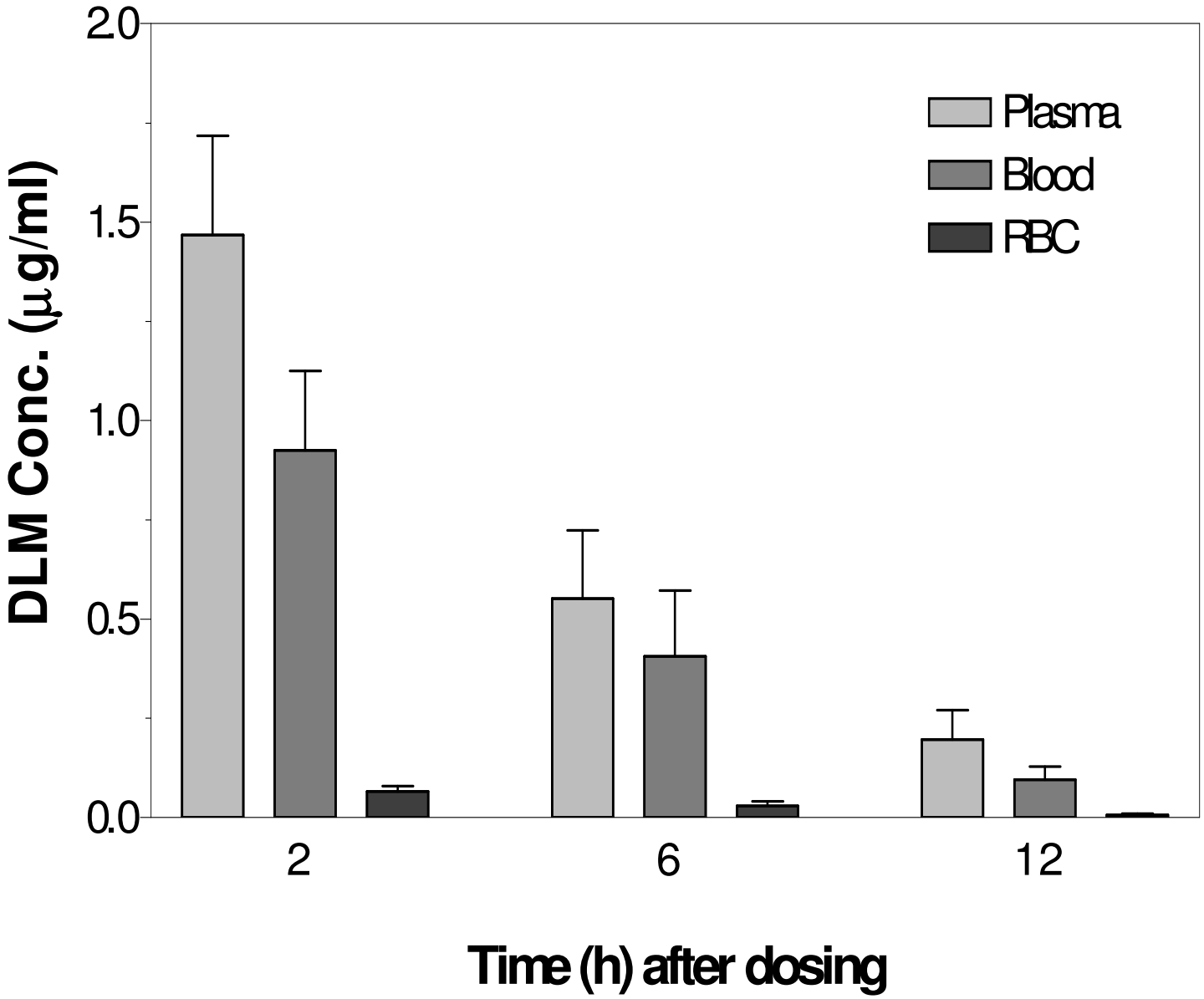


Figure 3-3. Mean plasma, blood and red blood cell (rbc) DLM concentrations in samples obtained 2, 6 and 12 h after male S-D rats were given 10 mg DLM/kg po. Bar heights represent mean concentrations in groups of 3 – 5 animals. Brackets indicate S.E.

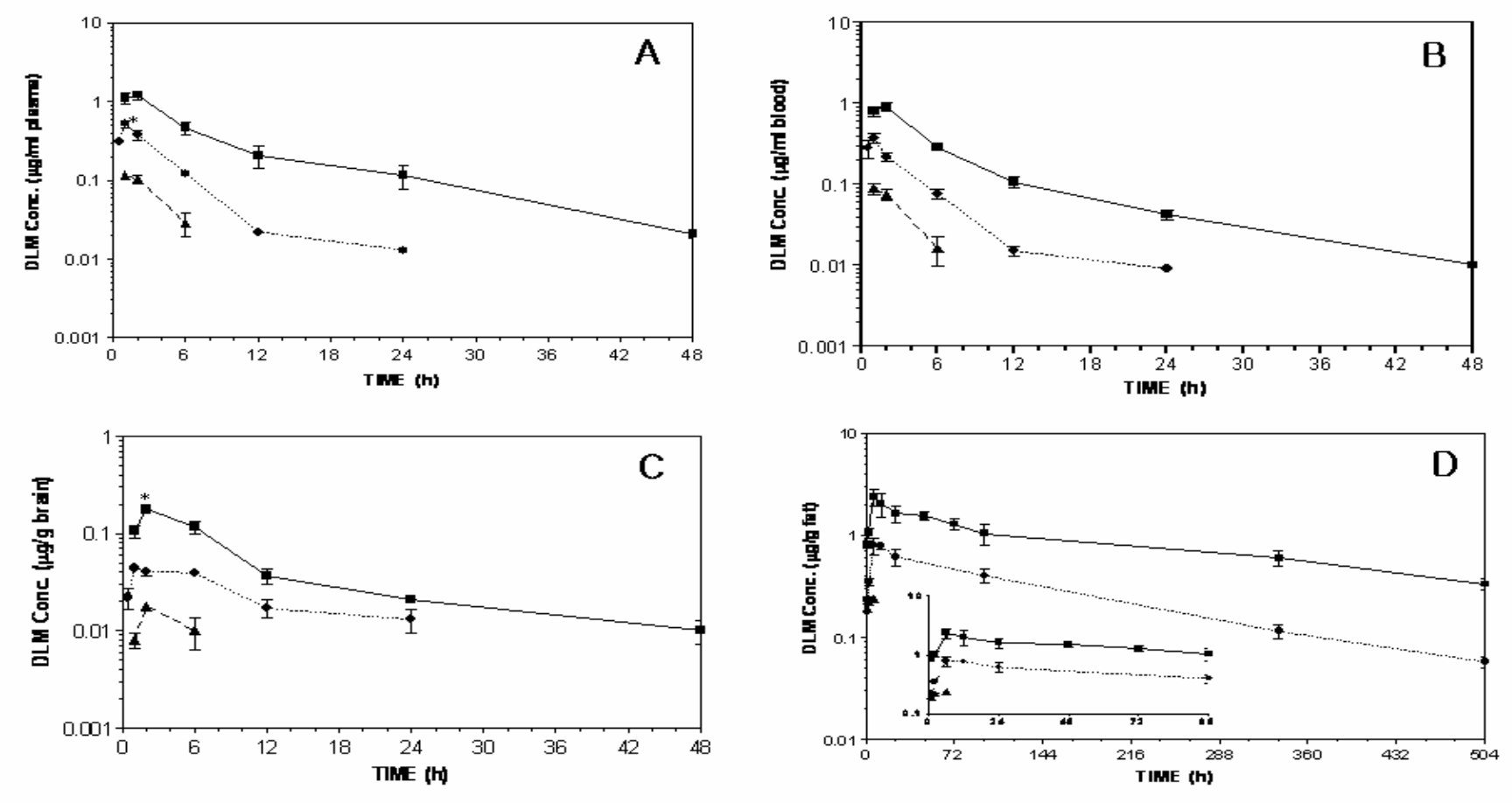


Figure 3-4. DLM uptake and elimination profiles of male S-D rats gavaged with 0.4 (▲), 2 (●) or 10 (■) mg DLM/kg. Serial plasma (A), blood (B), whole brain (C) and perirenal fat (D) samples were analyzed for their DLM content by HPLC. The early time-scale is expanded in D. Symbols represent means ± S.E. for groups of 3 – 5 rats. * Indicates C_{max} that is significantly different from the DLM concentrations at adjacent time-points ($p < 0.05$).

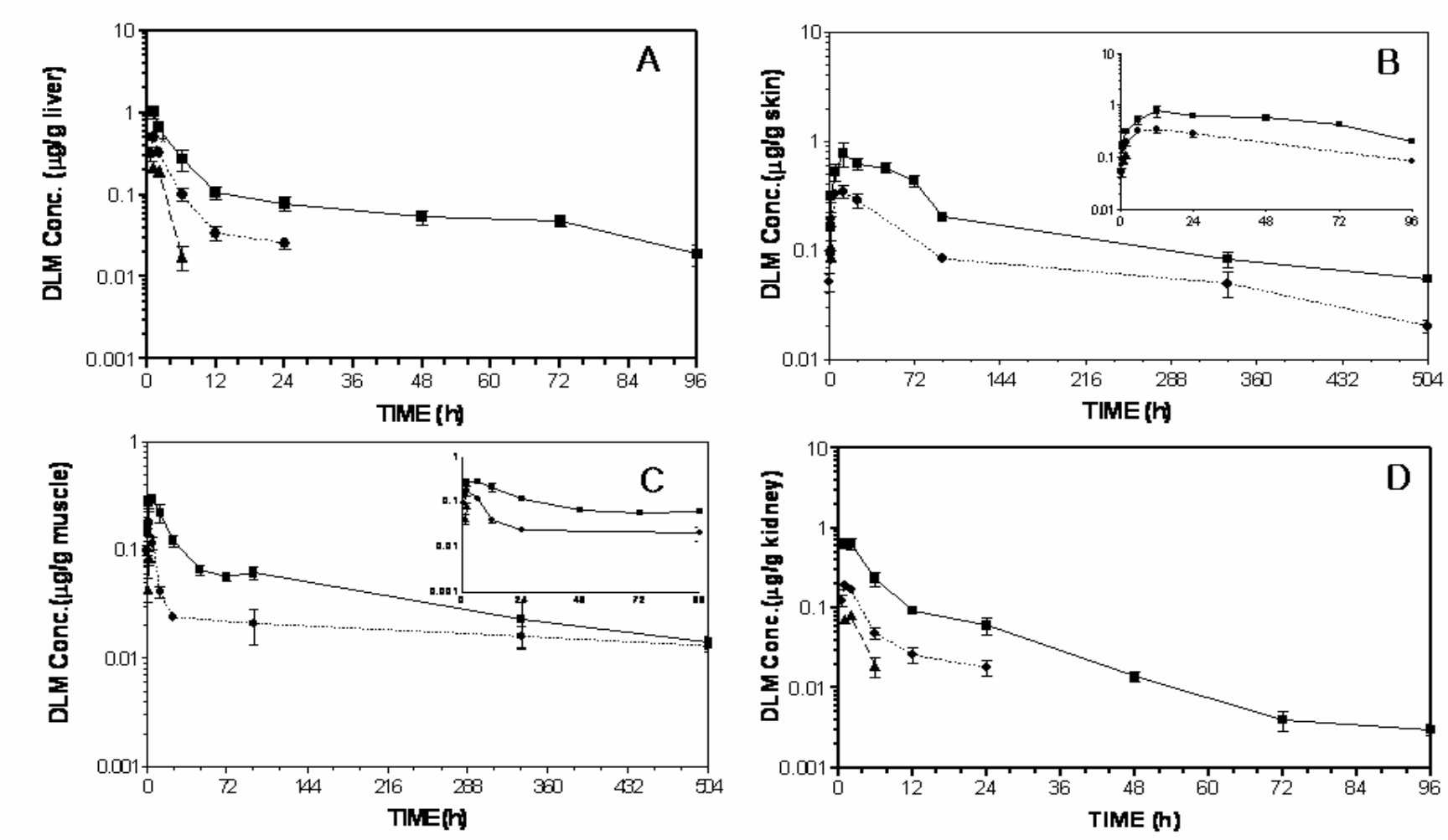


Figure 3-5. DLM uptake and elimination profiles of male S-D rats gavaged with 0.4 (▲), 2 (●) or 10 (■) mg DLM/kg. Serial liver (A), skin (B), skeletal muscle (C) and kidney (D) samples were analyzed for their DLM content by HPLC. The early time scales are expanded in B and C. Symbols represent means ± S.E. for group of 3 – 5 rats. * Indicates C_{max} that is significantly different from the DLM concentrations at adjacent time-points ($p < 0.05$).

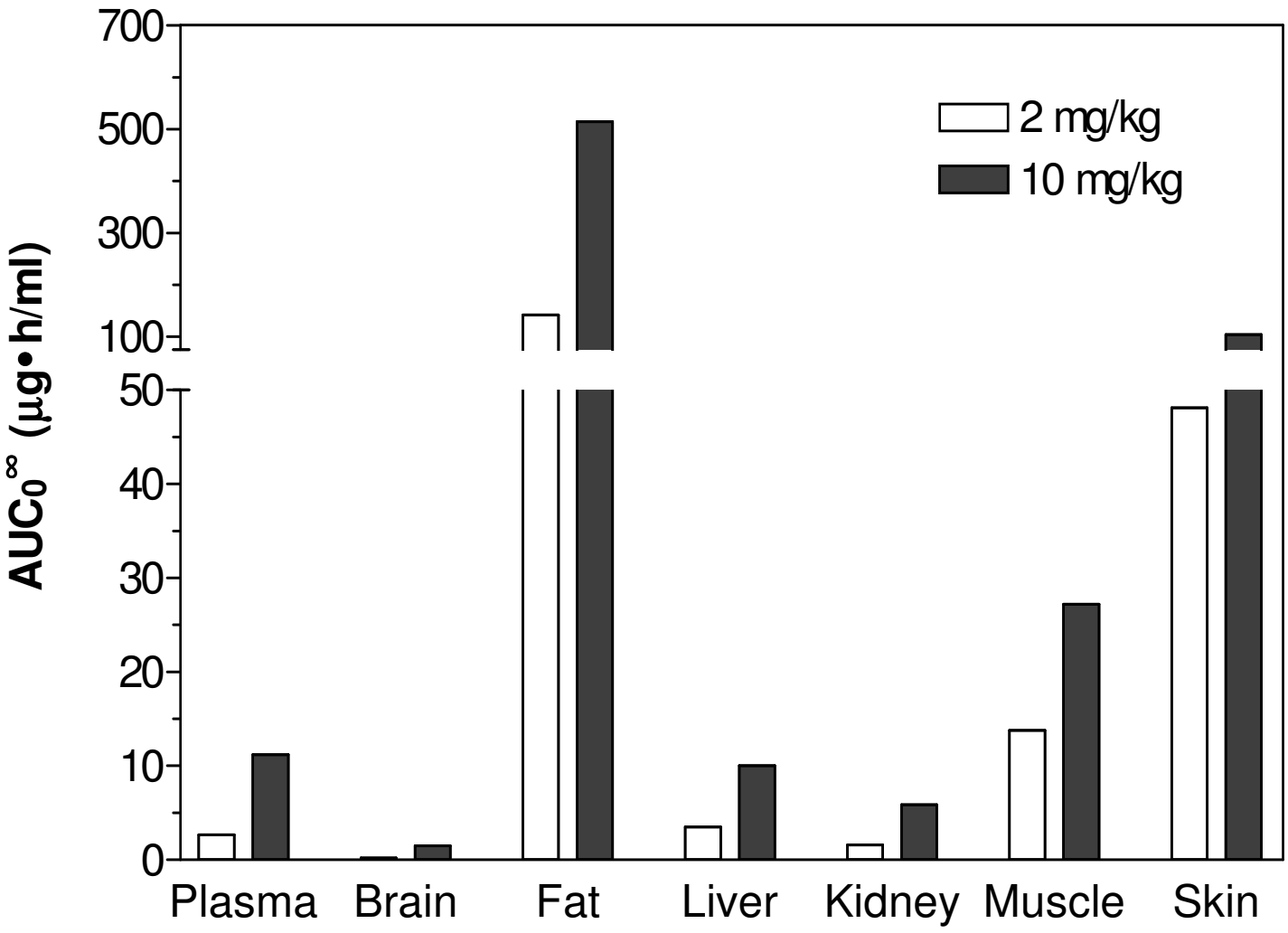


Figure 3-6. Comparison of AUC^{∞}_{0} ratios for male S-D rats administered 2 or 10 mg DLM/kg orally. AUCs were determined from time 0 to infinity using Winnonlin noncompartmental analysis (n = 3 – 5).

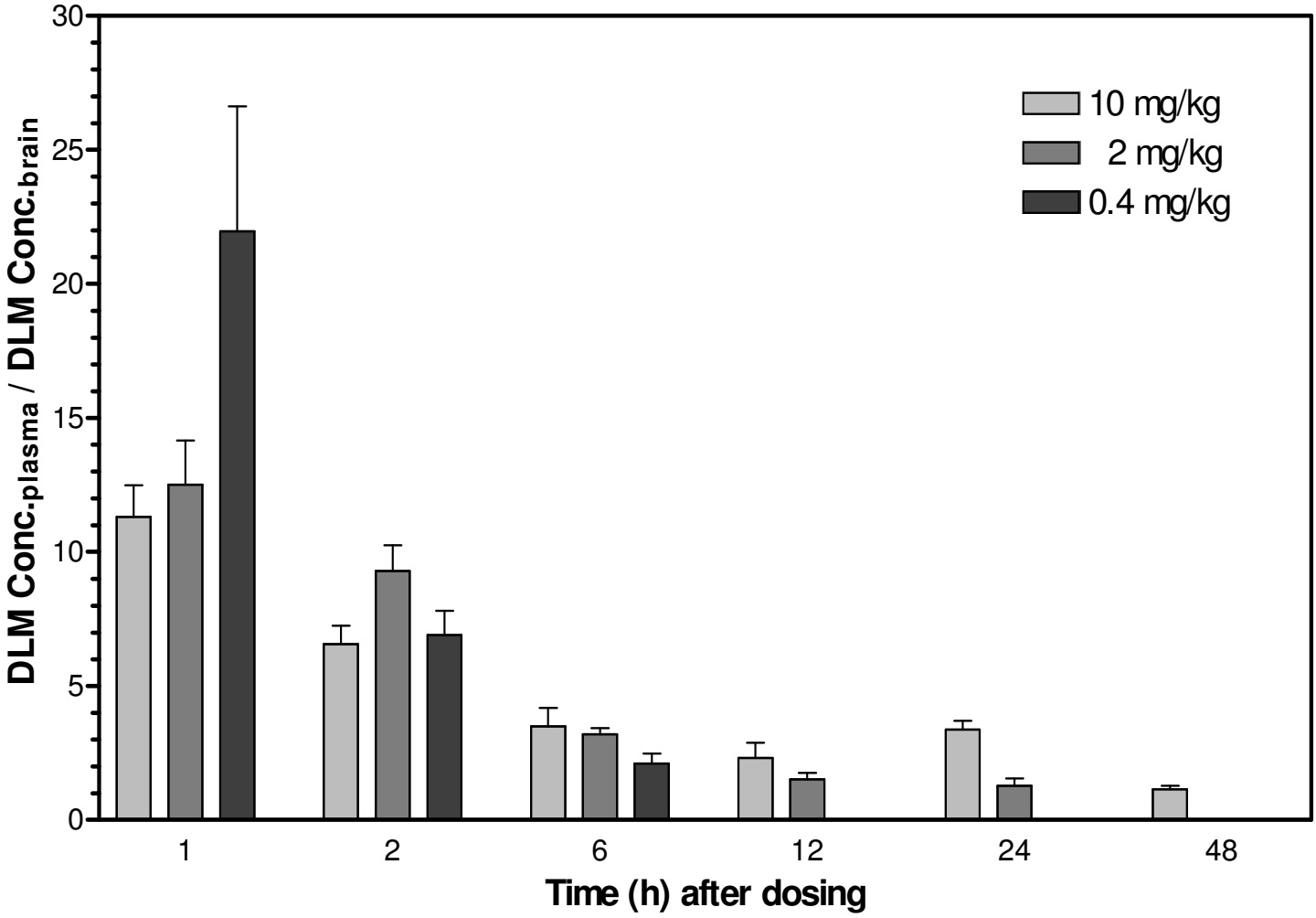


Figure 3-7. Plasma DLM Conc.$_{plasma}$/DLM Conc.$_{brain}$ ratios 1, 2, 6, 12, 24 and 48 h after oral administration of 0.4, 2 and 10 mg DLM/kg to male S-D rats. Bar heights and brackets represent x ± S.E. for groups of 3 – 5 animals.

CHAPTER 4

AGE AND DOSE DEPENDENCY OF TOXICOKINETICS AND TISSUE DISTRIBUTION OF DELTAMETHRIN IN IMMATURE SPRAGUE-DAWLEY RATS FOLLOWING ORAL ADMINISTRATION[1]

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[1]Kyu-Bong Kim, Sathanandam S. Anand, Hyo Jung Kim, Catherine A. White, Jeffrey W. Fisher and James V. Bruckner. To be submitted to *Drug Metab.Dispos.*

ABSTRACT

Deltamethrin (DLM) is a widely-used Type II pyrethroid insecticide with relatively potent neurotoxicty. Previous studies demonstrate that immature rats are more susceptible to acute DLM neurotoxicity than adults. The objective of this investigation was to determine whether the susceptibility of immature rats to acute DLM neurotoxicity is due to age-dependent deposition of DLM in the brain and whether there is dose-dependency of DLM toxicokinetics. DLM was administered via gavage at 0.4, 2 or 10 mg DLM/kg body weight to male Sprague-Dawley (S-D) rats of postnatal days (PND) 10, 21, and 40. Blood and selected tissues (whole brain, fat, liver, and muscle) were collected by timed sacrifice for up to 3 weeks post-dosing. DLM concentrations in plasma and tissues were determined by HPLC. Salivation and tremors were transient in PND 40 rats given 10 mg/kg. The signs were much more severe, leading to mortality in 10- and 21-day-olds by 8 and 12 h, respectively. The plasma $C_{max}s$ of DLM were 5.90 ± 1.36 µg/mL (PND 10), 1.89 ± 0.36 µg/mL (PND 21), and 1.63 ± 0.57 µg/mL (PND 40) at 10 mg DLM/kg. The AUC_0^{∞} (area under the plasma concentration of DLM post-dosing with 2 mg DLM/kg) of DLM in 10-day-old pups was about 6-times greater than that in 40-day-old rats. The brain AUCs and $C_{max}s$ were much lower than for plasma in each age-group. These results indicate that the increased susceptibility of immature rats to acute DLM neurotoxicity is primarily due to relatively high brain DLM concentrations over time. Fat and muscle showed high AUCs and long elimination half-lifves across age groups. Fat, muscle, and liver also varied inversely with age in rats given oral doses of 0.4 to 10 mg/kg. A dose dependency in the plasma toxicokinetics and tissue distributions of DLM administered to immature rats was also observed, though not directly proportional to dose. This age and dose dependency of toxicokinetic behavior has important implications for understanding of internal exposure to pyrethroid insecticides and

these data are useful for development of a physiologically-based pharmacokinetic model for children's risk assessments in future.

INTRODUCTION

Over a decade after Alar® and the well-known apple crisis of 1989, overall use of pesticides has continuously increased. There is a great deal of concern that exposure of infants and children to pesticides may result in relatively serious acute poisoning and in possible neurological dysfunction in adulthood. Many pesticides are generally believed to be more harmful to children than they are to adults (Pogoda and Preston-Martin, 1997; Guillette et al., 1998; Bruckner, 2000). There is a greater likelihood of higher exposures of infant and children due to greater intake of fruits and vegetables sprayed with pesticides (NRC, 1993), as well as crawling and hand to mouth activities that increase direct exposures (Gurunathan et al., 1998). Moreover, many sensitive, interrelated processes occur as nervous system rapidly grows and develops after birth. Many pesticides are designed to impair nervous system functions, and therefore may induce residual changes and dysfunctions (Casida and Quistad, 1998; Shafer et al., 2005). The dysfunction may elicit a loss of intelligence and/or alteration of normal behavior in adulthood. A study of Mexican children exposed to a mixture of agricultural chemicals showed impacts on motor skills, memory, attention, and learning (Guillette et al., 1998). Researches have clearly shown that immature rats are more sensitive than adults to the acute and subacute neurotoxicity of organophosphate cholinesterase inhibitiors (Atterberry et al., 1997; Moser and Padilla, 1998). An experiment by Sheets et al. (1994) revealed the same phenomenon for acute neurotoxic effects of high doses of deltamethrin (DLM), a pyrethroid insecticide.

Exposure to pyrethroids has been widely documented in potentially susceptible groups, including pregnant women, infants and children (Whyatt et al., 2002; Schettgen et al., 2002; Berkowitz et al., 2003; Heudorf et al., 2004). Heudorf et al. (2004) recently reported that

pyrethroid metabolites were found in 75% of an urban German population without occupational exposure to the insecticide. Levels in children were comparable to those in adults. Currently, 16 pyrethroids are registered for use in the U.S. in a variety of agricultural and consumer products (Bryant and Bite, 2003). Deltamethrin (DLM) (Figure 4-1), is the active ingredient in 27 products marketed in the U.S. (CALEPA, 2001). DLM is a widely-used Type II pyrethroid insecticide. Its mechanism of action involves binding to neuronal sodium channels, leading to depolization block and ensuring nerve inexitability (Forshaw et al., 1993). Typical Type II poisoning sysmptoms include profuse salivation without lacrimation and sinuous writhing (chorioathetosis). The parent compound is the proximate toxin (toxic moiety), as demonstrated by correlation of brain DLM levels with the onset of symptoms of poisoning in rats (Rikard and Brodie, 1985). Intracerebral injection of DLM induced signs of neurotoxicity within a minute or less in mice (Lawrence and Casida, 1982). Hydrolysis of molecule's ester linkage and P450-catalyzed oxidation resulted in biologically-inactive metabolites that are rapidly conjugated and excreted in the urine (Ruzo et al., 1978, 1979; Soderlund and Casida, 1977). Anand et al. (2006a) recently reported that adult male rat hepatic CYPs, notably CYP1A1 and CYP1A2, play a more important role than hepatic microsomal and plasma carboxylesterases (CaEs) in the biotransformation of DLM in vitro. Anand et al. (2006b) also showed that limited metabolic capacity of immature rats plays a significant role in their increased susceptibility to DLM. An objective of the current study is to elucidate the influence of limited DLM metabolism on target organ dosimetry.

Several studies demonstrated that immature rats are more susceptible to acute DLM neurotoxicity than adults and may exhibit residual effects later in life. Sheets et al. (1994) determined oral LD_{50}s for 11-, 21- and 72-day-old rats to be 5, 11 and 81 mg DLM/kg,

respectively. There have been studies of altered behavior and learning associated with changes in levels/binding of neurotransmitters, in the offspring of rats dosed with DLM (0.08 mg/kg) during pregnancy (Aziz et al., 2001; Lazarini et al., 2001). Eriksson and Fredriksson (1991) were among the first to demonstrate residual neurological effects following neonatal pyrethroid exposure. Ten-day-old male NMRI mice, given orally 0.7 mg DLM or bioallethrin/kg daily for 7 days, exhibited increased spontaneous motor activity and decreased density of muscarinic cholinergic receptors in the cerebral cortex at the adult age of 4 months. Oral administration of 7 mg DLM/kg to weanling rats on days 22-37 postpartum produced significant neurochemical and behavioral deficits (Husain et al., 1994).

The objective of this study was to determine whether the susceptibility of immature rats to acute DLM neurotoxicity is attributable wholly or in part to to age-dependent toxicokinetics of DLM. Limited toxicokinetic studies to date suggest that several maturational changes in DLM disposition may be factors in the vulnerability of immature animals. Anadon et al. (1996) found that gastrointestinal (GI) absorption of DLM was rapid, but incomplete in adult rats. The immature GI mucosa in neonatal animals may enhance DLM absorption. Sheet et al. (1994) reported significantly higher brain DLM concentrations in weanling rats than in adults given a low, behaviorally active oral dose (4 mg DLM/kg). However, no information was provided on the potential importance in age-dependent metabolism, blood-brain barrier efficiency, transporter-function, or other toxicokinetic differences.

MATERIALS AND METHODS

Chemicals. DLM [(*S*)-*α*-cyano-3-phenoxybenzyl-(1*R, cis*)-2,2-dimethyl-3-(2,2-dibromovinyl)-cyclopropane-1-carboxylate] (purity, 98.8%) was kindly provided by Bayer CropScience AG (Monheim, Germany). DLM's structure is shown in Fig. 4-1. Acetonitrile (HPLC grade) and glycerol formal (GF) were purchased from Sigma-Aldrich (St. Louis, MO). Methanol, sulfuric acid, and the deionized water (HPLC grade) were obtained from J.T. Baker (Phillipsberg, NJ). All other chemicals used were of the highest grade commercially available.

Animals and treatment. Pregnant Sprague-Dawley (S-D) rats were obtained from Charles River Breeding Laboratories (Raleigh, NC). S-D rats have been used previously for toxicokinetic studies in our laboratory (Kim et al., 2007). Pups were delivered and grown until the assigned postnatal day (PND 10, 21 and 40). The day of birth was defined as PND 0. The protocol for this study was approved by the University of Georgia Animal Use and Care Committee and rats were housed in polycarbonate cages on a 12-hr light/dark cycle at ambient temperature (22℃) and relative humidity (55±5%). Food (5001 Rodent Diet, PMI Nutrition International LLC, Brentwood, MO) and tap water were provided ad libitum.

All the immature rats were delivered in animal facilities located in College of Pharmacy of the University of Georgia. All pups were sexed and weanling pups were separated from their dam only after 21 days. Ten-day-old male rats were not fasted prior to administration of DLM, but 21-day- and 40-day-old male rats were fasted for 12 hr prior to DLM treatment and given food 3 hr after dosing, which was done between 0900 and 1000 hr. All immature rats were given a single dosage of 0.4, 2 or 10 mg DLM/kg bw by gavage GF. The averages and S.D.s of the bw by age group were 22.7 ± 2.0 g (PND 10), 43.5 ± 8.7 g (PND 21), and 189.2 ± 14.5 g (PND 40),

respectively at the time of dosing (Fig. 4-2). For sacrifice of 10-day-old rats, 4 animals in each time-point were decapitated 0.5, 1, 2, 4, 5 and 6 hr after dosing with 10 mg DLM/kg due to lethality. Three to five animals at each time point were sacrificed at 0.5 to 48 h after dosing with 2 and 0.4 mg DLM/kg. Additional groups of 2 and 3 weeks post-dosing with 2 mg DLM/kg were included to elucidate DLM's persistence in fat and muscle. Only two rats of 2 weeks group were available due to DLM toxicity. For sacrifice of 21-day-old rats, three to four animals at each group were decapitated 0.5, 1, 2, 4, 6 and 12 hr after dosing with 10 mg DLM/kg due to lethality. Five animals at each time-point were sacrificed 0.5 h to 3 weeks after dosing with 2 mg DLM/kg. Five animals at each time-point were sacrificed 0.5 to 24 h after dosing with 0.4 mg DLM/kg. Blood samples were collected in heparinized tubes. For sacrifice of 40-day-old rats, three animals at each time-point were euthenized by CO_2 asphyxiation 0.5, 1, 2, 4, 6, 12, 24, 48, 72, and 96 hr after dosing with 10 mg DLM/kg. Five animals at each time-point were sacrificed 0.5 h to 3 weeks after dosing with 2 mg DLM/kg. Five animals at each time-point were sacrificed 0.5 to 12 h after dosing with 0.4 mg DLM/kg. Blood samples were withdrawn from the inferior vena cava and collected in heparinized tubes.

Study design: dose and justification. The 0.4, 2, and 10 mg DLM/kg dosages were the same as those given adult rats, in order to compare the toxic effect and toxicokinetics of DLM in the different age-groups. The 10 mg/kg dose was chosen to because it caused modest toxic effects in adult rats. The 0.4 and 2 mg/kg doses were selected in order to determine whether the kinetics of DLM were liniear over a range of doses. Lower doses could not be given and complete time-courses obtained due to our limit of detection.

Sample collection and preparation. The DLM content of plasma and tissue samples was quantified by the HPLC procedure of Kim et al. (2006). To generate plasma, blood samples

collected at each time-point were centrifuged for 5 min at 13,000 rpm using a microcentrifuge (Microfuge 22R Centrifuge, Beckman Coulter, Fullerton, CA, USA). Sixty-five µl of plasma were added to microcentrifuge tubes containing 130 µl of acetonitrile. These were vigorously vortexed (Mini Vortexer, VWR, West Chester, PA, USA) for 30 s. The tubes were then centrifuged for 5 min at 13,000 rpm in the microcentrifuge, and 50 µl of the clear supernatant injected onto the column. The whole brain, fat, a part of the liver's median lobe, and thigh muscle were quickly isolated from the rats at each-time point, blotted dry and stored at -80°C for subsequent DLM analysis. Tissues were homogenized in 4 volumes of 50% (v/v) acetonitrile in distilled water with a Tissumizer (Tekmar, Cincinnati, OH, USA). Sixty-five µl of the tissue homogenates were added to microcentrifuge tubes containing 130 µl of acetonitrile. These tubes were vigorously mixed with a vortex mixer for 30 s and centrifuged for 5 min at 13,000 rpm in the microcentrifuge. Fifty µl of the clear supernatant were injected onto the column.

Analysis of DLM. DLM was measured in our laboratory by using a high-performance liquid chromatography (HPLC) system consisting of a Shimadzu HPLC (Shimadzu, Canby, OR, USA) equipped with a pump (LC-10AT), degasser (DGU-14A), auto-sampler (SIL-HT), detector (SPD-10AV) and computer with an EZStart 7.2 SP1 Rev B (Kim *et al.*, 2006). The analytical column was an Ultracarb 5 ODS (20) column (250 x 4.6 mm; 5 µm particle) (Phenomenex, Torrance, CA, USA), and the guard column was a Phenomenex fusion RP 4 mm x 3 mm (Torrance, CA). The mobile phase was 80% acetonitrile and 20% sulfuric acid (1%, v/v) (v/v). The flow rate was set at 1.0 ml/min. The eluate was monitored at 230 nm. Under these chromatographic conditions, DLM eluted at approximately 14.5 min.

Data analyses. Means and standard errors were calculated with Microsoft Excel (Microsoft Corp., Redmond, WA). Toxicokinetic parameters were estimated for plasma and tissues,

including area under the curve (AUC), T_{max}, C_{max}, and elimination half-life ($t_{1/2}$), using a Winnonlin (ver. 4.1) noncompartmental model analysis (Scientific Consulting, Inc., Cary, NC).

RESULTS

Clinical Signs

Developing male S-D rats (PND 10, 21 and 40) were administered a single oral dose (0.4, 2, or 10 mg/kg) of DLM. The highest dose (10 mg/kg) elicited lethality in PND 10 and 21 pups but not PND 40 rats. The PND 10 pups treated with 10 mg/kg lived up to 8 h post dosing, while PND 21 weanlings lived for up to 12 h. The dying animals showed severe tremors within 30 min of dosing. Although all the PND 40 rats treated with 10 mg/kg showed prominent salivation and tremors, these neurotoxic signs disappeared within 24 hrs. Lethality limited the length of time that plasma and tissues could be taken for DLM analysis from the PND 10 and 21 animals given 10 mg/kg. The intermediate dose of 2 mg/kg elicited pronounced tremors and some lethality (3/32) in PND 10 pups. PND 21 pups treated with 2 mg/kg showed less prominent salivation and tremors, while PND 40 rats did not show clinical signs. The developing animals treated with the lowest dose of 0.4 mg/kg did not exhibit clinical signs of poisoning.

Age dependency of toxicokinetics and tissue distribution of DLM

DLM concentrations in plasma and tissues (whole brain, fat, liver, and muscle) from immature rats 2 and 24 h following oral administration of 0.4, 2 or 10 mg DLM/kg bw showed age- and dose-dependency (Table 4-1). In PND 21 and 40 animals, peak plasma DLM concentrations (C_{max}) were reached soon (1 h) after dosing, but PND 10 plasma DLM C_{max}*s* were

manifest 2 or 6 h post dosing (Table 4-2). The 2- and 24-h plasma DLM concentrations, as well as those at the T_{max} decreased with increasing age, with the exception of PND 10 pups treated with 0.4 mg/kg (Table 4-1). Plasma toxicokinetic parameters are shown in Table 4-2. Elimination half-life ($t_{1/2}$) was estimated to be 15 to 20 h in PND 10 pups given 0.4 or 2 mg/kg, but the estimate of 7.6 h given 10 mg/kg was not reliable due to the limited sampling period. The $t_{1/2}s$ of PND 21 and 40 animals were estimated to be 17.7 and 9 h, respectively (Table 4-2). Area under the plasma DLM concentration curves from time 0 to infinity (AUC_0^{∞}) (69.8, 13.0, and 7.5 µg·hr/ml at 10 mg/kg; 13.6, 2.9, and 2.3 µg·hr/ml at 2 mg/kg; 2.0, 0.7, and 0.3 µg·hr/ml at 0.4 mg/kg in PND 10, 21 and 40 rats, respectively) clearly decreased with increasing age (Table 4-2 and Figure 4-6). $AUC_0^{6\ h}$ and $AUC_0^{12\ h}$ values were also compared, due to limited PND 10 and PND 21 data for rats treated with 10 mg/kg.

Brain DLM concentrations in immature rats were much lower than levels in plasma and other tissues 2 h and 24 h post dosing (Table 4-1). Brain DLM $C_{max}s$ in different age groups also were much lower than $C_{max}s$ of plasma and other tissues, though brain is DLM's target organ. $T_{1/2}s$ for brain were estimated to range from 23 – 33 h across all different age and dosage groups, based on reliable data points. Brain $C_{max}s$ and AUCs, however, definitely showed age dependency (Table 4-3 and Fig. 4-7). C_{max} values decreased from 0.38 to 0.14 µg DLM/g brain at 10 mg DLM/kg, and from 0.11 to 0.05 µg DLM/g brain at 2 mg DLM/kg, as age increased from 10 to 40 days. AUC_0^{∞} values decreased from 3.7 to 0.9 µg·hr/g at 2 mg DLM/kg and from 1.0 to 0.2 µg·hr/g at 0.4 mg DLM/kg, as age increased PND 10 to 40. The $AUC_0^{6\ h}$ at 10 mg DLM/kg decreased from 1.5 to 0.6 µg·hr/g with increasing age (Table 4-3). Fig. 4-7 visually shows this age-dependent AUC decrease as age increased.

Fat DLM concentrations and C_{max}s in immature rats were substantially higher than in plasma or other tissues 24 h post dosing, but not 2 h post dosing (Table 4-1). Fat DLM $t_{1/2}$s ranged from 218 – 354 h for the 2 mg DLM/kg PND 21 and 40 animals. These $t_{1/2}$s were calculated from reliable data points collected for up to 3 wks after dosing. C_{max}s and AUCs definitely showed age-dependency (Table 4-4 and Fig. 4-8). C_{max} decreased from 8.66 to 3.15 µg DLM/g fat at 10 mg DLM/kg, 9.47 to 0.65 µg DLM/g fat at 2 mg DLM/kg, and 1.09 to 0.31 µg DLM/g fat at 0.4 mg DLM/kg as age increased from 10 to 40 days. AUC_0^∞ also decreased 1690 to 129 µg·hr/g at 2 mg DLM/kg, as age increased 10 to 40 days. Fig. 4-8 visually shows the decrease in fat AUC at 2 mg DLM/kg as age increases.

Liver DLM concentrations in immature rats were shown 2 and 24 h post dosing (Table 4-1) in order to assess age- and dose-dependency. Liver $t_{1/2}$s ranged from 22 – 36 h across all age- and dosage-groups. These calculations were based on reliable data points. C_{max}s and AUCs definitely showed age-dependency (Table 4-5 and Fig. 4-9). C_{max} decreased from 2.66 to 0.64 µg DLM/g liver at 10 mg DLM/kg, 0.65 to 0.25 µg DLM/g liver at 2 mg DLM/kg, and 0.32 to 0.15 µg DLM/g liver at 0.4 mg DLM/kg, as age increased from 10 to 40 days. AUC_0^∞ decreased from 13.4 to 2.4 µg·hr/g at 2 mg DLM/kg and from 5.0 to 0.8 µg·hr/g at 0.4 mg DLM/kg, as age increased from 10 to 40 days. Fig. 4-9 visually shows age-dependent liver AUC decreases at 2 mg DLM/kg as age increases.

Muscle DLM concentrations in immature rats showed age-dependent decreases by 24 h post-dosing. These finding were not fully manifest 2 h post dosing (Table 4-1). Muscle DLM C_{max}s in different age groups were slightly higher than those for brain. The muscle T_{max} values were quite variable. $T_{1/2}$s in muscle were also quite variable and did not show consistent age- or dose-dependent patterns. Muscle C_{max}s and AUCs definitely showed age-dependency (Table 4-6

and Fig. 4-10). C_{max} values decreased from 1.70 to 0.34 µg DLM/g muscle at 10 mg DLM/kg, 0.38 to 0.15 µg DLM/g muscle at 2 mg DLM/kg, and 0.20 to 0.05 µg DLM/g muscle at 0.4 mg DLM/kg as age increased from 10 to 40 days. AUC_0^{∞} also decreased 104.7 to 23.5 µg·hr/g at 2 mg DLM/kg as age increased over this time-span. $AUC_0^{6\ h}$ at 10 mg DLM/kg decreased 5.2 to 1.7 µg·hr/g, AUC_0^{12h} at 0.4 mg DLM/kg decreased 1.5 to 0.5 µg·hr/g with increasing age (Table 4-6). Fig. 4-9 visually shows the age-dependent AUC decrease in muscle in rats given 2 mg DLM/kg.

Dose-dependency of toxicokinetics and tissue distributions of DLM

DLM concentrations in plasma and tissues in different ages of immature rats over time following a single oral bolus dose of DLM (0.4, 2, or 10 mg/kg) are shown Figs. 4-3 (PND 10), 4-4 (PND 21), and 4-5 (PND 40). Fig. 4-3A shows dose-dependent plasma DLM concentrations versus time profiles over 48 h in PND 10 rats. The DLM concentration versus time profile stops at 6 h in the 10 mg DLM/kg group due to lethality. Plasma DLM concentrations were biphasic with a distribution for 24 hr followed by a slow elimination after systemic transfer within 6 h post dosing in PND 10 treated with 0.4 or 2 mg DLM/kg (Fig. 4-3A). A similar biphasic tendency was found in brain and liver at same dose levels (Figs. 4-3B and 4-3D). The brain DLM concentration was lower than that in plasma and other tissues. Plasma, brain, and liver levels declined after $C_{max}s$ in PND 10 animals. In contrast, DLM levels in fat rose progressively, reaching a maximum at 48 h. DLM was still present 12 days later in the2 mg DLM/kg PND 10 pups that were monitored for 14 days (Fig. 4-3C). DLM levels in muscle rose progressively in this age group, reaching a maximum at 12 and 24 h in the preweanlings given 0.4 and 2 mg DLM/kg, respectively. The muscle levels remained elevated for a relatively long time (Fig. 4-

3E). These DLM concentration versus time profiles were clearly dose-dependent in the 0.4 to 10 mg DLM/kg dosage range (Figs. 4-3A – E).

Fig. 4-4A shows dose-dependent plasma DLM concentrations versus time profiles over up to 24 h in PND 21 pups. The DLM profile stops at 12 h for the 10 mg DLM/kg group due to lethality. The profile stopped at 6 h in 0.4 mg DLM/kg dosage group due to the analytical limit of detection. No DLM was measurable at the next (12-h) time-point. Plasma DLM concentrations were biphasic with a distribution for 12 hr followed by a slow elimination after systemic transfer within 1 h post dosing in PND 21 treated with 2 mg DLM/kg (Fig. 4-4A). A similar biphasic tendency was found in brain and liver at same dose levels (Figs. 4-4B and 4-4D). DLM remained in fat and muscle for a long time, in that it was detected up to 3 wks in PND 21 weanlings given 2 mg DLM/kg ($t_{1/2}s$ = 354 and 309 h, respectively) (Figs. 4-4C and 4-4E, Tables 4-4 and 4-6). DLM concentrations versus time profiles for plasma, brain, fat, liver, and muscle were dose-dependent in PND 21 rats in the dosage range of 0.4 to 10 mg DLM/kg.

PND 40 rats exhibited dose-dependent plasma DLM concentration versus time profiles over 48 h post dosing (Fig. 4-5A). These profiles ended at 6 and 24 h in the 0.4 and 2 mg DLM/kg dosage groups as DLM was not measurable at the next time-points (12 and 48 h, respectively). Plasma DLM concentrations peaked at 1 h across all doses (Fig. 4-5A). Plasma AUC_0^∞ increased from 0.3 to 7.5 µg·hr/g as the dosage increased from 0.4 to 10 mg DLM/kg) (Table 4-2). A similar tendency was observed in brain and liver (Figs. 4-5B and 4-5D). Brain AUC_0^∞ values progressively rose in the PND 40 groups (0.2, 0.9, and 2.2 µg·hr/g for 0.4, 2, and 10 mg DLM/kg, respectively) (Table 4-3). DLM in fat and muscle was present for a long time. The insecticide was detected for 3 wks in PND 40 rats given 2 mg DLM/kg ($t_{1/2}s$ = 218 and 361 h, respectively) (Figs. 4-5C and 4-5E, Tables 4-4 and 4-6), showing similarity of $t_{1/2}s$ in PND 21,

but lower DLM levels than those in PND 21 (Table 4-1). DLM concentrations versus time profiles for plasma, brain, fat, liver, and muscle in PND 40 showed dose-dependent increases with increasing dose levels of 0.4 to 10 mg DLM/kg.

DISCUSSION

Deltamethrin, a pyrethroid insecticide, is widely used in agriculture to protect crops, in the household to control pests, and in public health to control diseases caused by vectors or intermediate hosts (IPCS, 1990; Soderlund et al., 2002). Pyrethroids accounted for about 25% of the worldwide insecticide market in 1998 (Casida and Quistad, 1998). That percentage share has increased substantially over the last few years in the U.S. (Lee et al., 2002), as a result of the U.S. Environmental Protection Agency's (EPA's) restrictions on household and agricultural use of organophosphates. Pyrethroids' popularity also stems from their insecticidal potency, slow development of pest resistance, and relatively low toxicity of most congeners in mammals. Widespread exposure of the general population to pyrethroids necessitates risk assessments. Toxicokinetic data for pyrethroids are essential for health risk assessments, but relatively little is known about toxicokinetics of DLM or other pyrethroids in humans or animals. Pyrethroids exposures have been widely documented in humans, including pregnant women, infants, and children (Berkowitz et al., 2003; Heudorf et al., 2004; Schettgen et al., 2002; Whyatt et al., 2002). It had been reported that immature animals are more sensitive to the acute neurotoxicity of pyrethroids than adults. The susceptibility of rat pups to permethrin and cypermethrin was found to be more pronounced the younger the pup (Cantalamessa, 1993). Sheets et al. (1994) also reported that LD_{50} values of DLM in 11-, 21-, and 72-day-old rats were 5.1, 11, and 81 mg

DLM/kg, respectively, showing the much greater sensitivity of preweanling pups than adults to acute lethality. Anand et al. (2006b) characterized the ontogeny of hepatic and plasma metabolism of DLM in vitro and concluded that limited metabolic capacity of immature rats contributed to increased systemic exposure and ensuing acute neurotoxic effects of DLM. In the present study, age- and dose-dependency of DLM toxicokinetics in immature rats was characterized to establish whether the susceptibility of immature rats could be attributed to increased target organ dosimetry. This proved to be the case. These data should also be useful for the children's risk assessments by providing an empirical base for development of a PBPK model.

Clinical signs were observed after the DLM treatments (0.4, 2, or 10 mg DLM/kg) of PND 10, 21, and 40 rats consistent with age-dependent neurotoxicity and lethality. In PND 10 and 21 pups, the highest dose of 10 mg DLM/kg produced 100% lethality within 8 and 12 h post dosing, respectively. In 11- and 21-day-old Long-Evans rats, ~ 10 mg DLM/kg induced 100% and 50% mortality, and the LD_{50} were estimated to be 5.1 and 11 mg DLM/kg, respectively (Sheets et al., 1994). DLM's acute neurotoxicity in our hands was in accordance with that observed by Sheets et al. (1994) in PND 10 pups, but was not in PND 21 weanlings. Oral administration of 10 mg DLM/kg to PND 40 juveniles did not induce lethality, but did cause prominent neurotoxic effects of salivation and tremors. Sheets et al. (1994) noted that at the time of death, DLM concentration (0.129 ± 0.015 µg/g brain) in brain of PND 21 weanlings given 12 mg DLM/kg was comparable to that (0.145 ± 0.063 µg/g brain) in PND 72 adults given 80 mg DLM/kg. Table 4-3 shows that brain C_{max}s of PND 10 and 21 pups were 0.38 ± 0.12 and 0.25 ± 0.04 µg/g brain at 10 mg DLM/kg. These values are higher than those of Sheets et al. (1994). The DLM concentration of 0.145 µg/g brain induced lethality in adult rats (Sheets et al., 1994),

whereas the C_{max} of our PND 40 rats was 0.14 ± 0.07 μg/g brain at 10 mg DLM/kg. However, our animals showed acute neurotoxic signs of salivation and tremors, but recovered. This discrepancy might be caused by different animal strains (S-D vs. Long-Evans), vehicles (glycerol formal vs. corn oil), and analytical methods (HPLC vs. GC). Rickard and Brodie (1985) reported that DLM brain concentration of 0.191 μg/g brain elicited choreoathetosis when adult rats were given 8 mg DLM/kg ip. These animals did not die, but recovered in 3 – 3.5 h. This DLM brain concentration and toxic symptom was comparable to the current result of relation between DLM brain concentration and clinical signs. The lowest dose of 0.4 mg DLM/kg did not induced any clinical signs of salivation and tremors in PND 10 to 40. However, age-dependency was also observed in $C_{max}s$ (0.04, 0.05, and 0.02 μg/g brain in PND 10, 21, and 40) and AUCs (1.0, 0.7, and 0.2 μg·h/g brain in PND 10, 21, and 40) of brain (Table 4-3). Brain C_{max} of PND 10 was lower than that of PND 21. When 10-day-old rats were administered DLM, they were not fasted, as they were housed with their dam. But, PND 21 and 40 were fasted before treatment. This is thought to lower $C_{max}s$ of plasma and brain and also extend $T_{max}s$ in PND 10 given by 0.4 mg DLM (Tables 4-2 and 4-3). Sheets (2000) documented that the age-dependent sensitivity of pyrethoids is apparent only at high acute doses (not at low doses) and concluded that infants and children are protected by existing food tolerances, without the need for an additional uncertainty factor. We did not see age-dependent differences in behavioral toxicity in pups given a low dose of 0.4 mg DLM/kg, but age-dependent differences in toxicokinetic parameters ($C_{max}s$ and AUCs in plasma and brain) were observed. And, these parameters also showed dose-dependent differences in each age groups (Tables 4-2 and 4-3). These results imply that an additional uncertainty factor may be warrented to protect infants' and children's health from low-dose effects of pyrethoids, should these be found to occur.

Table 4-1 briefly showed age- and dose-dependency of plasma and tissue DLM concentrations taken 2 and 24 h post dosing. One or 2 h was the T_{max} for rapidly-perfused tissues and 12 to 48 h was the T_{max} for slowly-perfused tissues. It is hard to directly compare plasma AUCs of PND 10 to 40 rats due to the incomplete time-courses due to DLM lethality and the detection limit of DLM. It is reasonable to compare plasma AUC_0^{∞} of PND 10, 21 and 40 rats given 2 mg DLM/kg, because of data points extended to 24 h and longe in PND 21 and 40 rats. Plasma AUC_0^{∞} of PND 10 pups was 4.7- and 5.9-fold higher than that of PND 21 and 40 rats, respectively. The PND 10 plasma $AUC_0^{6\ h}$ (21.0 μg·h/ml) was 2.8-fold higher than that of the PND group 21 (7.4 μg·h/ml) at the acutely toxic dose of 10 mg DLM/kg (Table 4-2). This ratio agrees fairly well with the ratio (2.2) of LD_{50}s which were determined in PND 11 and PND 21 rat pups by Sheets et al. (1994). Plasma $T_{max}s$ (2 - 6 h) of PND 10 pups were longer than those (1 h) of the PND 21 and 40 animals. The PND 10 plasma C_{max} was lower than those of the PND 21 and 40 groups, because the PND 10 pups were not fasted. The plasma DLM concentrations were measurable up to 48 h in PND 10 pups after the lowest dose of 0.4 mg DLM/kg, but DLM was detected only up to 6 h post dosing in the PND 21 and 40 groups. The higher plasma DLM concentrations in younger rats were attributed to the limited metabolic detoxification capacity in immature pups (Anand et al., 2006b).

Brain is the target organ of DLM in mammals and insects. Brain DLM concentration-versus-time profiles showed dose-dependency in each age group (Figs 4-3B, 4-4B, and 4-5B). With the PND 10 group, the AUC_0^6 ratio between 2 and 0.4 mg DLM/kg was 2.5, and that between 10 and 2 mg DLM/kg was 3. In PND 21, the AUC_0^{12} ratio between 2 and 0.4 mg DLM/kg was 1.5 and that between 10 and 2 mg DLM/kg was 3.5. In PND 40 juveniles, the AUC_0^{12} ratio between 2 and 0.4 mg DLM/kg was 3, and that between 10 and 2 mg DLM/kg was 3.3. These similar AUC

ratios suggest that DLM access to brain is not age-dependent. It should be recognized that $t_{1/2}$s (23 – 33 h) in brain were somewhat longer than $t_{1/2}$s (9 – 20 h) in plasma. Brain DLM concentrations were much lower than in plasma and tissues. In contrast, Anadon et al. (1996) reported DLM concentrations in most regions of the brain far exceeded plasma concentrations of adult Wistar rats receiving 26 mg/kg po. Other investigators' (Rickard and Brodie, 1985; Gray and Rickard, 1982) findings on brain:blood ratios agreed with our results. DLM was found to be slowly and poorly metabolized in vitro in rat brain homogenate (Rickard and Brodie, 1985). Substantial P-gp expression and other transporters in blood brain barrier (BBB) may serve as an effective efflux transporter for DLM (Choudhuri et al., 2003; Leslie et al., 2005), but this has not been confirmed. In rats, the closure of the BBB correlates with the formation of the astrocytic pericapillary sheath, as well as with the development of tight junctions between barrier endothelial cells (Laterra et al., 1998). This closure of BBB occurs postnatally, during the third week after birth (Jacobson, 1991; Laterra et al., 1998). Brain AUCs of PND 10 pups were much higher than those of PND 21 and 40 rats. However, the AUC_{brain}/AUC_{plasma} ratios were quite similar across age groups. There were not dramaticly increased ratios in the youngest pups, but the ratios did show increasing tendency at the younger age at the lowest dose (0.4 mg DLM/kg). These results indicate that high plasma concentrations of DLM due to limited metabolic capacity in immature rats may play the major role in DLM neurotoxicity, rather than maturation of BBB. Further studies of the BBB's role in DLM neurotoxicity are needed.

Fat and muscle DLM $C_{max}s$ and AUCs showed clear age- and dose-dependency in PND 10 to PND 40 animals (Tables 4-4 and 4-5). There have been no previous data for comparison of fat and muscle DLM disposition in immature rats. The high C_{max} and long elimination half-lives (218 – 353 h) of DLM in fat are directly attributable to the lipid solubility of the compound.

DLM has a log Kow of 4.53 (Laskowski, 2002). Marei et al. (1982) reported similar findings with DLM and several other pyrethroids in adult rats. The higher AUCs in fat from younger pups might be ascribed to highly systemic DLM concentrations and low fat content. Fat DLM concentrations in PND 10 pups consistently increased up to the last sacrifice time at each dosage-level (Fig. 4-3C). The fat:plasma distribution ratios (AUC_{Fat}/AUC_{Plasma}) were somewhat variable values of 22 to 56 from PND 10 to 40 animals. These characteristics of long half-lives, high AUCs, and high distribution ratios in fat might demonstrate that fat is a amjor storage tissue for DLM in immature and mature (Kim et al., 2007) rats. DLM also remained in muscle for a long time. Its elimination half-lives (168 - 361 h) are comparable to those of fat tissue. Age-dependent differences in $C_{max}s$ and AUCs might be attributable to high systemic exposure of DLM and low muscle contents. Muscle thus serves as another major storage site of DLM in addition to fat. Muscle, of course, constitutes a relatively large percentage of the total body weight of rats (Brown et al., 1997).

Liver is known as the major organ to metabolize DLM in rats. The two major enzymes, CYP450s and carboxylesterases, exhibit age-dependent differences in DLM metabolism (Anand et al., 2006). In liver, DLM $C_{max}s$ and AUCs also decreased as the age of animals increased (Table 4-5). The liver:plasma distribution ratio (AUC_{Liver}/AUC_{Plasma}) of PND 10 pups at 0.4 mg DLM/kg was much higher than that of PND 21 and 40. Low hepatic metabolic function might be ascribed to the higher distribution ratio to plasma in PND 10.

In conclusion, the present study has demonstrated that immature (PND 10, 21, and 40) rats orally dosed with 0.4, 2, or 10 mg DLM/kg showed age- and dose-dependent differences in toxicokinetics. Evidence of age-dependency in plasma DLM $C_{max}s$ and AUCs was observed, and may be explained largely by the limited metabolic capacity of the younger pups (Anand et al.,

2006b). Evidence of age-dependency in brain DLM C_{max}s and AUCs was observed at the low dose of 0.4 mg DLM/kg, as well as the acutely neurotoxic dose of 10 mg DLM/kg. This is contrary to the report by Sheets (2000), who argued that the age-dependent sensitivity of pyrethroids is apparent only at high neurotoxic doses and concluded that infants and children are protected by existing tolerances, without the need for an additional uncertainty factor. However our results imply that an additional uncertainty factor should be considered to protect infants' and children's health from pyrethoids. Evidence of age- and dose-dependency in brain also suggests that DLM toxicokinetics in brain might be responsible for the increased sensitivity of immature rats. This age- and dose-dependency of toxicokinetic behavior in immature rats has important implications for understanding internal exposure to pyrethroid insecticides. These data are also necessary for development of a physiologically-based pharmacokinetic model for children's risk assessment in future.

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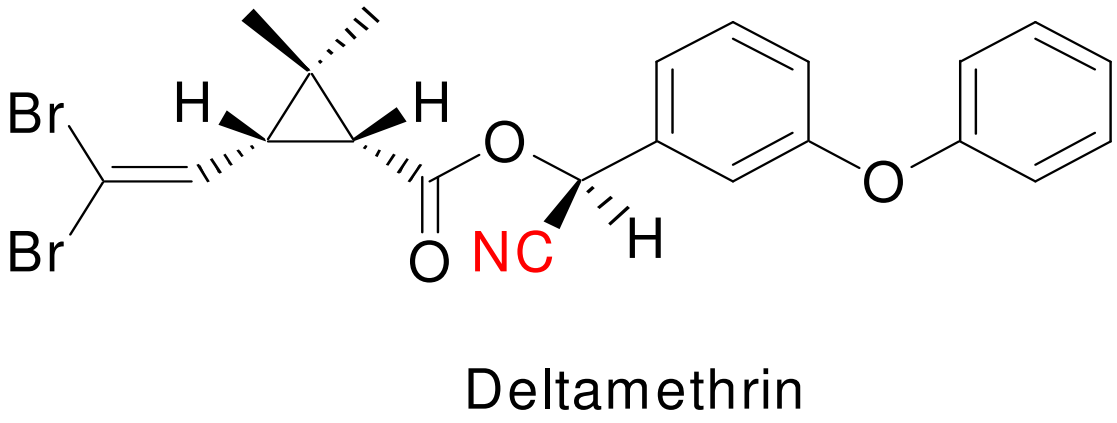

Figure 4-1. Chemical structure of deltamethrin (DLM)[*(S)*-α-cyano-3-phenoxybenzyl-(1*R*, *cis*-2,2-dimethyl-3-(2,2-dibromovinyl)-cyclopropane-1-carboxylate].

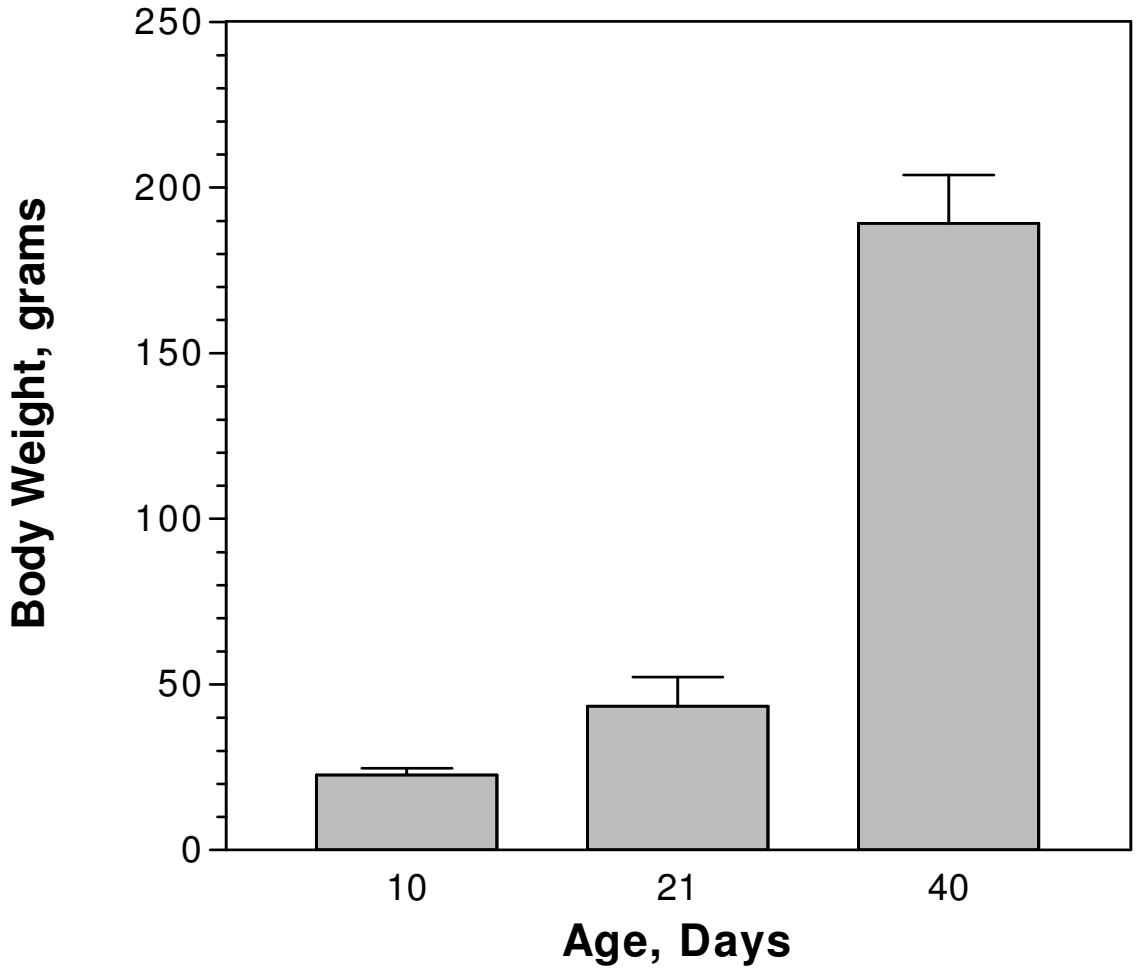

Figure 4-2. Body weights at time of administration to immature rats used in this study. Bar heights and brackets represent means ± S.D.

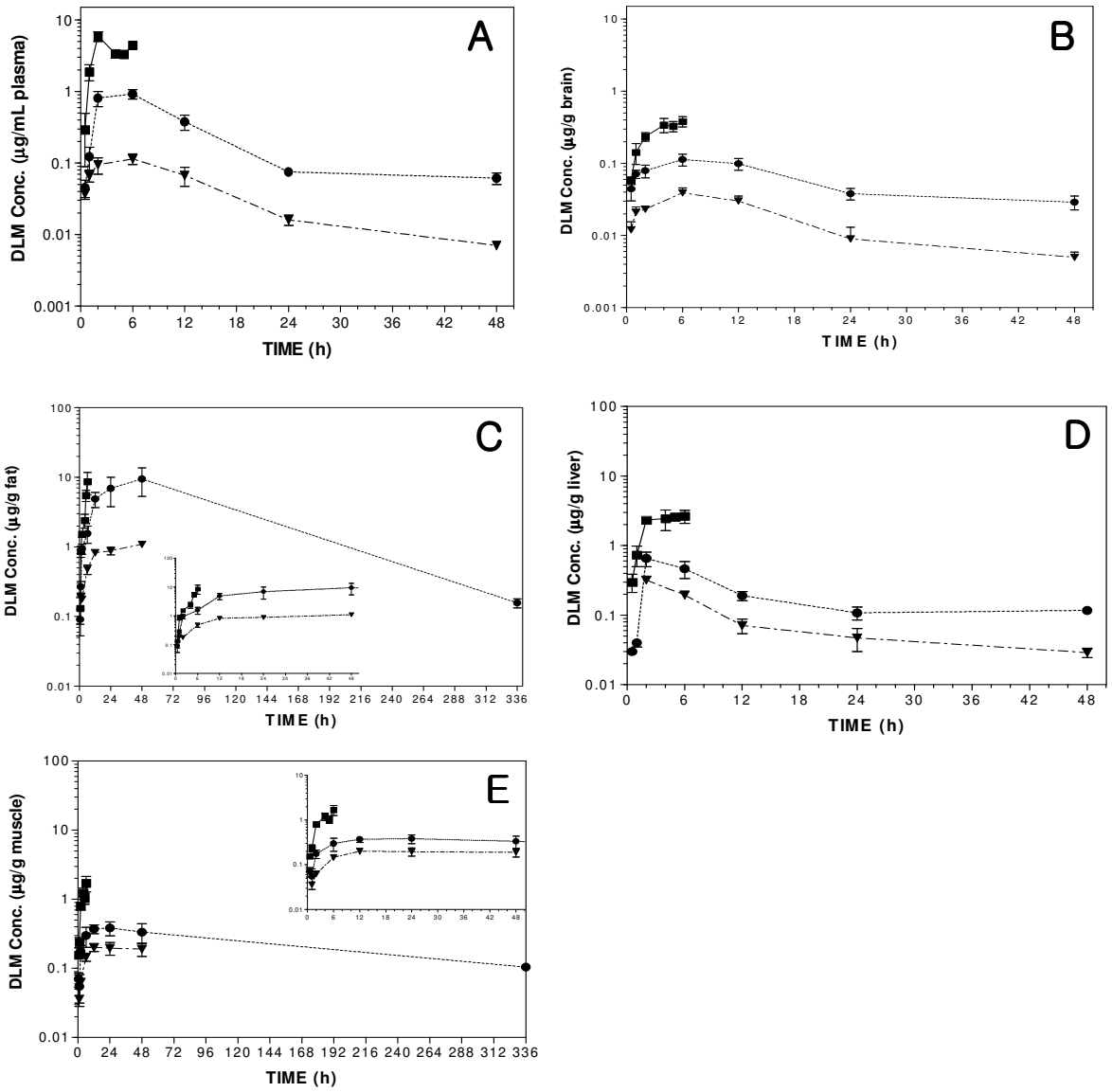

Figure 4-3. DLM uptake and elimination profiles of male PND 10 rats gavaged with 0.4 (▼), 2 (●) or 10 (■) mg DLM/kg. Serial plasma (A), whole brain (B), fat (C), liver (D), and muscle (E) samples were analyzed for their DLM content by HPLC. The early time-scale is expanded in C and D. Symbols represent means ± S.E. for groups of 3 – 5 rats.

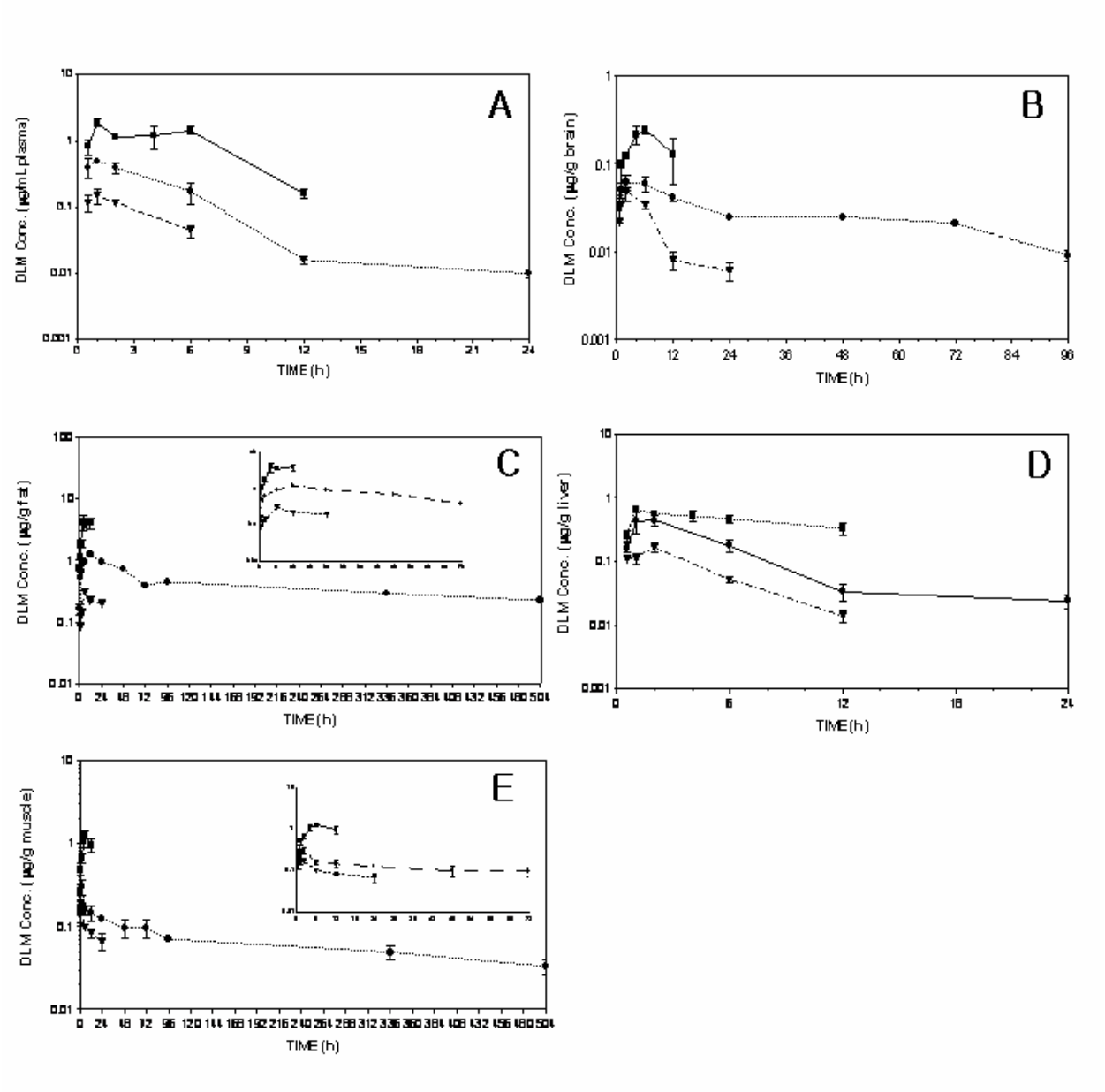

Figure 4-4. DLM uptake and elimination profiles of male PND 21 rats gavaged with 0.4 (▼), 2 (●) or 10 (■) mg DLM/kg. Serial plasma (A), whole brain (B), fat (C), liver (D), and muscle (E) samples were analyzed for their DLM content by HPLC. The early time-scale is expanded in C and D. Symbols represent means ± S.E. for groups of 3 – 5 rats.

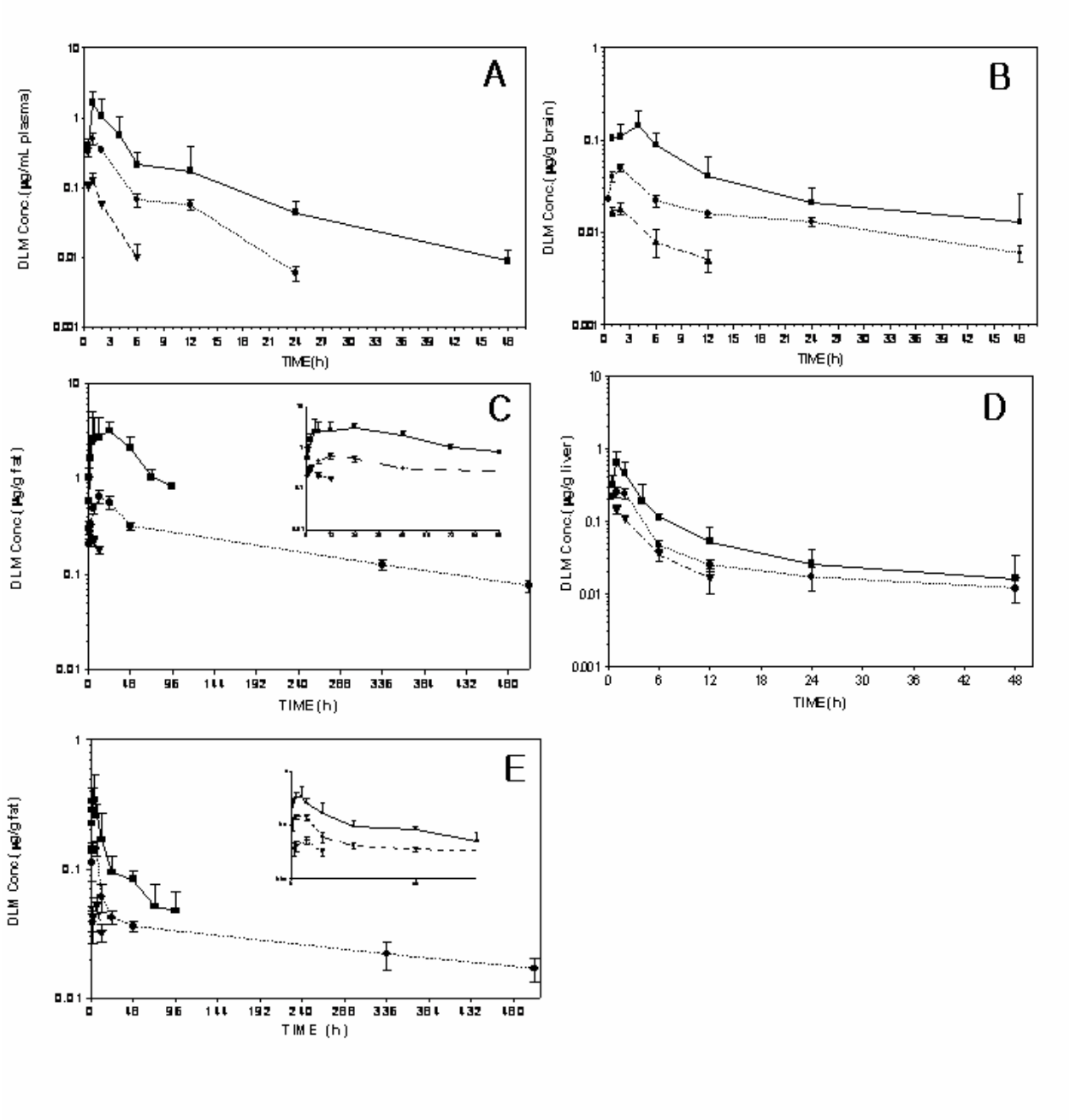

Figure 4-5. DLM uptake and elimination profiles of male PND 40 rats gavaged with 0.4 (▼), 2 (●) or 10 (■) mg DLM/kg. Serial plasma (A), whole brain (B), fat (C), liver (D), and muscle (E) samples were analyzed for their DLM content by HPLC. The early time-scale is expanded in C and D. Symbols represent means ± S.E. for groups of 3 – 5 rats.

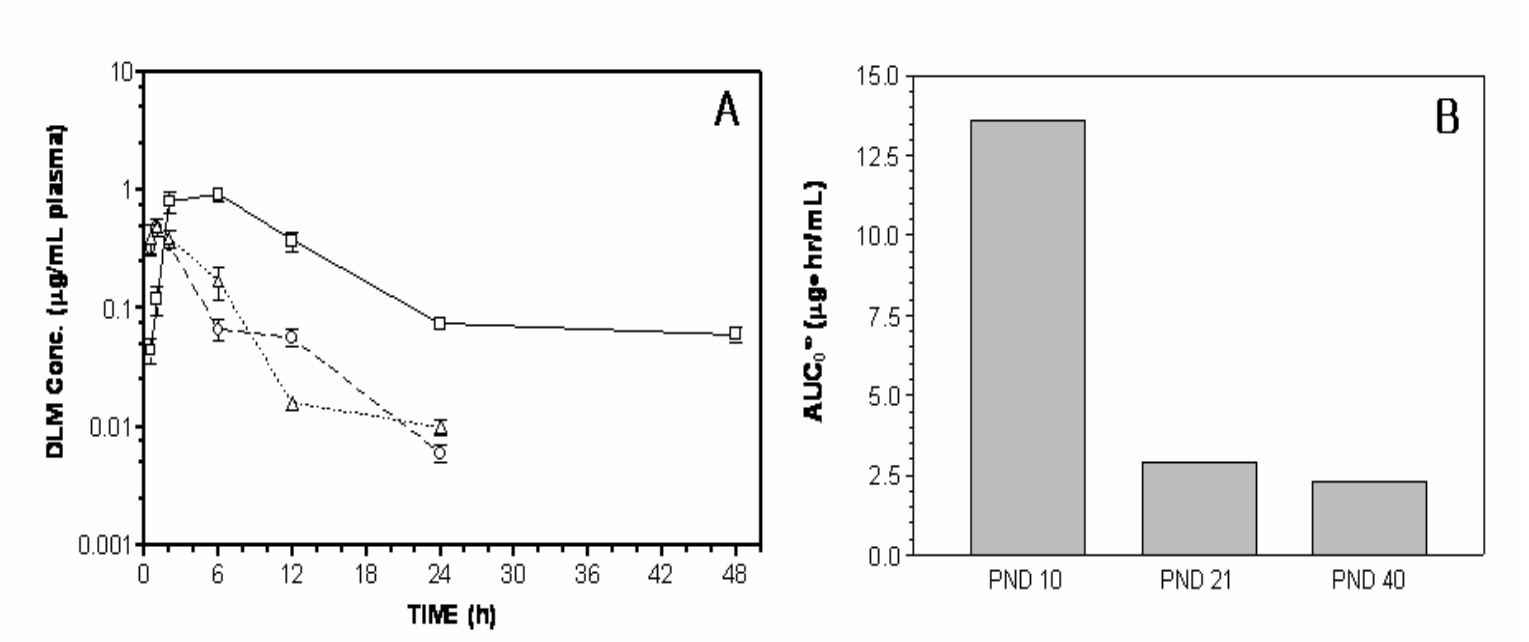

Figure 4-6. Age-dependent plasma deltamethrin (DLM) time-courses (A) after an oral dose of 2 mg DLM/kg to PND 10 (□), 21 (Δ) and 40(○) rats. Right panel (B) shows calculated plasma AUC values. Data are means ± SD for 3-5 animals.

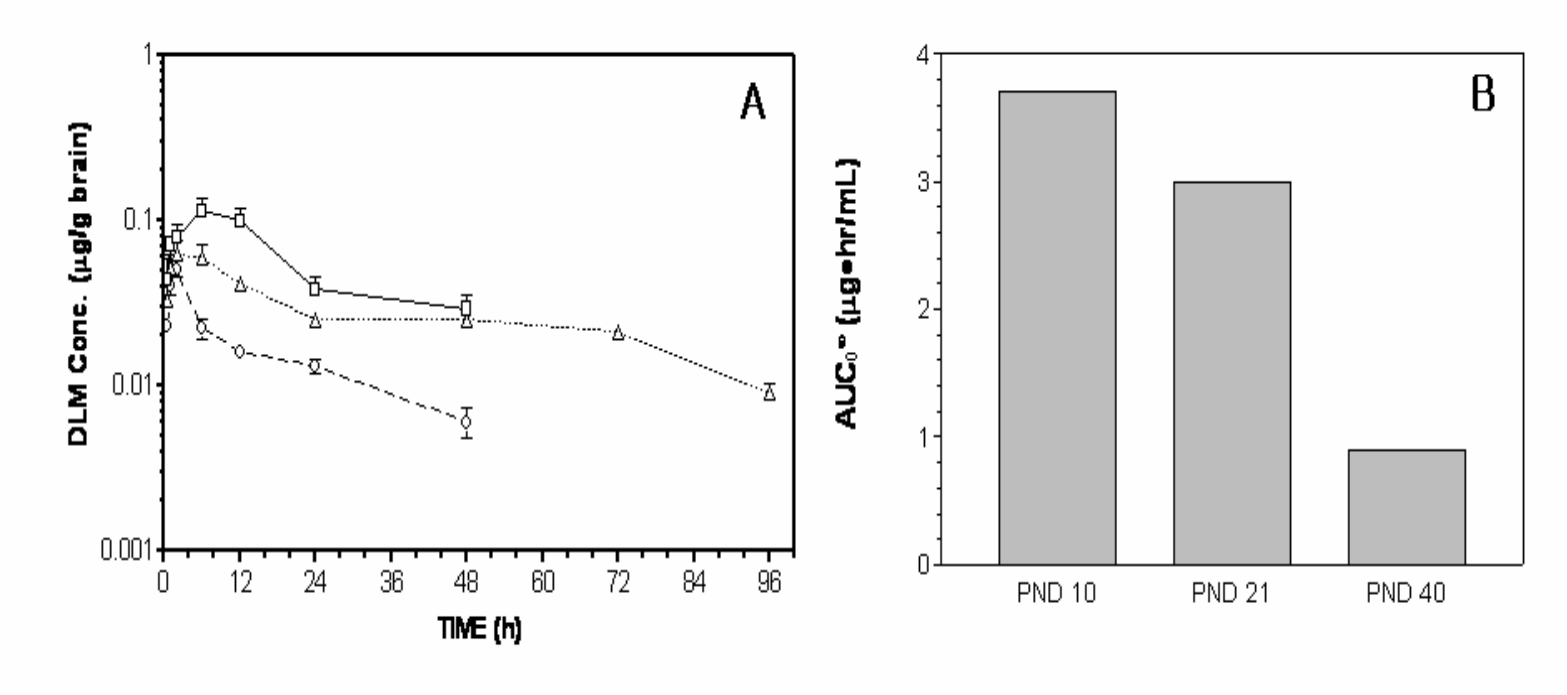

Figure 4-7. Age-dependent brain deltamethrin (DLM) time-courses (A) after an oral dose of 2 mg DLM/kg to PND 10 (□), 21 (Δ) and 40 (○) rats. Right panel (B) shows calculated brain AUC values. Data are means ± SD for 3-5 animals.

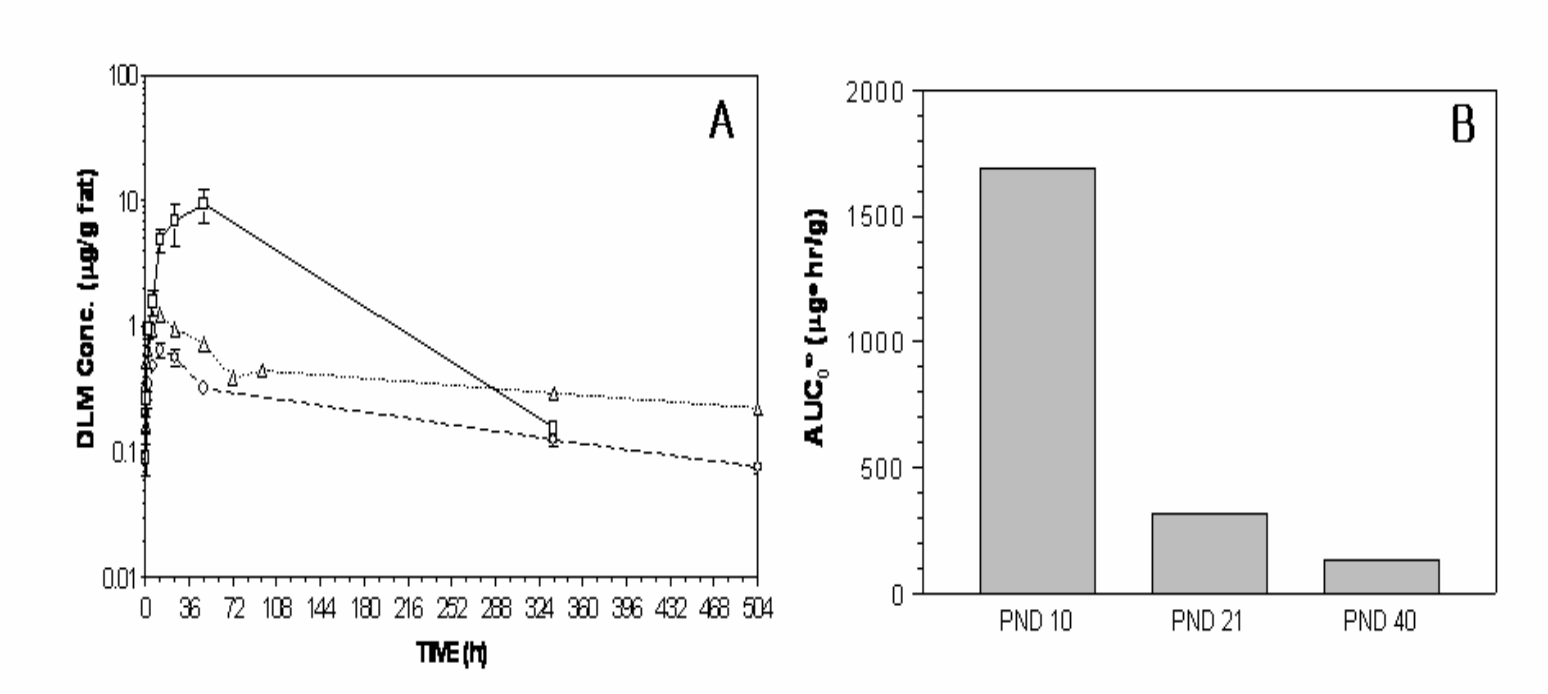

Figure 4-8. Age-dependent fat deltamethrin (DLM) time-courses (A) after an oral dose of 2 mg DLM/kg to PND 10 (□), 21 (Δ) and 40 (○) rats. Right panel (B) shows calculated fat AUC values. Data are means ± SD for 3-5 animals.

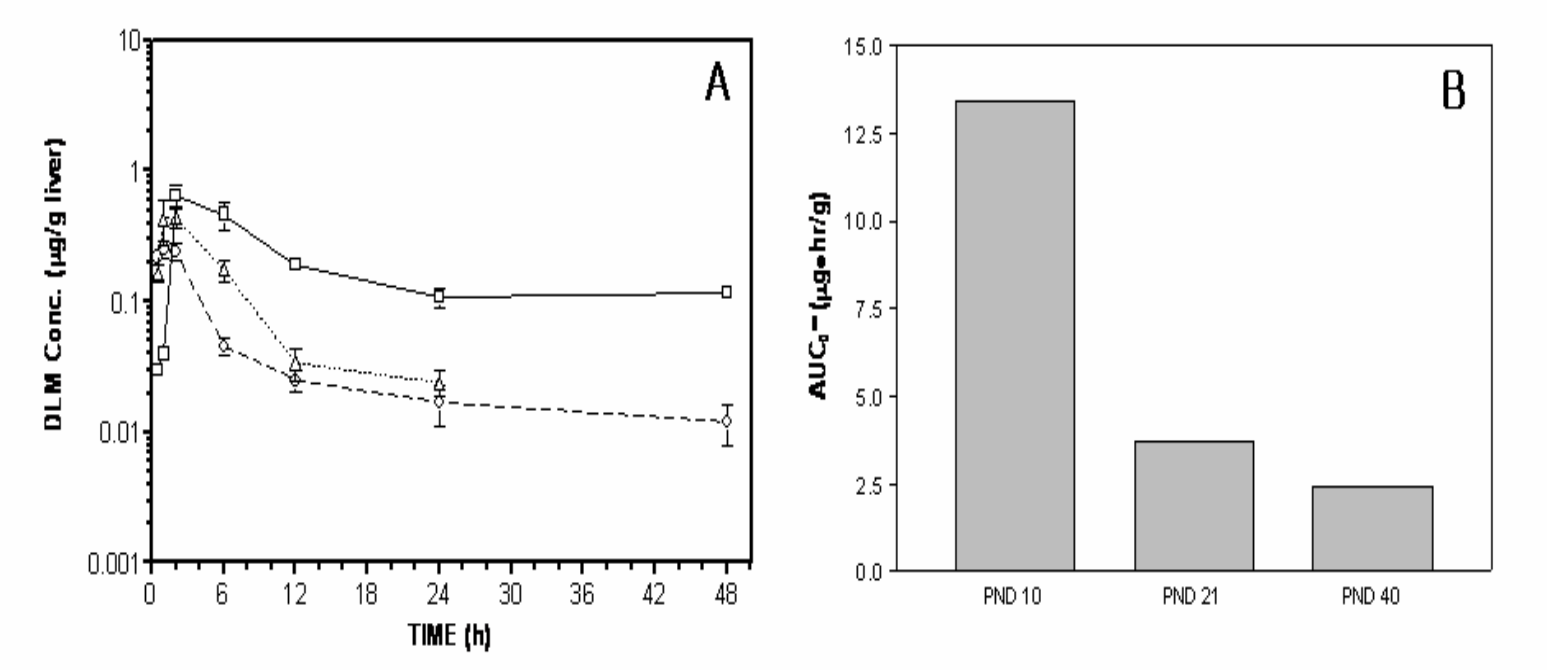

Figure 4-9. Age-dependent liver deltamethrin (DLM) time-courses (A) after an oral dose of 2 mg DLM/kg to PND 10 (□), 21 (Δ) and 40 (○) rats. Right panel (B) shows calculated liver AUC values. Data are means ± SD for 3-5 animals.

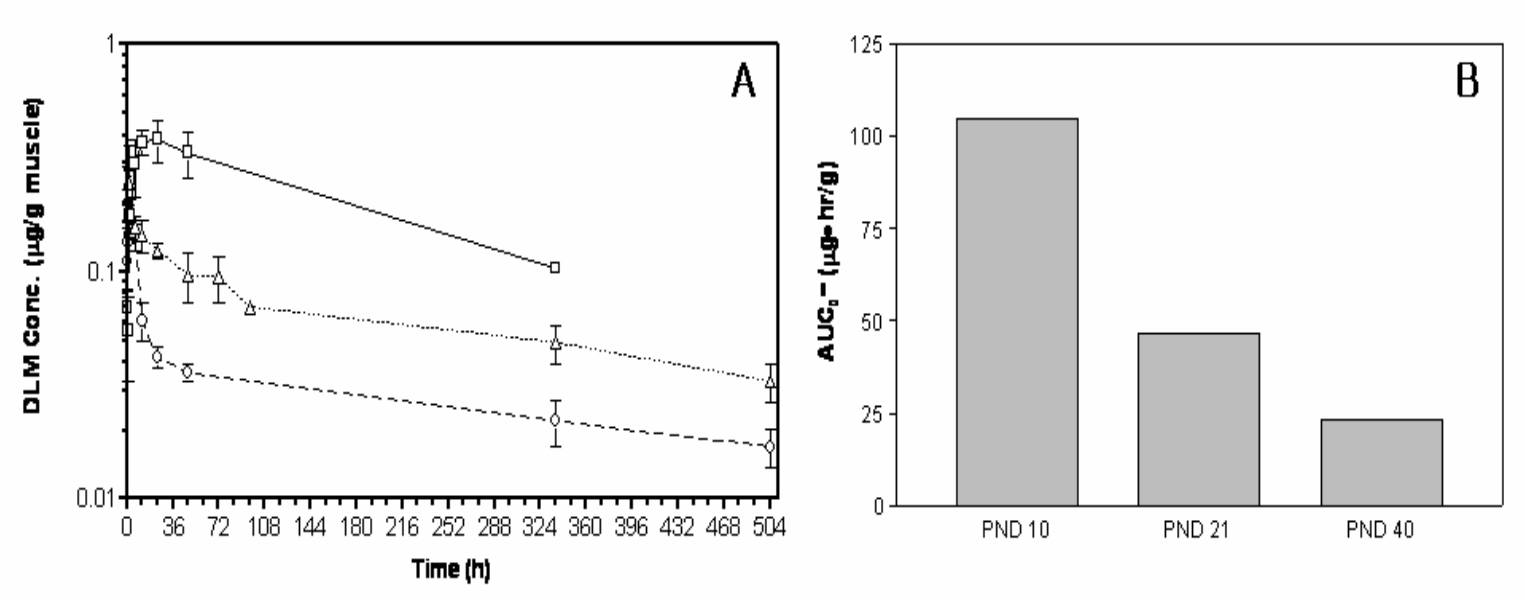

Figure 4-10. Age-dependent muscle deltamethrin (DLM) time-courses (A) after an oral dose of 2 mg DLM/kg to PND 10 (□), 21 (Δ) and 40 (○) rats. Right panel (B) shows calculated muscle AUC values. Data are means ± SD for 3-5 animals.

TABLE 4-1

Plasma and Tissue DLM Concentrations (mean ± SE, n= 3-5) Following Oral Administration of 0.4, 2 or 10 mg DLM/kg bw to PND-10, -21, and -40 Rats

Age (PND)	Dosage (mg/kg)	Plasma		Brain		Fat		Liver		Muscle	
		2 h post-dose	24 h post-dose	2 h post-dose	24 h post-dose	2 h post-dose	24 h post-dose	2 h post-dose	24 h post-dose	2 h post-dose	24 h post-dose
10	0.4	0.09 ± 0.05	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.18 ± 0.02	0.88 ± 0.22	0.32 ± 0.07	0.05 ± 0.04	0.06 ± 0.01	0.20 ± 0.09
	2	0.81 ± 0.28	0.08 ± 0.01	0.08 ± 0.03	0.04 ± 0.01	0.94 ± 0.22	6.91 ± 4.41	0.65 ± 0.22	0.11 ± 0.04	0.18 ± 0.06	0.38 ± 0.14
	10	5.90 ± 1.36	NA[a]	0.23 ± 0.06	NA[a]	1.53 ± 0.26	NA[a]	2.33 ± 0.31	NA[a]	0.81 ± 0.20	NA[a]
21	0.4	0.12 ± 0.02	ND	0.05 ± 0.03	0.01 ± 0.00	0.14 ± 0.02	0.20 ± 0.04	0.17 ± 0.06	ND	0.17 ± 0.03	0.07 ± 0.03
	2	0.40 ± 0.16	0.01 ± 0.00	0.06 ± 0.03	0.03 ± 0.01	0.66 ± 0.10	0.95 ± 0.18	0.44 ± 0.19	0.02 ± 0.01	0.30 ± 0.14	0.12 ± 0.02
	10	1.13 ± 0.13	NA[a]	0.12 ± 0.01	NA[a]	1.88 ± 0.40	NA[a]	0.56 ± 0.05	NA[a]	0.66 ± 0.15	NA[a]
40	0.4	0.06 ± 0.00	NA[b]	0.02 ± 0.01	NA[b]	0.31 ± 0.01	NA[b]	0.11 ± 0.01	NA[b]	0.04 ± 0.02	NA[b]
	2	0.34 ± 0.06	0.01 ± 0.00	0.05 ± 0.01	0.01 ± 0.00	0.35 ± 0.03	0.57 ± 0.18	0.24 ± 0.08	0.02 ± 0.01	0.15 ± 0.03	0.04 ± 0.01
	10	1.06 ± 0.68	0.04 ± 0.02	0.11 ± 0.04	0.02 ± 0.01	1.61 ± 0.52	3.15 ± 0.62	0.45 ± 0.18	0.03 ± 0.01	0.33 ± 0.08	0.09 ± 0.03

All data expressed as µg DLM/mL or g.
NA[a]:Data were not available due to DLM lethality; NA[b]: Data were not available due to no sample collection; ND: DLM was not detected.

TABLE 4-2

Toxicokinetic Parameters for DLM for Plasma Following Oral Administration of 0.4, 2 or 10 mg DLM/kg bw to PND-10, -21, and -40 Rats

Age (PND)	Dosage (mg/kg)	Plasma Toxicokinetic Parameters					
		Cmax[a] (µg/ml)	Tmax (h)	Half-life (h)	AUC_{0}^{∞} (µg·hr/ml)	$AUC_{0}^{6\,h}$ (µg·hr/ml)	$AUC_{0}^{12\,h}$ (µg·hr/ml)
10	0.4	0.11 ± 0.04	6	20.1	2.0	0.5	1.1
	2	0.92 ± 0.19	6	15.6	13.6	4.0	7.8
	10	5.90 ± 1.36	2	7.6	69.8	21.0	NA[b]
21	0.4	0.15 ± 0.07	1	2.8	0.7	0.5	ND[c]
	2	0.49 ± 0.12	1	17.7	2.9	1.9	2.5
	10	1.89 ± 0.36	1	3.3	13.0	7.4	12.3
40	0.4	0.13 ± 0.07	1	1.6	0.3	0.3	ND
	2	0.50 ± 0.18	1	4.9	2.3	1.5	1.9
	10	1.63 ± 0.57	1	8.9	7.5	4.3	5.5

[a]Means ± SE, n= 3-5.
[b]NA:Data were not available due to DLM lethality; [c]ND: Data were not available due to no detection of DLM

TABLE 4-3

Toxicokinetic Parameters for DLM for Brain Following Oral Administration of 0.4, 2 or 10 mg DLM/kg bw to PND-10, -21, and -40 Rats

Age (PND)	Dosage (mg/kg)	Brain Toxicokinetic Parameters						
		Cmax[a] (µg/g)	Tmax (h)	Half-life (h)	AUC $^{0-\infty}$ (µg·hr/g)	AUC $^{0-6\ h}$ (µg·hr/g)	AUC $^{0-12\ h}$ (µg·hr/g)	$\frac{AUC_{Brain}}{AUC_{Plasma}}$
10	0.4	0.04 ± 0.02	6	9.0	1.0	0.2	0.4	0.5
	2	0.11 ± 0.04	6	22.5	3.7	0.5	1.1	0.3
	10	0.38 ± 0.12	6	NA[b]	NA	1.5	NA	0.1[c]
21	0.4	0.05 ± 0.02	2	28.9	0.7	0.2	0.4	0.4[c]
	2	0.06 ± 0.03	2	32.6	3.0	0.3	0.6	0.4[e]
	10	0.25 ± 0.04	6	6.3	3.2	1.0	2.1	0.2[d]
40	0.4	0.02 ± 0.01	2	8.8	0.2	0.1	0.1	0.3[c]
	2	0.05 ± 0.01	2	23.5	0.9	0.2	0.3	0.4
	10	0.14 ± 0.07	4	22.9	2.2	0.6	1.0	0.3

[a]Means ± SE, n= 3-5.

[b]NA:Data were not available due to DLM lethality

[c] $AUC_0{}^{6}{}_{Brain}/AUC_0{}^{6}{}_{Plasma}$; [d] $AUC_0{}^{12}{}_{Brain}/AUC_0{}^{12}{}_{Plasma}$; [e] $AUC_0{}^{24}{}_{Brain}/AUC_0{}^{24}{}_{Plasma}$

TABLE 4-4

Toxicokinetic Parameters for DLM for Fat Following Oral Administration of 0.4, 2 or 10 mg DLM/kg bw to PND-10, -21, and -40 Rats

Age (PND)	Dosage (mg/kg)	Fat Toxicokinetic Parameters						
		Cmax[a] (µg/g)	Tmax (h)	Half-life (h)	AUC $^{0-\infty}$ (µg·hr/g)	AUC $^{0-6\ h}$ (µg·hr/g)	AUC $^{0-12}_{h}$ (µg·hr/g)	$\frac{AUC_{Fat}}{AUC_{plasma}}$
10	0.4	1.09 ± 0.16	48	NA[c]	NA[c]	1.6	5.5	21.5[f]
	2	9.47 ± 4.11	48	53.3	1690.0	5.7	25.0	24.0[f]
	10	8.66 ± 4.99	6	NA[b]	NA[b]	16.4	NA[b]	0.8[d]
21	0.4	0.31 ± 0.06	6	31.8	14.5	1.1	2.7	20.7
	2	1.24 ± 0.14	12	353.5	313.8	4.0	10.6	108.2
	10	4.23 ± 1.70	4	NA[b]	NA[b]	16.1	39.2	5.3[e]
40	0.4	0.31 ± 0.01	2	19.2	7.6	1.5	2.7	25.3
	2	0.65 ± 0.20	12	218.2	129.0	2.2	5.6	56.1
	10	3.15 ± 0.62	24	71.8	270.4	11.0	26.9	36.1

[a]Means ± SE, n= 3-5.

[b]NA: Data were not available due to DLM lethality; NA[c]: Data were not available due to no sample collection

[d] $AUC_0{}^{6}{}_{Fat}/AUC_0{}^{6}{}_{Plasma}$; [e] $AUC_0{}^{12}{}_{Fat}/AUC_0{}^{12}{}_{Plasma}$; [f] $AUC_0{}^{48}{}_{Fat}/AUC_0{}^{48}{}_{Plasma}$

TABLE 4-5

Toxicokinetic Parameters for DLM for Liver Following Oral Administration of 0.4, 2 or 10 mg DLM/kg bw to PND-10, -21, and -40 Rats

Age (PND)	Dosage (mg/kg)	Liver Toxicokinetic Parameters						
		Cmax[a] (µg/g)	Tmax (h)	Half-life (h)	AUC $^{0-\infty}$ (µg·hr/g)	AUC $^{0-6\ h}$ (µg·hr/g)	AUC $^{0-12}_{h}$ (µg·hr/g)	$\frac{AUC_{Liver}}{AUC_{plasma}}$
10	0.4	0.32 ± 0.07	2	28.7	5.0	1.3	2.1	2.5
	2	0.65 ± 0.22	2	25.6	13.4	2.6	4.6	1.0
	10	2.66 ± 1.04	6	NA[b]	NA	11.8	NA	0.7
21	0.4	0.17 ± 0.06	2	3.2	0.9	0.7	0.9	1.4[c]
	2	0.44 ± 0.18	2	23.9	3.7	1.9	2.5	1.3
	10	0.64 ± 0.11	1	12.9	11.4	2.9	5.3	0.4[d]
40	0.4	0.15 ± 0.04	1	5.5	0.8	0.5	0.6	1.7
	2	0.25 ± 0.01	1	35.5	2.4	1.0	1.2	1.0
	10	0.64 ± 0.26	1	22.4	3.8	1.8	2.3	0.5

[a]Means ± SE, n= 3-5.

[b]NA:Data were not available due to DLM lethality

[c] $AUC_0{}^6{}_{Liver}/AUC_0{}^6{}_{Plasma}$; [d] $AUC_0{}^{12}{}_{Liver}/AUC_0{}^{12}{}_{Plasma}$

TABLE 4-6

Toxicokinetic Parameters for DLM for Muscle Following Oral Administration of 0.4, 2 or 10 mg DLM/kg bw to post-natal day (PND)-10, -21, and -40 Rats

Age (PND)	Dosage (mg/kg)	Muscle Toxicokinetic Parameters						
		Cmax[a] (µg/g)	Tmax (h)	Half-life (h)	AUC $^{0-\infty}$ (µg·hr/g)	AUC $^{0-6\ h}$ (µg·hr/g)	AUC $^{0-12\ h}$ (µg·hr/g)	$\frac{AUC_{Muscle}}{AUC_{plasma}}$
10	0.4	0.20 ± 0.05	12	454.5	133.1	0.5	1.5	4.7[e]
	2	0.38 ± 0.14	24	167.9	104.7	1.1	3.1	7.7
	10	1.70 ± 0.74	6	NA[b]	NA	5.2	NA	0.2[c]
21	0.4	0.17 ± 0.02	2	32.9	5.4	0.8	1.3	7.7
	2	0.30 ± 0.14	2	308.8	46.7	1.3	2.2	16.1
	10	1.27 ± 0.22	6	14.8	32.0	4.9	11.5	0.9[d]
40	0.4	0.05 ± 0.02	1	8.6	0.9	0.2	0.5	3.0
	2	0.15 ± 0.03	2	361.0	23.5	0.8	1.4	10.2
	10	0.34 ± 0.18	4	64.8	13.9	1.7	3.0	1.9

[a]Means ± SE, n= 3-5.

[b]NA: Data were not available due to DLM lethality

[c] $AUC_0{}^{6}{}_{Muscle}/AUC_0{}^{6}{}_{Plasma}$; [d] $AUC_0{}^{12}{}_{Muscle}/AUC_0{}^{12}{}_{Plasma}$; [e] $AUC_0{}^{48}{}_{Muscle}/AUC_0{}^{48}{}_{Plasma}$

SUMMARY

Deltamethrin (DLM), the compound chosen for the current study, is a widely-used Type II pyrethroid insecticide and a relatively potent neurotoxicant. The overall objective of this research was to characterize the absorption, systemic/tissue distribution and elimination of DLM over a range of doses (0.4 to 10 mg DLM/kg) in adult rats, and also to determine the age- and dose-dependency of TK and tissue distribution in developing rats.

Although the acute toxicity of DLM is well characterized, there has been a lack of published simple and reliable analytical methods to support TK studies of this compound. Therefore, prior to TK study, such a method for quantitation of DLM in plasma and tissues was developed and validated following U.S. FDA and ICH guidelines. To avoid laborious steps, DLM was extracted from plasma by vortexing it in twice its volume of acetonitile for 30 s, and then the aliquot of supernatant was injected onto a HPLC column without taking the samples through an evaporation process. This procedure produced high recoveries, showed good linearity, precision and accuracy within the range of 0.05 – 4.0 µg/ml. The LOD and LOQ were 0.01 and 0.05 µg/ml, respectively. This method has enabled our laboratory to characterize the plasma and tissue disposition of DLM in developing and mature rats.

Particle size is an important determinant of systemic absorption of hydrophobic drugs and other chemicals from the GI lumen. Reducing the size of particulates increases their surface area, enhancing a compound's opportunity for dissolution and diffusion across GI epithelial membranes. DLM is a solid (powdered) hydrophobic chemical at room temperature. Oral dosage vehicle can have a marked effect on the acute toxicity of DLM. Oral LD_{50} values published for DLM ranged from 87 mg/kg (in corn oil) to > 5,000 mg/kg (in a 1% methylcellulose aqueous

suspension). Glycerol formal (GF) and Alkamuls® (AL) were used to investigate the influence of different formulations on bioavailability, TK and acute neurotoxicity of orally-administered DLM. GF, a binary solvent, is used to solubilize a wide variety of hydrophobic and hydrophilic chemicals and AL (formerly, Emulphor®), a polyethoxylated vegetable oil, is widely used to prepare stable aqueous emulsions of aliphatic and aromatic hydrocarbons of low to moderate molecular weight. Bioavailability and C_{max} were significantly lower when DLM was given by gavage as an aqueous AL suspension than when dissolved in GF. Our AL formulation proved to be an unstable aqueous suspension, in that the DLM particles settled soon after vigorous shaking. Some 22% of the DLM particles in the suspension were < 5 µm, but the size distribution of even smaller particles could not be determined. Although systemic uptake from our AL suspension was quite limited, T_{max} and ka, two indices of the rate of absorption, were not significantly different in the AL and GF groups. Thus, it seems reasonable to assume that much less DLM was available for systemic uptake in the AL animals, but the compound that was available (i.e., dissolved) was absorbed from the gut at a comparable rate as that dissolved in GF. The vehicle in which DLM is administered orally has a pronounced influence on the compound's particle size, dissolution, absorption and bioavailability, as well as target and storage tissue deposition. Therefore, GF which dissolves DLM completely, was used for all TK studies of DLM in our laboratory.

Relatively little is known about the TK of DLM or other pyrethroids in humans or other mammals, despite frequent exposures of the populace to this relatively new class of insecticide. The time-course data and TK parameter estimates presented here provide a relatively comprehensive overview of the systemic uptake, disposition and elimination of iv and oral DLM in rats. A key contribution of the work to toxicity risk assessment of DLM is information on the

relationships among administered dose, internal dose (i.e., blood/plasma levels) and target organ dose (i.e., brain C_{max} and AUC). DLM appeared to be rapidly but incompletely absorbed from the GI tract of fasted adult rats. Peak blood levels were manifest in 1 – 2 h with each dosage level, but bioavailability was only 15%. DLM was apparently not well absorbed from the GI tract, even though it is highly lipophilic compound. A review of the literature revealed bioavailability was also low for a number of other highly lipid-soluble, slowly-metabolized chemicals including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, benzo(*a*)pyrene and other polyaromatic hydrocarbons. In recent experiments, we found ~ 40% of a 10 mg DLM/kg oral dosage to be eliminated in the feces of rats. DLM was not detectable in the feces following iv injection, indicating that the chemical does not reenter the GI lumen via biliary excretion or by passive diffusion from mesenteric blood. P-glycoprotein (P-gp) is located on the GI luminal brush border membrane and may diminish DLM uptake in rats by acting as an efflux transporter. There has been little evidence reported to date, however, that DLM or other pyrethroids are P-gp substrates. Unexpectedly low concentrations of DLM were present in its target organ. We had theorized that substantial amounts of DLM would accumulate in the brain, due to its relatively high lipid content. Based upon the brain DLM C_{max} of 0.18 µg/g at the T_{max} (2 h) and an assumed brain weight of 2 g in a 388-g rat, it was calculated that only 0.29 % of the total body burden of rats given 10 mg/kg was present in the whole brain. This unanticipated phenomenon remains to be explained. The brain's largely phospholipid composition may be a factor in limited partitioning of DLM. DLM readily exits the blood and enters the other organs and tissues studied, but may be limited by the blood-brain barrier. The substantial amounts of P-gp in the blood-brain and cerebrospinal barriers may serve as effective efflux transporters. This supposition is supported by DLM's relatively short $t_{½}$ (12.5 – 19.5 h) in brain, though as noted previously, the pyrethroid

is yet to be shown to be a P-gp substrate. Rat brain is reported to slowly metabolize DLM to a very limited extent. Adipose tissue, skin and skeletal muscle were found to be the major depots for DLM. T_{max} values were relatively long for these poorly-perfused tissues. Perirenal fat exhibited substantially higher concentrations than other tissues/organs. DLM levels in skin were lower, but the skin's relatively large volume and similarly long $t_{½}$, resulted in it being a major repository. Muscle contained modest DLM concentrations, but its mass (40.4% of total bw) and slow elimination rate resulted in it serving as the third major depot for the parent compound. It is not clear at the present time whether DLM binds to muscle, and if so whether this and slow perfusion account for the chemical's long half-life there. It is clear, however, that fat, skin and muscle are important factors in DLM's potential for systemic accumulation and its delayed elimination and prolonged duration of action.

Clinical signs were observed after DLM treatment (0.4, 2, or 10 mg DLM/kg) of PND 10, 21, and 40 rats. The severity of neurotoxicity and lethality varied inversely with age. In PND 10 and 21 pups, the highest dose (10 mg DLM/kg) elicited lethality within 8 and 12 h post dosing, respectively. DLM brain concentration and magnitude of toxic symptoms paralleled one another. The lowest dose (0.4 mg DLM/kg) did not produce salivation or tremors in PND 10 to 40 rats. Age-dependency was also observed in brain $C_{max}s$ (0.04, 0.05, and 0.02 µg/g brain) and AUCs (1.0, 0.7, and 0.2 µg·h/g brain) in PND 10, 21, and 40 rats. Although 0.4 mg DLM/kg did not produce recognizable clinical signs, age-dependent differences in the TK parameters C_{max} and AUC in plasma and brain were observed. These parameters also showed dose-dependent differences in each age group. Brain AUCs of PND 10 pups were much higher than those of PND 21 and 40. However, the ratios of AUC_{brain}/AUC_{plasma} were similar from 0.1 to 0.5 across age-groups. There were not dramatic increased ratios in youngest pups, but the ratios showed a

tendency towards this at 0.4 mg DLM/kg. These results imply that high plasma concentrations of DLM, due to limited metabolic capacity in immature rats, may play a major role in DLM neurotoxicity, rather than maturation of the BBB. The present study has demonstrated that immature (PND 10, 21, and 40) rats orally treated with 0.4, 2, or 10 mg DLM/kg showed age- and dose-dependent differences in toxicokinetics. Evidence of age-dependency of brain DLM C_{max}s and AUCs was manifest at all three dosages utilized in the investigation. This is contrary to the previous report (Sheets et al., 2000) that the age-dependent sensitivity of pyrethroids is apparent only at high neurotoxic doses and the implication that infants and children are protected by existing food tolerances, without the need for an additional uncertainty factor. However our results imply that an additional uncertainty factor should be considered to protect infants' and children's from pyrethoids. Evidence of age- and dose-dependency in brain concentrations also suggests that DLM TK in the brain may contribute to the increased sensitivity of immature rats. This age- and dose-dependency of TK behavior of DLM in immature rats has important implications for understanding internal exposure to pyrethroid insecticides. These data are essential for development of a physiologically-based pharmacokinetic model for children's risk assessment.