AGE-, DOSE- AND TIME-DEPENDENCY OF PLASMA AND TISSUE DISTRIBUTION OF DELTAMETHRIN IN IMMATURE RATS

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SHORT TITLE: Deltamethrin Kinetics in Immature Rats

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ABSTRACT

The major objective of this project was to characterize the systemic disposition of the pyrethroid, deltamethrin (DLT), in immature rats, with emphasis on the age-dependence of target organ (brain) dosimetry. Postnatal day (PND) 10, 21, and 40 male Sprague-Dawley rats received 0.4, 2 or 10 mg DLT/kg by gavage in glycerol formal. Serial plasma, brain, fat, liver and skeletal muscle samples were collected for up to 510 h and analyzed for DLT and/or 3-phenoxybenzoic acid (PBA) content by HPLC. Toxicokinetic (TK) data from previous experiments of the same design with young adult (PND 90) rats (Kim *et al.*, 2008) were used to compare to immature rat data. Plasma and tissue DLT levels were inversely related to age. Preweanlings and weanlings showed markedly elevated brain concentrations and pronounced salivation, tremors and eventual fatalities. Plasma DLT levels did not reliably reflect brain levels over time. Plasma:brain ratios were time- and dose-dependent, but apparently not age-dependent. Brain levels were better correlated with the magnitude of CNS effects than plasma levels. Hepatic intrinsic clearance of DLT progressively increased during maturation, as did the hepatic extraction ratio. Thus, limited capacity to metabolically inactivate DLT appeared primarily responsible for the inordinately high target organ doses and acute neurotoxicity in pups and weanling rats. Hepatic blood flow was not rate limiting in any age-group. Limited DLT hydrolysis was manifest *in vivo* in the pups by relatively low plasma PBA levels. Elevated exposure of the immature brain to a pyrethroid may prove to be of consequence for long-, as well as short-term neurotoxicity.

KEY WORDS: Pyrethroid, deltamethrin, toxicokinetics, metabolic inactivation, children's risk assessment, neurotoxicity

INTRODUCTION

There has been concern for the last two decades that children may be more vulnerable than adults to harmful effects of pesticides and other chemicals. The National Research Council formed a committee to address exposures and risks posed to infants and children by dietary pesticides, as well as pertinent regulatory policies (Bruckner, 2000). Recommendations in the committee's report (NRC, 1993) had some far-reaching consequences. The Food Quality and Protection Act (FQPA) of 1996, for example, dictated that an additional 10X uncertainty factor be used in risk assessments of pesticides, when toxicokinetic (TK) and toxicity data for children were unavailable. Two 3.16X components of the 10X factor were assumed to provide for potential age-dependent TK and toxicodynamic (TD) differences (Renwick *et al.*, 2000). After years of proposed rules and court delays, it was signed into law in December, 2003. TK data for pyrethroids in immature subjects are apparently limited to blood levels of parent compound in developing rats following administration of one oral dose of deltamethrin (DLT) (Anand et al., 2006a). Information on the TD of this relatively new class of insecticides is quite limited. A second consequence of the NRC report was the Pediatric Research Equity Act of 2003. It required companies seeking FDA approval of new drugs prescribed for substantial numbers of children to assess the drugs' safety and efficacy in pediatric patients.

The developing nervous system may be particularly sensitive to some chemicals, as neuronal replication, migration, differentiation, myelination and synapse formation must occur correctly in proper sequence (Adams *et al.*, 2000; Rice and Barone, 2000). Some research with mature animals suggests that repeated, low-level exposure to chlorpyrifos and certain other organophosphates (OPs) may adversely affect neurodevelopment in children (Eskenazi *et al.*, 1999; Samsam *et al.*, 2005; Slotkin, 2004). This was generally attributed to deficient OP metabolic inactivation, though findings of Timchalk *et al.* (2006) indicate that preweanling rats' susceptibility to chlorpyrifos was due to both age-dependent TK and TD differences. Concern led to

agreements between the U.S. EPA and manufacturers to phase out the sale of diazinon, chlorpyrifos and many other OPs for residential and agricultural uses.

Pyrethroids, synthetic derivatives of naturally-occurring pyrethrins, have enjoyed increasing use in the U.S. and Europe with the decline of OPs. The number of OP exposure incidents in the U.S. has decreased substantially since 2001, while pyrethroid incidents have increased (Sudakin, 2006). Pyrethroids are widely used in agriculture and households for pest control, so human exposures are becoming increasingly common. Heudorf *et al.* (2004) discovered pyrethroid metabolites in the urine of 1,177 persons without apparent exposure in Germany. Exposures to pyrethroids have been well documented in several potentially sensitive populations, including pregnant women, infants and children (Berkowitz *et al.*, 2003; Lu et al., 2006; Morgan *et al.*, 2007; Tulve et al., 2006; Whyatt *et al.*, 2002). The acute neurotoxic potency, signs of poisoning, and apparently mechanisms of action can differ from one pyrethroid to another (Soderlund et al., 2002). There have been reports that certain pyrethroids can cause adverse neurodevelopmental effects in rodents, but evidence to date is inadequate (Shafer *et al.*, 2005).

No data on the disposition of pyrethroids in their target organ (brain), liver, storage sites, or other tissues of immature animals are available for use in health risk assessments for infants and children. DLT is one of the most potent neurotoxicants of the pyrethroids (Choi and Sutherlund, 2006; Wolansky et al., 2006). As illustrated in Figure 1, it is metabolically detoxified by liver cytochrome P450s (CYPs) and liver and plasma esterases. Anand et al. (2006b) found that CYPs 1A2, 1A1 and C11, in decreasing order quantitatively, oxidized DLT. Carboxylesterases (CaEs) were primarily responsible for catalyzing DLT hydrolysis in rats. The capacity of these enzymes to metabolize DLT *in vitro* increases substantially during maturation of rats (Anand et al., 2006a,b). This observation helped explain an early finding of Sheets et al. (1994) that preweanling rats succumbed to lower DLT doses than did adults. Studies are needed to account for any

additional effects of age-dependent changes in liver size and blood flow on systemic disposition and clearance. Although Anand et al. (2006a) described blood time-courses in preweanling, weanling and adolescent rats, it is not clear whether blood levels reflect brain levels, or how brain dosimetry varies with age or dose. Previous experiments with adult rats revealed that skeletal muscle and fat were two primary storage sites for DLT (Kim et al., 2008). It would be useful to gain understanding of these depots' roles in DLT kinetics, as body composition changes during maturation. Such data on DLT and other pyrethroids should be useful to risk assessors, by providing some scientific TK basis for making decisions when dealing with child-specific mandates of the FQPA.

A major objective of the current study was to characterize the systemic disposition of DLT in immature rats, with particular emphasis on the age-dependence of target organ (i.e., brain) dosimetry. A goal was to ascertain whether blood DLT concentrations were a reliable index of brain concentrations over time. A related aim was to determine whether blood or brain DLT concentrations were better correlated with the magnitude of CNS effects. The final objective was to obtain comprehensive time-course data, including liver and storage depot profiles, with which to develop a PBTK model for the pyrethroid in rats during their maturation.

Materials and Methods

Chemicals. Deltamethrin (DLT) ([*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 (Monhein, Germany). The structures of the parent compound and some of its major metabolites are shown in Figure 1. HPLC-grade acetonitrile (ACN) and glycerol formal (GF) were purchased from Sigma-Aldrich (St. Louis, MO). Methanol, sulfuric acid and deionized water (HPLC-grade) were obtained from J.T. Baker Co. (Phillipsberg, NJ). All other chemicals were of the highest grade commercially available.

Animals and Treatment. Pregnant Sprague-Dawley (S-D) rats were purchased from Charles River Breeding Laboratories (Raleigh, NC). They were housed individually in shoebox cages with corncob bedding (PharmaServ, Framingham, MA) in an AAALAC-approved animal care facility maintained at 22° ± 2° C and 55 ± 5% relative humidity with light from 0600 – 1800 h. Food (5001 Rodent Diet®, PMI Nutrition International LLC, Brentwood, MO) and tap water were available *ad libitum*. Following delivery, the pups remained with their mother until weaning on postnatal day (PND) 21. Only male pups were utilized in this investigation. Mean body weights ± SD of the three age-groups at the time of dosing were as follows: 23.7 ± 2.0 g (PND 10); 56.4 ± 8.7 g (PND 21), and 176.7 ± 14.5 g (PND 40). Subgroups of each age were gavaged between 0900 and 1000 h with 0.4, 2 or 10 mg DLT/kg in 2 ml/kg of GF. Although PND 10 pups were not fasted prior to dosing, PND 21 and 40 rats were fasted for 12 h and allowed access to food again 3 h post dosing.

One aim of the study was to assess the dose-dependence of DLT disposition in rats of different maturational stages. This proved to be somewhat difficult with this particular pyrethroid. Ten mg/kg caused only transient neurotoxic signs in adult rats (Kim et al., 2008). All the PND 10 and 21 pups given 10 mg/kg, however, died within 6 - 8 and 12 - 16 h, respectively. Most TK parameters could not be calculated from these abbreviated time-courses. The analytical limits of detection and quantitation limited the length of time 0.4 mg/kg DLT profiles could be monitored in the more mature animals with relatively high metabolic capacity. Plasma and tissue DLT time-course data from young adult (PND 90) rats, gavaged with 0.4, 2 and 10 mg DLT/kg (Kim et al., 2008), are compared with the immature rat data obtained in the current study.

Sample Collection and Preparation. Groups of immature rats were sacrificed at selected times post dosing for collection of biological specimens. The PND 10 and 21 pups were killed by decapitation and blood collected in heparinized tubes. The PND 40 adolescents were euthanized with CO_2 and blood samples withdrawn from the inferior vena cava. The plasma was processed for DLT analysis as described by Kim et al.

(2008). Selected tissue specimens (whole brain, perirenal fat, liver and thigh muscle) were rapidly excised, blotted dry and processed according to the procedure of Kim et al. (2008).

Analysis of DLT and PBA. DLT was quantified by the isocratic high-performance liquid chromatography (HPLC) technique of Kim *et al.* (2006). The limits of quantitation and detection for DLT in biological specimens were 0.01 and 0.05 μg/ml, respectively. In a limited series of experiments, a somewhat different HPLC method was used to quantify both DLT and 3-phenoxybenzoic acid (PBA) in the same plasma samples. The mobile phase was ACN and deionized water adjusted to pH 2.4 with phosphoric acid. These latter chromatography conditions were described by Ding et al. (2004). DLM eluted in ~ 14.5 min, while PBA eluted in ~ 26 min.

Assessment of Neurotoxicity. The objective of this experiment was to monitor manifestations of DLT-induced neurotoxicity as a function of age, for subsequent correlation with blood and brain dose metrics. Ten, 21- 40- and 90-day-old SD rats received 0.4, 2 or 10 mg DLT/kg by gavage. Analogous findings were seen in a previous experiment of the same design by Anand et al. (2006b), in which the magnitudes of salivation and tremors were each subjectively scored hourly for 6 h on a scale of 0 to 3 for members of each age-group. The hourly scores were summed to give a total score for each of the end points. These previously determined scores were utilized to correlate with plasma and brain AUCs measured in each age-group in the current investigation.

Age- and Pathway-Dependent Intrinsic Clearance. Age-dependent differences in *in vitro* intrinsic clearance of DLM in rats reported by Anand *et al.* (2006a) were refined by adjusting them for age-dependent liver weight and blood flow. Hepatic intrinsic clearance (Cl_h) was obtained by multiplying the age-specific clearance (per g basis) by the liver weight for the corresponding age (Mirfazaelian *et al.*, 2007). The hepatic extraction ratio (E) was defined as the reduction in the blood concentration of DLT by the liver.

Equation (1) implicitly considers blood flow to the liver:

$$E = \frac{Cin - Cout}{Cin} \qquad (1)$$

It can be shown (Gabrielson and Weiner, 2000) that E is related to both clearance (Cl_h) and blood flow (Q_h) by the following relationship:

$$E = \frac{fu \cdot Cl\ int}{Qh + fu \cdot Cl\ int} \qquad (2)$$

where fu is the fraction of unbound DLT. Thus, if fu=1 (all DLT is free) E reduces to:

$$E = \frac{Clint}{Qh + Clint} \qquad (3)$$

Based on a study of the binding of permethrin (a structural analog to deltamethrin) in human serum albumin (Abu-Donia *et al.*, 2002), we applied an estimate 0.91 for fu in calculating E (Eq. 2). To compute Qh in E, we estimated age-dependent Q_h in young rats using ¾ power allometric scaling according to Delp *et al.* (1991).

Data Analyses. Means and SEs calculated with Microsoft Excel (Microsoft Co., Redmond, WA). TK parameters, including area under the DLT concentration versus time curve (AUC), observed time of maximum plasma concentration (T_{max}), maximum plasma concentration (C_{max}) and apparent half-life ($t½$) were estimated for plasma and tissues using WinNonlin (ver. 4.1) noncompartmental analysis (Pharsight, Cary, NC). AUC from 0 to the last measured time-point was calculated using the log/linear trapezoidal rule. AUC from the last

time-point to ∞ was determined as the plasma concentration at the last data point divided by the elimination rate constant. It was not possible to calculate SEs for t½ and AUC values, as sample collection required terminal sacrifice. The statistical significance of differences in mean C_{max} values and plasma/brain concentrations ratios was assessed with a one-way ANOVA, followed by Duncan's Multiple Range Test, Tukey's Studentized Range Test, and Newman-Keul's Test using a Modstat statistical package (Bellingham, WA).

RESULTS

Age-Dependency of Clinical Signs

The magnitude of severity of DLT-induced neurotoxicity was inversely related to age. The PND 10 and 21 rats given 10 mg/kg po initially exhibited pronounced salivation, followed by tremors of increasing severity over the first 6 h after dosing. All of the 10 mg/kg 10- and 21-day-old pups died between 6 and 8 h and 12 and 16 h, respectively. The 40-day-old adolescents only showed mild to moderate salivation for 3 h, while the 90-day-old adults experienced mild salivation for 2 h. The intermediate (2 mg/kg) dose elicited severe salivation and tremors, with deaths of 3 of 32 PND 10 pups. These clinical signs were moderate in the PND 21 pups. This dosage did not produce clinical signs in the PND 40 or 90 rats. The lowest dose of DLT (0.4 mg/kg) failed to adversely affect any age-group.

Systemic DLT Disposition Profiles

DLT concentration time-courses measured in plasma and tissues of immature and mature rats are shown in Figures 2 – 4. Due to fatalities, profiles do not extend beyond 6 and 12 h for the 10 mg/kg PND 10 and 21 pups, respectively. DLT concentrations quickly fell below the LOQ in the PND 40 and 90 rats given 0.4 mg/kg, due to their relatively high DLT metabolic capacity (Fig. 2). Thus, the profiles for the different age-group are

most complete and readily distinguishable from one another at the 2 mg/kg dosage (Fig. 3). Visual inspection of these profiles reveals that DLT levels in the plasma and liver soon peak (i.e., within 1 – 2 h post dosing) in the three older age-groups and drop rapidly thereafter. Plasma, brain and liver concentrations in the youngest (PND 10) pups rise more slowly to higher levels, followed by more gradual declines during the first 12 – 24 h post dosing. DLT concentrations increase even more slowly in skeletal muscle and most slowly of all in fat, notably in the PND 10 pups. Peak fat concentrations 48 h post dosing in these preweanlings are 10-fold higher than in the other age-groups. The rank order of DLT concentrations over time in all biological specimens is as follows: PND 10 > PND 21 > PND 40 ~ PND 90. This pattern of age-dependency can be most clearly seen in Figure 3, but is manifest at the 0.4 (Fig. 2) and 10 (Fig. 4) mg/kg doses as well. In most instances, plasma and tissue time-courses in the adolescent (PND 40) and young adult (PND 90) rats closely resemble one another.

Dose- and Age-Dependency of Systemic DLT TK Parameter Estimates

The influence of dose and maturation on DLT TK parameter estimates can be seen in Tables 1 and 2. Plasma C_{max} AUC^{6}_{0} and AUC^{∞}_{0} values were dose-dependent for each age-group (Table 1). Half-lives did not appear to vary with dose, but data of sufficient duration were not available for many groups, due to lethality or lack of DLT detection at very low concentrations. It is evident in Figures 2A and 3A that the youngest rats given 0.4 and 2 mg/kg exhibited substantially higher plasma levels over time and as a result, larger AUC^{6}_{0} and AUC^{∞}_{0} values than the other age groups (Table 1). C_{maxs} and T_{maxs} were also higher/longer in the PND 10 pups than in the older animals. Weanlings (PND 21) exhibited values of intermediate magnitude. C_{max} and AUC^{∞}_{0} values were usually lowest and comparable to one another in the PND 40 and 90 groups. There were usually inadequate time-course data to assess age-dependency of t½ values. The influence of maturation on liver DLT time-profiles can be seen in Figures 2D, 3D and 4D. It is evident for each of the three dosages that hepatic DLT concentrations in the youngest pups were substantially higher than in other age-groups for the last 46 h of the

48-h monitoring interval. Mean C_{max}, AUC^{6}_{0} and AUC^{∞}_{0} increase with dose for each age-group, though the increases are frequently not proportional to increases in dose (Table 1). In most instances the measures AUC^{6}_{0} and AUC^{∞}_{0} are inversely related to age in the PND 10, 21 and 40 groups, as are C_{maxs}. Liver t½ and AUC^{∞}_{0} appear to be somewhat longer/larger in adults than in PND 40 adolescents. This discrepancy may be due to the longer duration of monitoring during the terminal elimination phase in the adults (i.e., 96 h versus 48 h in the PND 40 group).

Brain DLT concentration versus time profiles also proved to be age- and dose-dependent. It can be seen in Figure 3B that the youngest rats exhibited the highest brain concentrations over time following ingestion of 2 mg DLT/kg. Corresponding brain DLT levels, as well as AUC^{6}_{0} and AUC^{∞}_{0} values (Table 2) for the PND 21 weanlings were only slightly lower. The PND 40 and 90 brain profiles and AUC^{∞}_{0} values were substantially lower and comparable to one another. It should be recalled the PND 10 and 21 pups experienced moderate to severe poisoning by 2 mg/kg, but the older rats were asymptomatic. C_{maxs} were generally inversely related to age from PND 10 – 40, though intersubject variability sometimes precluded statistically significant differences. Dose-dependent increases in C_{max}, though most pronounced in the 10-day-old pups, were far from proportional to increases in DLT dose. Observed brain T_{maxs} were quite variable and long, relative to plasma T_{maxs}. The brain t½s were consistently longer than plasma t½s (Tables 1 and 2). Brain t½ values did not appear to vary much with animal age or dose.

The age-dependency of fat and skeletal muscle DLT profiles is evident in Figures 2 – 4E and C. The 10-day-old pups exhibit far higher quantities of DLT in their adipose tissue for up to 48 h after dosing than do the other age groups. Skeletal muscle DLT levels exhibit the following rank order: PND 10 > 21 > 40 ≅ 90. DLT appears to be cleared more rapidly from muscle and fat of the preweanlings, though t½ and AUC^{∞}_{0} values

were not calculated due to a lack of data beyond 12 h. Incomplete time-courses make assessment of the age-dependency of muscle and fat profiles difficult for the 0.4 and 10 mg/kg dosage groups.

DLT Tissue and Body Burdens

DLT concentrations in plasma, brain, skeletal muscle, fat and liver in PND 10, 21 and 40 rats measured 6 and 24 h after ingestion of 0.4, 2 and 10 mg DLT/kg are included in Tables 3, 4 and 5, respectively. As noted in the tables' footnotes, it was possible to calculate the burden, or total amount of DLT present in the selected tissues at these two time-points. It was also possible to express the amount of DLT in each tissue as % of the measured body burden (i.e., % of the total amount of DLT found in monitored tissues). The five tissues monitored in the study constituted ~ 51 – 61 % of total body weight.

The tissue disposition of DLT was somewhat different from what would be anticipated for a highly lipophilic compound (Tables 3 – 5). Plasma DLT consistently exceeded brain DLT, whether expressed in terms of concentrations, total tissue burden or % of measured body burden. Although the brain is the primary target organ, its contribution to the measured body burden varied from just 0.2 – 1.8% across the range of ages, doses and time-points. In almost all instances, each measure of DLT in plasma, brain, muscle, fat and liver was inversely related to age. Skeletal muscle and fat were the two major depositories of the parent compound. DLT levels in muscle were significantly lower than in fat 6 h post ingestion. The 6-h muscle burden and % measured body burden values, however, were often higher or comparable to fat burdens, since skeletal muscle comprises a substantially greater % of body weight than does fat. Muscle DLT levels remained relatively constant, or declined modestly between 6 and 24 h. In contrast, fat levels generally rose during this time-frame. Thus, the majority of the insecticide in the body $\geq$ 24 h was present in adipose tissue. Distribution between adipose tissue and muscle did not appear to vary during maturation. Tissue disposition in PND 90 young adults (data not shown) was quite similar to that in PND 40 adolescents.

Plasma Versus Brain DLT Levels and Effects

Plasma DLT levels were not a reliable internal dosimeter for target organ (i.e., brain) levels or adverse effects. Plasma and liver profiles resemble one another most closely in Figure 5, though the plasma levels increased and decreased more rapidly. This became more pronounced with increase in dose. In contrast, brain DLT levels rose quite slowly, plateaued at relatively low concentrations, and then diminished slowly. As anticipated, correlation between plasma and brain DLT concentrations was poor. Correlation coefficients for the PND 10, 21 and 40 groups were 0.65, 0.59 and 0.47 (plots of brain versus blood levels not shown). Plots of plasma:brain concentration ratios revealed certain patterns (Fig. 6). The ratios for many groups tended to diminish over time post dosing. The ratios generally increased with dose during the initial 6 h after exposure. Ratios of plasma AUC^{∞}_{0}:brain AUC^{∞}_{0}, showed little evidence of age-dependency, except for relatively high PND 10 ratios at 2 and 6 h at the highest dose. Brain DLT levels in immature rats showed much better concordance with severity of neurotoxicity signs than did blood levels (Fig. 7). There was excellent correlation between brain AUC^{6}_{0} values and magnitude of salivation ($r^2 = 0.95$) and tremors ($r^2 = 0.99$). Lower correlation coefficients were obtained when relating blood AUC^{6}_{0} values to the extent of salivation ($r^2 = 0.57$) and tremors ($r^2 = 0.79$) (Fig. 7B).

Age-Dependent Hepatic Metabolic Clearance. The potential influence of changes in liver weight and blood flow during maturation of rats were taken into account to better understand the physiological basis of alterations in DLT kinetics during development. A PBPK model (Mirazaelian *et al.*, 2006) was used to estimate that metabolism accounted for ~ 75.5 – 83.1% of the elimination of administered doses of DLT in adult SD rats. The current focus was on hepatic clearance, as about 91% of the total metabolism of DLT occurs in the liver. Age- and pathway-specific (i.e., CYP- and CaE-mediated) V_{max} and K_m values for liver microsomes were obtained from Anand *et al.* (2006a). Hepatic intrinsic clearance (Cl_h) for different age groups of SD rats was

estimated by multiplying *in vitro* intrinsic clearance by their liver weight (Mirfazaelian *et al.*, 2007). Cl_h progressively increased with increase in age from PND 10 – 90 (Table 6). DLT liver extraction ratios were calculated as described in the Materials and Methods, by accounting for Cl_h, liver blood flow and the fraction of DLT not bound to plasma proteins. The liver extraction ratio progressively increased with age, from 0.02 in PND 10 pups to 0.34 in PND 90 young adults (Table 6).

Systemic PBA Profiles

Blood PBA concentration versus time profiles were monitored and compared to DLT profiles as indices of DLT metabolism *in* vivo during maturation. It is apparent in Figure 8A that PBA levels rise more slowly and are lower at most time-points in PND 10 pups than in the other age-groups given 10 mg DLT/kg po. This is reflected in the youngest animals' relatively low AUC_{PBA}:AUC_{DLT} ratios for 0 – 6 h (Fig. 8B). The other age-group PBA time-courses are difficult to discern from one another in Figure 8A. PBA was detectable only at 6 h in the PND 10 animals. The PND 21 AUC_{PBA}:AUC_{DLT} ratio is somewhat lower than that for the adolescents (PND 40) and young adults (PND 90) gavaged with 2 mg DLT/kg (Fig. 8D). Interpretation of these PBA data should be tempered by the knowledge that the magnitude of CYP-mediated oxidation of DLT exceeds its CaE-mediated hydrolysis to PBA. Hydroxylated metabolites of DLT are not available commercially for use as analytical standards.

DISCUSSION

There is little, if any, information available on the TK of DLT or other synthetic pyrethroids in infants and children, despite their frequent exposures to this relatively new class of insecticide. The current report is, to our knowledge, the first to provide a comprehensive description of the systemic uptake, tissue distribution and elimination of a pyrethroid in an immature animal model. Kim *et al.* (2008) recently utilized a similar

experimental protocol to characterize the TK of the same doses of DLT in young, male adult (90-day-old) S-D rats. Some of these PND 90 data are included in the current report to facilitate comparisons of absorption and disposition in mature and immature animals.

DLT was not as well absorbed from the immature GI tract as might be anticipated for a lipophilic compound. Plasma DLT levels rose more slowly in PND 10 than in more mature animals. Nevertheless, despite its relatively rapid absorption in young adults, oral bioavailability was only 18% (Kim *et al.*, 2008). It was not possible to calculate bioavailability in the younger rats, due to difficulty in giving pups iv injections. P-glycoprotein (P-gp) or other efflux transporters in enterocytes may oppose systemic uptake of DLT, though it is not clear whether pyrethroids are P-gp substrates.

Compilation of comprehensive plasma and tissue DLT time-course data in different age-groups made it possible to ascertain the influence of maturation on tissue dosimetry. Brain deposition is of key interest, as the CNS is the target of the neurotoxic parent compound. It is clear in Table 2 that brain C_{max} and AUC^{∞}_{0} values are substantially higher in PND 10 than in PND 40 or 90 groups given 2 mg DLT/kg. The PND 21 brain AUC^{∞}_{0} is also relatively large, despite a relatively modest plasma AUC^{∞}_{0}. This pattern was also manifest in most instances at the other two dosage levels (Tables 1 and 2). Salivation and tremors elicited by 2 mg/kg were severe in the preweanlings and moderate in the weanlings, but PND 40 and 90 group members were asymptomatic. Even the 10 mg/kg dose elicited only mild, transient symptoms and equivalent brain AUCs in these two groups. It can be concluded that target organ deposition and ensuing adverse effects of DLT were most pronounced in the least mature rats, but that 40-day-old rats achieved adult status in these respects.

Plasma DLT concentrations proved to be an unreliable index of brain concentrations over time following dosing. Plasma levels showed much more pronounced changes during the uptake and elimination phases than did brain levels. This can be clearly seen in Figure 5. As would be anticipated, plots of plasma versus brain

concentrations yielded low correlation coefficients. Patterns in plasma:brain concentration ratios revealed effects of time and dosage on distribution of DLT between the two compartments. The rapid rise in plasma levels in the first h post dosing resulted in relatively high plasma:brain ratios at the earlier time-points (Fig. 6). These ratios progressively diminished, due to the relatively rapid clearance from the bloodstream. The plasma:brain ratios typically increased with increase in DLT dosage for the first 6 h following ingestion. In most instances age had little apparent influence on the distribution of DLT between plasma and brain.

It was of particular interest to learn whether brain or plasma DLT concentrations were better correlated with acute neurotoxicity, in view of the dissimilar kinetics of the chemical in the two body compartments. Very high correlations of brain AUC^{6}_{0} values with severity of salivation and tremors during the initial 6 h post dosing were observed. This supports conclusions by other investigations that the parent compound is the putative neurotoxicant (Lawrence and Casida, 1982; Casida *et al.*, 1983; Rickard and Brodie, 1985). The latter researchers recorded levels of ^{14}C-DLT in different brain regions and in blood at the time of onset of clinical signs in adult rats injected ip. Their results showed a clear correlation between DLT levels and CNS effects, but it was not established which biological sample was the better index. As in the current investigation, Rickard and Brodie's (1985) blood:brain ratios were relatively high and decreased with time following dosing. We found lower correlation between plasma AUC^{6}_{0} and CNS effects than was the case for brain AUC^{6}_{0} in immature rats. These plasma level versus CNS effects r^2 values were somewhat lower than previously reported by Anand *et al.* (2006b) in young adult rats.

Information obtained to date suggests that the markedly higher brain DLT levels in rat pups are primarily the result of correspondingly high plasma levels, which in turn are attributable to inefficient metabolic inactivation of the parent compound. Unfortunately, very little is known about CNS uptake of DLT or other pyrethroids from the blood and vice versa. The relatively low brain concentrations suggest that the blood-brain

(BBB) may limit uptake of DLT despite its high degree of lipophilicity. Deposition of other very lipophilic compounds of relatively high molecular weight in the brain has also been reported to be quite limited. Diliberto *et al.* (1996) for example, found only 0.02 and 0.03% of 1 nmol iv and po doses of 2,3,7,8-tetrachloroadibenzo-*p*-dioxin (TCDD) in the brain of rats 3 days post exposure. Relatively low brain levels have similarly been described for PCBs (Lee *et al.*, 2002), DDT and a variety of related chlorinated hydrocarbons (Lakshmanan *et al.*, 1979). Lipid extracts from the brain of Inuit people in Greenland contained lower levels of 11 organochlorines and 14 PCB congeners than did lipid extracts from their liver, omental fat, and subcutaneous fat (Dewailly *et al.*, 1999). The brain's substantial phospholipid content may be a factor in limiting partitioning and accumulation of such highly lipophilic compounds in the CNS.

It might be anticipated that an immature BBB in pups may allow more DLT into the CNS, but PND 10 plasma:brain ratios were usually not significantly different from those in older animals. Nevertheless, the relatively slow uptake of pyrethroid into the brain enhanced plasma:brain ratios suggests there is a limiting uptake process of some type regardless of maturity. Immaturity of p-glycoprotein (P-gp) or other BBB efflux transporters could be a candidate for allowing accumulation of a disproportionately large amount of DLT in the pup brain. Two findings argue against this: (1) the absence of age-dependency in plasma:brain ratios; and (2) the increase, rather than decrease in plasma:brain ratios with increase in dose to potentially saturating concentrations. Higher doses would be expected to saturate efflux transporters, resulting in a decreased plasma:brain ratio. It has not yet been clearly established whether DLT or other pyrethroids are P-gp substrates. Plasma protein binding of pyrethroids could account for their relatively high circulating levels and limit availability for uptake into the brain and other tissues. Abu-Qare and Abou-Donia (2002) reported that permethrin did not bind significantly to human serum albumin. Cui *et al.* (2006), however, demonstrated that

cypermethrin binds strongly to bovine serum albumin and may bind to some extent to hemoglobin. Kim *et al.* (2008) reported that ~ 83% of DLT in blood of adult rats given 10 mg/kg was present in plasma.

Limited capacity to metabolically detoxify the neurotoxic parent compound appears to be primarily responsible for the markedly higher systemic/target organ levels and toxicity of DLT in preweanling and weanling rats. Plasma levels of PBA, a product of esterase-catalyzed hydrolysis, were relatively low in the 10-day-old pups during the 6-h monitoring period after ingestion of 10 mg/kg in the present study (Fig. 9A). PBA was detected at just one sampling time in PND 10 rats given 2 mg/kg (Fig. 9C). Anand *et al.* (2006b) clearly demonstrated that metabolism by hepatic microsomal CYPs and plasma CaEs *in vitro* progressively rose during maturation. Liver weight during development was also taken into account in calculation of liver intrinsic clearance. This metabolic parameter was very low at PND 10, but progressively increased to a substantially higher value by PND 90 (Table 6). Hepatic extraction ratios (Es) were calculated to learn whether metabolism or blood flow to the liver of young rats was the limiting factor. Table 6 showed an E of 0.02 for PND 10 rats; hence, it can reasonably be concluded that the youngest animals experienced elevated DLT levels due to inadequate metabolism. Under these conditions, clearance is capacity- (V_{max}) limited, not flow-limited. E rose steadily with age (Table 6), indicating maturation of the pertinent enzyme systems (e.g., CYPs and CaEs), though it did not exceed 0.8, indicating that even the young adults (E = 0.34) do not experience flow-limited kinetics. It is important to place *in vitro* intrinsic clearance into proper physiological context by considering chemical delivery to the liver and liver size (Lipscomb and Poet, 2008).

In summary, the systemic disposition of ingested DLT was characterized at several critical developmental stages in the rat (i.e., pup, weanling, adolescent and young adult). The most notable finding was the strikingly high DLT levels in plasma, brain and other tissues of the 10-day-old pups. Age-dependent differences were less pronounced, except in the brain, at weaning (PND 21). Inefficient DLT metabolism in the

liver and plasma was a major contributor to the elevated brain levels and neurotoxicity in the more immature subjects. This was confirmed by the finding of progressive increases in hepatic intrinsic clearance with age. Estimation of E also revealed age-dependent increases, though hepatic blood flow was not rate limiting in any age group. Immaturity of brain CYPs and esterases could also contribute to the high levels, though no data on this topic were found. Little is yet known about potential roles of the BBB and its influx or efflux transporters in regulating movement of DLT or other pyrethroids between the blood and CNS. Plasma:brain DLT ratios were time- and dose-dependent, but apparently not age-dependent. Plasma DLT levels did not reliably reflect brain levels over time. As anticipated, brain DLT levels were better correlated with acute CNS effects. Elevated exposure of the immature brain may prove to be of consequence for long-term as well as acute neurotoxicity, in view of reports of potential neurodevelopmental effects of pyrethroids (Shafer *et al.*, 2005; Nasuti *et al.*, 2007). When considering the relevance of the current findings to humans, it should be remembered that rodents are much less mature at birth, and may thus exhibit more pronounced differences in susceptibility from adults than do infants and children (NRC, 1993). The TK data in the present report are being used to develop a PBTK model for DLT in immature rats. It should be possible with a validated model to predict dosage adjustments necessary to achieve equivalent brain dosimetry and to assess whether the 3.16 TK component of the 10-fold child uncertainty factor is adequate for protection against DLT neurotoxicity.

FUNDING INFORMATION

This work was supported by the U.S Environmental Protection Agency (STAR Grant R830800). This does not signify that the contents necessarily reflect the views and policies of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

ACKNOWLEDGEMENT

The authors appreciate the skilled assistance of Ms. Libby Rice in preparation of this manuscript.

REFERENCES

Abu-Qare, A.W., and Abou-Donia, M.B. (2002). Binding of pyridostigmine bromide N,N-diethyl-m-toluamide and permethrin, alone and in combinations, to human serum albumin. *Arch. Toxicol.* **76**, 203-208.

Adams, J., Barone, S., Jr., LaMantia, A., Philen, R., Rice, D.E., Spear, L., and Susser, E. (2000). Workshop to identify critical windows of exposure for children's health: Neurobehavioral work group summary. *Environ. Health Perspect.* **108**, 535-544.

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

Anand, S.S., Bruckner, J.V., Haines, W.T., Muralidhara, S., Fisher, J.W., and Padilla, S. (2006b). Characterization of deltamethrin metabolism by rat plasma and liver microsomes. *Toxicol. Appl. Pharmacol.* **212**, 156-166.

Berkowitz, G.A., Obel, J., Deych, E., Lapinski, R., Godbold, J., Liu, Z., Landrigan, P.J., and Wolff, M.S. (2003). Exposure to indoor pesticides during pregnancy in a multiethnic, urban cohort. *Environ. Health Perspect.* **111**, 79-84.

Bruckner, J.V. (2000) Differences in the sensitivity of children and adults to chemical toxicity: The NAS panel report. *Regul. Toxicol. Pharmacol.* **31**, 280-285.

Casida, J.E., Gammon, D.W., Glickman, A.H., and Lawrence, L.J. (1983). Mechanisms of selective action of pyrethroid insecticides. *Ann. Rev. Pharmacol. Toxicol.* **23**, 413-438.

Choi, J.-S., and Soderlund, D.M. (2006). Structure-activity relationships for the action of 11 pyrethroid insecticides on rat Na v 1.8 sodium channels expressed in *Xenopus* oocytes. *Toxicol. Appl. Pharmacol.* **211**, 233-244.

Cui, Y., Guo, J., Xu, B., and Chen, Z. (2006). Binding of chlorpyrifos and cypermethrin to blood proteins. *Pest. Biochem. Physiol.* **85**, 110-114.

Delp, M.D., Manning, R.O., Bruckner, J.V., and Armstrong, R.B. (1991). Distribution of cardiac output during diurnal changes in activity in rats. *Am. J. Physiol.* **261**, H1487-H1493.

Dewailly, E., Mulvad, G., Pederson, H.S., Ayotte, P., Demers, A., Weber, J.P., and Hansen, J.C. (1999). Concentration of organochlorines in human brain, liver, and adipose tissue autopsy samples from Greenland. *Environ. Health Perspect.* **107**, 823-828.

Diliberto, J.J., Jackson, J.A., and Birnbaum, L.S. (1996). Comparison 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) disposition following pulmonary, oral, dermal, and parenteral exposures to rats. *Toxicol. Appl. Pharmacol.* **138**, 158-168.

Ding, Y., White, C.A., Muralidhara, S., Bruckner, J.V., and Bartlett, M.G. (2004). Determination of deltamethrin and its metabolite 3-phenoxybenzoic acid in male rat plasma by high performance liquid chromatography. *J. Chromatogr.* B **810**, 221-227.

Eskenazi, B., Bradman, A., and Castorina, R. (1999). Exposures of children to organophosphate pesticides and their potential adverse health effects. *Environ. Health Perspect.* **107 (Suppl 3)**, 409-419.

Gabrielsson, J., and Weiner, D. (2000). Pharmacokinetics and Pharmacodynamics Data Analysis: Concepts and Applications, 3rd ed., Swedish Pharmaceutical Press, Stockholm, Sweden.

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

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

Kim, K.-B., Bartlett, M.G., Anand, S.S., Bruckner, J.V., and Kim, H.J. (2006). Rapid determination of the synthetic pyrethroid insecticide, deltamethrin, in rat plasma and tissues by HPLC. *J. Chromatogr.* B **834**, 141-148.

Lakshmanan, F.L., Pommer, A., and Patterson, O. (1979). Chlorinated hydrocarbon insecticide residues in tissues of rats before and after reduction of body fat by dieting restriction. *J. Agric. Food Chem.* **27**, 720-725.

Lawrence, L.J., and Casida, J.E. (1982). Pyrethroid toxicology: Mouse intracerebral structure-toxicity relationships. *Pestic. Biochem. Physiol.* **18**, 9-14.

Lee, S.K., Ou, Y.C., and Yang, R.S.H. (2002). Comparison of pharmacokinetic interactions and physiologically based pharmacokinetic modeling of PCB 153 and PCB 126 in nonpregnant mice, lactating mice, and suckling pups. *Toxicol. Sci.* **65**, 26-34.

Lipscomb, J.C., and Poet, T.S. (2008). In vitro measurements to metabolism for application in pharmacokinetic modeling. *Pharmacol. Therap.* **118**, 82-103.

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

Mirfazaelian, A., Kim, K.-B., Anand, S.S., Kim, H.J., Tornero-Velez, R., Bruckner, J.V., and Fisher, J.W. (2006). Development of a physiologically based pharmacokinetic model for deltamethrin in the adult male Sprague-Dawley rat. *Toxicol. Sci.* **93**, 432-442.

Mirfazaelian, A., Kim, K.-B., Lee, S., Kim, H.J., Bruckner, J.V., and Fisher, J.W. (2007). Organ growth functions in maturing male Sprague-Dawley rats. *J. Toxicol. Environ. Health Part A* **70**, 429-438.

Morgan, M.K. Sheldon, L.S., Croghan, C.W., Jones, P.A., Chuang, J.C., and Wilson, N.K. (2007). An observational study of 127 preschool children at their homes and daycare centers in Ohio: Environmental pathways to *cis*- and *trans*-permethrin. *Environ. Res.* **104**, 266-274.

Nasuti, C., Gabbianelli, R., Falcioni, M.L., Di Stefano, A., Sozio, P., and Cantalamessa, F. (2007). Dopaminergic system modulation, behavioral changes, and oxidative stress after neonatal administration of pyrethroids. *Toxicology* **229**, 194-205.

(NRC) National Research Council (1993). *Pesticides in the Diets of Infants and Children*. Ch. 3, National Academies Press, Washington, DC.

Renwick, A.G., Dorne, J.L., and Walton, K. (2000). An analysis of the need for an additional uncertainty factor for infants and children. *Regul. Pharmacol. Toxicol.* **31**, 286-296.

Rice, D., and Barone, S., Jr. (2000). Critical periods of vulnerability for the developing nervous system. *Environ. Health Perspect.* **108** (Suppl 3): 511-533.

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

Samsam, T.E., Hunter, D.L., and Bushnell, P.J. (2005). Effects of chronic dietary and repeated acute exposure to chlorpyrifos on learning and sustained attention in rats. *Toxicol. Sci.* **87**, 460-468.

Shafer, T.J., Meyer, D.A., and Crofton, K.M. (2005). Developmental neurotoxicity of pyrethroid insecticides: Critical review and future research needs. *Environ. Health Perspect.* **113**, 123-136.

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

Slotkin, T.A. (2004). Guidelines for developmental neurotoxicity and their impact on organophosphate pesticides: A personal view from an academic perspective. *Neurotoxicology* **25**, 631-640.

Soderlund, D.M., Clark, J.M., Sheets, L.P., Mullin, L.S., Piccirillo, V.J., Sargent, D., Stevens, J.T., and Weiner, M.L. (2002). Mechanisms of pyrethroid neurotoxicity: Implications for cumulative risk assessment. *Toxicology* **171**, 3-59.

Sudakin, D.L. (2006). Pyrethroid insecticides: Advances and challenges in biomonitoring. *Clin. Toxicol.* **44**, 31-37.

Timchalk, C., Poet, T.S., and Kousba, A.A. (2006). Age-dependent pharmacokinetic and pharmacodynamic response in preweanling rats following oral exposure to the organophosphorus insecticide chlorpyrifos. *Toxicology* **220**, 13-25.

Tulve, N.S., Jones, P.A., Nishioka, M.G., Fortmann, R.C., Croghan, C.W., Zhou, J.Y., Fraser, A., Cavel, C., and Friedman, W. (2006). Pesticide measurements from the first national environmental health survey of child care centers using a multi-residue GC/MS analysis method. *Environ. Sci. Technol.* **40**, 6269-6274.

Whyatt, R.M., Camann, D.E., Kinney, P.L., Reyes, A., Ramirez, J., Dietrich J., Diaz, D., Holmes, D., and Perera, F.P. (2002). Residential pesticide use during pregnancy among a cohort of urban minority women. *Environ. Health Perspect.* **110**, 507-514.

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

TABLE 1

Plasma & Liver DLT Toxicokinetic Parameter Estimates in Orally-Dosed PND 10, 21, 40 & 90 Rats

	Age (PND)	Dosage (mg/kg)	C_{max} (μg/ml)	T_{max} (h)	Half-life (h)	AUC_0^6 (μg·h/ml)	AUC_0^∞ (μg·h/ml)
Plasma	10	0.4	0.11 ± 0.04^a	2 – 6	20.1	0.5	1.7
		2	$0.92 \pm 0.19^{b*}$	2 – 6	15.6	4.0	18.3
		10	$5.90 \pm 1.36^{c*}$	2	NA	21.0	24.2
	21	0.4	0.15 ± 0.07^a	1	NA	0.5	0.7
		2	0.49 ± 0.12^b	1	17.7	1.9	2.9
		10	1.89 ± 0.36^c	1	NA	7.4	12.1
	40	0.4	0.13 ± 0.07^a	1	NA	0.3	0.3
		2	0.50 ± 0.18^b	1	NA	1.5	2.0
		10	1.63 ± 0.57^c	1	8.9	4.3	4.9
	90	0.4	0.12 ± 0.01^a	1	NA	ND	NA
		2	0.53 ± 0.14^b	1	15.8	ND	2.7
		10	1.22 ± 0.36^c	2	10.7	ND	6.5
Liver	10	0.4	$0.32 \pm 0.02^{a*}$	2	28.7	1.3	4.7
		2	$0.65 \pm 0.15^{b**}$	2	25.6	2.6	19.7
		10	$2.66 \pm 0.42^{c*}$	2 – 6	NA	11.8	32.3
	21	0.4	0.17 ± 0.03^a	2	NA	0.7	0.8
		2	$0.44 \pm 0.07^{b*}$	2	23.9	1.9	2.7
		10	$0.64 \pm 0.06^{c*}$	1	NA	2.9	11.2
	40	0.4	0.15 ± 0.04^a	1	NA	0.5	0.8
		2	0.25 ± 0.01^b	1	20.5	1.0	3.1
		10	0.64 ± 0.10^c	1	22.4	1.8	4.4
	90	0.4	0.21 ± 0.03^a	1	NA	ND	NA
		2	0.51 ± 0.12^b	1	31.0	ND	3.5
		10	1.02 ± 0.37^c	1	32.4	ND	10.0

C_{max} values are means ± SE. All others are means. n= 3 – 5

NA – Sufficient time-course data not available to estimate parameter due lethality or lack of DLT detection.

ND – Not determined.

C_{max} group means for each age-group with different letters are significantly different from one another at $p \leq 0.05$.

* Significantly different from PND 21, 40 and 90 values at the same dosage ($p \leq 0.05$)

** Significantly different from PND 40 value at the same dosage ($p \leq 0.05$).

Values for PND 90 rats are from Kim *et al.* (2008).

Table 2

Brain DLT Toxicokinetic Parameter Estimates in Orally-Dosed PND 10, 21, 40 and 90 Rats

	Age (PND)	Dosage (mg/kg)	C_{max} (µg/ml)	T_{max} (h)	Half-life (h)	AUC_0^6 (µg·h/ml)	AUC_0^∞ (µg·h/ml)
Brain	10	0.4	0.04 ± 0.02^a	2 – 6	28.3	0.2	0.8
		2	0.11 ± 0.04^a	2 – 6	22.5	0.5	3.6
		10	$0.38 \pm 0.12^{b*}$	2 – 6	NA	1.5	NA
	21	0.4	0.05 ± 0.02^a	2	28.9	0.2	0.5
		2	0.06 ± 0.03^a	2	32.6	0.3	3.0
		10	0.25 ± 0.04^b	4 – 6	NA	1.0	NA
	40	0.4	0.02 ± 0.01^a	2	NA	0.1	0.2
		2	0.05 ± 0.01^a	2	23.5	0.2	0.9
		10	0.14 ± 0.07^b	4	22.9	0.6	2.5
	90	0.4	0.02 ± 0.00^a	2	NA	ND	NA
		2	0.04 ± 0.01^a	1	12.5	ND	0.8
		10	0.18 ± 0.02^b	2	19.5	ND	2.2

C_{max} values are means ± SE. All others are means. n = 3 – 5

NA – Sufficient time-course data not available to estimate parameter due lethality or lack of DLT detection.

ND – Not determined.

C_{max} group means for each age-group with different letters are significantly different from one another at $p \leq 0.05$.

Significantly different from PND 40 and 90 values at the same dosage ($p \leq 0.05$).

Values for PND 90 rats are from Kim *et al.* (2008).

TABLE 3

Age-dependent DLT Disposition in Rat Plasma and Tissues 6 and 24 h After 0.4 mg DLT/kg po

Age (days)	Tissue	% Body Weight[a]	6 h DLT Conc.[b] (μg/ml or g)	6 h Total Tissue Burden[c] (μg)	6 h Measured Body Burden[d] (%)	24 h DLT Conc.[b] (μg/ml or g)	24 h Total Tissue Burden[c] (μg)	24 h Measured Body Burden[d] (%)
10	Plasma	4.6	0.11 ± 0.04	0.12	5.4	0.02 ± 0.01	0.02	0.7
	Brain	4.1	0.04 ± 0.02	0.04	1.8	0.01 ± 0.01	0.01	0.3
	Muscle	33.1	0.15 ± 0.04	1.13	50.6	0.20 ± 0.09	1.50	49.0
	Fat	7.5	0.48 ± 0.15	0.82	36.8	0.88 ± 0.22	1.50	49.0
	Liver	2.6	0.20 ± 0.05	0.12	5.3	0.05 ± 0.04	0.03	1.0
	Total	51.9		2.23	100.0		3.06	100.0
21	Plasma	5.8	0.04 ± 0.02	0.10	3.2	ND	-	-
	Brain	2.6	0.03 ± 0.01	0.03	1.0	0.01 ± 0.00	0.01	-
	Muscle	41.0	0.10 ± 0.02	1.78	57.8	0.07 ± 0.03	1.25	-
	Fat	8.1	0.31 ± 0.06	1.09	35.4	0.20 ± 0.04	0.7	-
	Liver	3.5	0.05 ± 0.01	0.08	2.6	ND	-	-
	Total	61.0		3.08	100.0			
40	Plasma	4.2	0.01 ± 0.01	0.08	1.2	NA	-	-
	Brain	1.0	0.01 ± 0.01	0.02	0.3	NA	-	-
	Muscle	42.8	0.05 ± 0.02	4.64	59.2	NA	-	-
	Fat	5.8	0.22 ± 0.05	2.41	35.3	NA	-	-
	Liver	4.8	0.03 ± 0.01	0.27	3.9	NA	-	-
	Total	50.6		6.82	100.0			

ND – Not detected; NA – Not available, as no sample collected. Tissue DLT concentrations are means ± SE. n = 3 – 5

[a]Each age-group's tissue % bw values were obtained from Brown et al. (1997) and Mirfazaelian et al. (2007).

[b]Concentrations are corrected for tissue-specific % recovery determined by Kim et al. (2006).

[c]Total tissue burdens were calculated by multiplying each tissue's % bw X mean bw (22.7, 53.5 & 189.0 g for PND 10, 21 & 40 rats) X the mean tissue DLT conc.

[d]Disposition is also expressed as % of the body burden actually measured.

TABLE 4

Age-dependent DLT Disposition in Plasma and Tissues 6 and 24 h After 2 mg DLT/kg po

Age (days)	Tissue	% Body Weight[a]	6 h DLT Conc.[b] *(μg/ml or g)*	6 h Total Tissue Burden[c] *(μg)*	6 h Measured % Body Burden[d] (%)	24 h DLT Conc.[b] *(μg/ml or g)*	24 h Total Tissue Burden[c] *(μg)*	24 h Measured Body Burden[d] (%)
10	Plasma	4.6	0.92 ± 0.19	0.96	15.4	0.08 ± 0.01	0.08	0.5
	Brain	4.1	0.11 ± 0.04	0.10	1.6	0.04 ± 0.01	0.04	0.3
	Muscle	33.1	0.30 ± 0.15	2.25	36.0	0.38 ± 0.14	2.86	19.4
	Fat	7.5	1.56 ± 0.61	2.66	42.5	6.91 ± 4.41	11.76	79.4
	Liver	2.6	0.47 ± 0.19	0.28	4.5	0.11 ± 0.04	0.06	0.4
	Total	51.9		6.25	100.0		14.80	100.0
21	Plasma	5.8	0.17 ± 0.12	0.43	6.2	0.01 ± 0.00	0.03	0.5
	Brain	2.6	0.06 ± 0.03	0.07	1.0	0.03 ± 0.01	0.03	0.5
	Muscle	41.0	0.16 ± 0.05	2.85	40.8	0.12 ± 0.02	2.14	38.5
	Fat	8.1	0.95 ± 0.19	3.35	48.1	0.95 ± 0.18	3.35	60.0
	Liver	3.5	0.18 ± 0.07	0.27	3.9	0.02 ± 0.01	0.03	0.5
	Total	61.0		6.97	100.0		5.58	100.0
40	Plasma	4.2	0.07 ± 0.03	0.56	3.2	0.01 ± 0.00	0.08	0.8
	Brain	1.0	0.02 ± 0.01	0.04	0.2	0.01 ± 0.00	0.02	0.2
	Muscle	42.8	0.14 ± 0.04	11.32	64.2	0.04 ± 0.01	3.24	33.5
	Fat	5.8	0.48 ± 0.12	5.26	29.8	0.57 ± 0.18	6.25	63.7
	Liver	4.8	0.05 ± 0.02	0.45	2.6	0.02 ± 0.01	0.18	1.8
	Total	50.6		17.63	100.0		9.97	100.0

Tissue DLT concentrations are means ± SE. n = 3 – 5

[a]Each age-group's tissue % bw values were obtained from Brown et al. (1997) and Mirfazaelian et al. (2007).

[b]Concentrations are corrected for tissue-specific % recovery determined by Kim et al. (2006).

[c]Total tissue burdens were calculated by multiplying each tissue's % bw X mean bw (22.7, 53.5 & 189.0 g for PND 10, 21 & 40 rats) X the mean tissue DLT conc.

[d]Disposition is also expressed as % of the body burden actually measured.

TABLE 5

Age-dependent DLT Disposition in Plasma and Tissues 6 and 24 h After 10 mg DLT/kg po

Age (days)	Tissue	% Body Weight[a]	6 h DLT Conc.[b] *(μg/ml or g)*	6 h Total Tissue Burden[c] *(μg)*	6 h Measured Body Burden[d] (%)	24 h DLT Conc.[b] *(μg/ml or g)*	24 h Total Tissue Burden[c] *(μg)*	24 h Measured Body Burden[d] (%)
10	Plasma	4.6	4.44 ± 1.03	4.64	13.6	NA	-	-
	Brain	4.1	0.38 ± 0.12	0.35	1.0	NA	-	-
	Muscle	33.1	1.71 ± 0.74	12.85	37.6	NA	-	-
	Fat	7.5	8.66 ± 5.00	14.74	43.2	NA	-	-
	Liver	2.6	2.66 ± 1.04	1.57	4.6	NA	-	-
	Total	51.9	-	34.15	100.0	-	-	-
21	Plasma	5.8	1.46 ± 0.25	3.68	9.2	NA	-	-
	Brain	2.6	0.25 ± 0.04	0.28	0.7	NA	-	-
	Muscle	41.0	1.27 ± 0.22	22.65	56.6	NA	-	-
	Fat	8.1	3.61 ± 0.16	12.72	31.8	NA	-	-
	Liver	3.5	0.46 ± 0.10	0.70	1.7	NA	-	-
	Total	61.0	-	40.03	100.0	-	-	-
40	Plasma	4.2	0.22 ± 0.09	1.75	3.4	0.04 ± 0.02	0.32	0.8
	Brain	1.0	0.09 ± 0.03	0.17	0.3	0.02 ± 0.01	0.04	0.1
	Muscle	42.8	0.26 ± 0.05	21.03	40.4	0.09 ± 0.03	7.28	17.2
	Fat	5.8	2.57 ± 1.47	28.17	54.0	3.15 ± 0.62	34.53	81.3
	Liver	4.8	0.11 ± 0.01	1.00	1.9	0.03 ± 0.01	0.27	0.6
	Total	50.6	-	6.82	100.0	-	42.44	100.0

NA – Not available, as no sample collected. Tissue DLT concentrations are means ± SE. n = 3 – 5

[a]Each age-group's tissue % bw values were obtained from Brown et al. (1997) and Mirfazaelian et al. (2007).

[b]Concentrations are corrected for tissue-specific % recovery determined by Kim et al. (2006).

[c]Total tissue burdens were calculated by multiplying each tissue's % bw X mean bw (22.7, 53.5 & 189.0 g for PND 10, 21 & 40 rats) X the mean tissue DLT conc.

[d]Disposition is also expressed as % of the body burden actually measured.

TABLE 6

Age- and Pathway-Dependent Intrinsic Clearance

Age (PND)	Liver Weight[a] (g)	Hepatic CYP[b]			Hepatic Esterase[b]			Liver Intrinsic Clearance[c] (ml/h)	Liver Blood Flow[d] (ml/h)	Liver Extraction Ratio[e] (0-1)
		Vmax (nmol/h) per g liver	Km (nmol/ml)	Intrinsic Clearance (ml/h)	Vmax (nmol/h) per g liver	Km (nmol/ml)	Intrinsic Clearance (ml/h)			
10	0.55	185.30	37.79	2.7	23.78	73.69	0.18	2.87	125.6	0.02
21	2.08	381.33	23.40	33.9	114.25	74.76	3.2	37.1	282.9	0.11
40	6.98	1231.73	34.15	224	296.65	118.78	17.4	269.2	588.4	0.21
90	13.6	2611.30	74.90	474	1981.80	172.5	156.3	630.4	1093	0.34

[a] Liver weights were are from Mirfazaelian et al. (2007).
[b] Metabolic rate constants are from Anand et al. (2006a). Intrinsic clearance is computed on a whole liver basis.
[c] Total hepatic intrinsic clearance (Cl_h) is sum of CYP and CaE intrinsic clearance.
[d] Liver blood flows (Q_h) were estimated as a fixed fraction (17%) of cardiac flow (QC) (Delp et al., 1991). The QC (L/h) was based on body weight (kg): 14.1 x $kg^{0.75}$ (Delp et al., 1991). Body weights (g) were 19.6, 57.9, 153.7, and 351, respectively, for PND10, 21, 40, and 90 rats.
[e] Calculated according to Gabrielsson and Weiner (2000).

FIGURE LEGENDS

Figure 1. Metabolic inactivation of deltamethrin (DLT). DLT is detoxified by cytochrome P450(CYP450)-mediated hydroxylation (dotted arrows) and carboxylesterase-catalyzed hydrolysis (solid arrows), followed by conjugation with glucuronide and sulfate (dashed arrows).

Figure 2. DLT uptake and elimination profiles of male postnatal day (PND) 10 (□), 21 (▲) 40 (○) and 90 (♦) rats gavaged with 0.4 mg DLT/kg in glycerol formal (GF). Serial plasma, whole brain, skeletal muscle, liver and fat samples were analyzed for their DLT content by HPLC and expressed as µg DLT/ml on the Y axes. Time (h) post dosing is shown on the X axes. Symbols represent mean ± SE for groups of 3 – 5 rats.

Figure 3. DLT uptake and elimination profiles of male PND 10 (□), 21 (▲), 40 (○) and 90 (♦) rats gavaged with 2 mg DLT/kg in GF. Serial plasma, whole brain, skeletal muscle, liver and fat samples were analyzed for their DLT content by HPLC and expressed as µg DLT/ml on the Y axes. Time (h) dosing is shown on the X axes. Symbols represent mean ± SE for groups of 3 – 5 rats.

Figure 4. DLT uptake and elimination profiles of male PND 10 (□), 21 (▲), 40 (○) and 90 (♦) rats gavaged with 10 mg DLT/kg in GF. Serial plasma, whole brain, fat, liver and skeletal muscle samples were analyzed for their DLT content by HPLC and expressed as µg DLT/ml on the Y axes. Time (h) post dosing is shown on the X axes. Symbols represent mean ± SE for groups of 3 – 5 rats.

Figure 5. Plasma, liver and brain DLT concentrations over time in PND 40 rats gavaged with 0.4 (A), 2 (B) or 10 (C) mg DLT/kg. Symbols represents mean ±SE for groups of 3 – 5 rats. SEs are too small to be apparent for some data points.

Figure 6. Plasma:brain DLT concentration ratios presented as a function of age and time after oral administration of (A) 0.4, (B) 2 or (C) 10 mg DLT/kg. Bar heights represent mean values for groups of 2 – 5 rats. Group values with different letters are significantly different from one another at $p \leq 0.05$.

Figure 7. Correlation of magnitude of (A) brain dose (AUC^{6}_{0}) and (B) blood dose (AUC^{6}_{0}) with magnitude of DLT-induced salivation and tremors in orally-dosed PND 10, 21, 40 and 90 rats.

Figure 8. Time-course of 3-phenoxybenzoic acid (PBA) in PND 10, 21, 40 and 90 rats gavaged with (A) 10 mg DLT/kg or (C) 2 mg DLT/kg. $(AUC_{PBA})^{6}_{0}$:$(AUC_{DLT})^{6}_{0}$ ratios for each age-group are presented as bar graphs for the 10 and 2 mg/kg doses in (B) and (D), respectively.