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The effects of dose and route on the toxicokinetics and disposition of 1-butyl-3-methylimidazolium chloride (Bmim-Cl) in male F-344 rats and female B6C3F1 mice

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Running Title: The Disposition of 1-butyl-3-methylimidazolium chloride in rats and mice

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$AUC_{[0-\infty]}$	area under the blood concentration–time curve from time zero to infinity
Bmim-Cl	1-butyl-3-methylimidazolium chloride
CL_b	clearance from whole blood
DMF	dimethylformamide
HPLC	high pressure liquid chromatography
IL, ILs	ionic liquid(s)
JVC	jugular vein cannula
LD_{50}	lethal dose for 50 percent of the population
LOD	limit of detection
LOQ	limit of quantification
MS	mass spectrometry
m/z	mass to charge ratio
OCT	organic cation transporters
$t_{1/2\alpha}$	distribution half-life
$t_{1/2\beta}$	elimination half-life
V_{SS}	volume of distribution at steady-state

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ABSTRACT

These studies characterize the effect of dose and route of administration on the disposition and elimination of the ionic liquid, 1-butyl-3-methylimidazolium chloride (Bmim-Cl). Following IV (5 mg/kg) or oral (50 mg/kg) administration to male F-344 rats [^{14}C] Bmim-Cl detected in blood decreased rapidly. Clearance rates from the blood following IV and oral administration were similar (7.4 and 11.9 mL/min, respectively). Systemic bioavailability was determined to be 62.1% of a 50 mg/kg dose in rats. Urinary excretion of parent compound by rats was the major route of elimination (IV: 91% in 24 h; oral: 55-74% in 24 h). The rates and routes of elimination were not affected by escalation of dose (0.5-50 mg/kg) or repeated oral administration (5 daily administrations, 50 mg/kg) and were similar in male rats and B6C3F1 female mice (86-95% of dose eliminated in 24 h). Apparent systemic exposure to Bmim-Cl following dermal administration was dependent upon vehicle, as assessed by the percent of dose eliminated in urine after application in a particular vehicle (water: 1%, ethanol/water: 3%, dimethylformamide/water: 13% of dose). Regardless of gender, species, dose, route, or number of exposures, HPLC-UV/Vis-radiometric analyses of urine samples showed a single peak that co-eluted with the Bmim-Cl standard. These studies illustrate that systemic bioavailability of Bmim-Cl is high, tissue disposition and metabolism are negligible, and absorbed compound is extensively extracted by the kidney and eliminated in the urine as parent compound.

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INTRODUCTION

Ionic liquids (ILs) comprise a class of organic salts that are distinguished by a range of useful properties such as negligible vapor pressure, thermal stability, non-flammability, high ionic conductivity and variable solubility properties (Welton, 1999). The structural characteristics of ILs feature an organic ammonium cation and an exchangeable anion. This anion can range from a simple halide to complex polyatomic ions (Sheldon, 2001). Commonly used ILs are based on imidazolium, pyridinium or pyrrolidinium cations, a number of which are commercially available (Freemantle, 2003). Because they are generally non-volatile and stable at standard temperature and pressure, ILs may supplant or reduce the reliance on classical volatile organic solvents currently employed in industrial chemistry (Integrated Laboratory Systems, 2003).

Room temperature ionic liquids possess a number of physical and chemical properties that explain their potential for industrial and laboratory applications. There is growing interest in developing cleaner technologies across the chemical industry, for both economical and environmental reasons, with the search for alternatives to conventional solvents being foremost (Seddon, 1997). A major reason is that while organic solvents are used in massive quantities, their use is generally restricted to a single use, as the high vapor pressure of these solvents promotes loss into the environment as air pollutants. Low vapor pressures of ILs would result in limited evaporative and environmental losses (Huddleston *et al*, 1998). High volume use of solvents for large scale synthesis is a standard procedure for industry, but tailored physical properties for specific processes and reactions coupled with improved recapture and reuse of IL-based solvents could reduce these volumes and decrease waste (Marsh *et al*, 2002). Also, intrinsically low vapor pressures of ILs allow for use in

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high vacuum reaction systems without solvent loss. Ionic liquids can be designed for both organic and inorganic reactions, a unique property that could be exploited to bring particular chemicals together in the same phase. Furthermore, ILs can be adapted to be immiscible with particular solvents, resulting in non-aqueous alternatives for two phase reaction systems (Welton, 1999).

Rogers and Seddon (2003) noted that while some ILs are toxic, the theoretical structural versatility of ILs could result in the design of numerous nontoxic and chemically useful ILs. The environmental impact of these compounds has not yet been determined and little data are available on the mammalian toxicity, metabolism, and disposition of these chemicals. The studies reported here represent the first comprehensive studies on the absorption, distribution, metabolism and elimination of a model IL, 1-butyl-3-methylimidazolium chloride (Bmim-Cl). They describe the toxicokinetics of Bmim-Cl after IV or oral dosing, the extent of its systemic bioavailability after oral or dermal administration, and assess its metabolism. Studies were also designed to characterize the elimination profile after dermal application and repeated oral administration.

MATERIALS AND METHODS

CHEMICALS

[^{14}C]-labeled Bmim-Cl in water (Lot # 9719-75) was received from RTI International (Research Triangle Park, NC) and stored at 8°C. The [^{14}C] label was located on the α-carbon of the butyl side chain, and is indicated by an asterisk (*) in Figure 1. An unlabeled Bmim-Cl reference standard (98% Bmim-Cl) was obtained from EMD Chemicals Inc. (Gibbstown, NJ, USA). The radiochemical purity of [^{14}C] Bmim-Cl was 97.5%, as determined by reversed-phase HPLC-radiometric detection. The specific

activity was reported to be 27.5 mCi/mmol. Soluene-350® and Solvable® tissue solubilization solvents and Pico-Flour 40 scintillation cocktail solution were obtained from Perkin Elmer (Torrance, Ca). Dimethylformamide (DMF) was obtained from J.T. Baker (Phillipsburg, NJ). Hydrogen peroxide (30%) was obtained from VWR. All reagents used in these experiments were HPLC or analytical grade.

ANIMAL STUDIES

Animals

Male Fischer-344 (F-344) rats (8-9 weeks of age; 161-200 g) without surgical alteration (conventional) or with in-dwelling jugular vein cannula (JVC) were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN). Female B6C3F1 mice (8-10 weeks old; 17-20 g) were obtained from Harlan Sprague Dawley, Inc (Indianapolis, IN). Animals were maintained in The University of Arizona Animal Care facility which is accredited by the Association for Assessment and Accreditation for Laboratory Animal Care. The animals were maintained in a temperature controlled (25°C) room under a 12 h light/12 h dark cycle. Conventional animals were acclimated for 5-7 days after receipt. Those animals with indwelling JVC were acclimated for only 1 day after arrival to ensure the patency of cannula. The rats and mice were allowed food and water *ad libitum* except as noted. Animals used in oral- and IV-dose administered disposition and toxicokinetic studies were fasted for 12 h before administration of Bmim-Cl. Food was returned 2 h after dosing. Animals used in the dermal and repeated oral administration studies were not fasted. Food (NTP 2000, Harlan Teklad; Madison, WI) was provided as a powder except to the animals in dermal administration studies. Food particles were separated from feces during collection in these studies.

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Dose selection

The selection of doses was based on the acute oral LD_{50} of 550 mg/kg bodyweight in female F-344 rats reported by Landry *et al* (2005). In the studies reported herein, sub-toxic doses of 50 mg/kg (0.1 x LD_{50}), 5 mg/kg (0.01 x LD_{50}) and 0.5 mg/kg (0.001 x LD_{50}) were chosen to assess the effect of dose on the rate and route of excretion following oral administration. For repeated doses studies 50 mg/kg was administered daily by oral gavage for 5 days. The dose of 5 mg/kg was selected for the IV route of administration. Landry *et al* (2005) reported that a dermal application of 2,000 mg/kg Bmim-Cl (95% in water) was not toxic to F-344 rats but did induce dermal irritation at the site of application. However, 2,000 mg/kg Bmim-Cl (75% in DMF) was toxic to B6C3F1 mice. A preliminary dermal study in rats that utilized a 50 mg/kg dose of [^{14}C] Bmim-Cl (1,900 µg/cm^2 in methanol/water (1.8:1)) also resulted in irritation at the site of application; therefore, a dose of 5 mg/kg, (190 µg/cm^2) was used for the dermal application studies. All doses provided 50 µCi/kg [^{14}C] Bmim-Cl unless otherwise indicated.

Sample collection and preparation

Following dosing, the animals were maintained in Nalgene® metabolism cages for collection of urine and feces. In single dose studies, urine was collected at 6, 12, 24, 36, 48 and 72 h; feces were collected at 12, 24, 36, 48, and 72 h. In the repeated dose studies, urine was collected at 6, 12, and 24 h after each dose, while feces were collected at 12 and 24 h after each administration. The metabolism cages were rinsed with deionized water (approximately 15 mL) after the collection of urine. Radioactivity recovered in cage rinses was added to that determined for urine.

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Following collections, aliquots of urine and cage rinse (3 x 100 µL) were directly analyzed for [^{14}C] radioactivity content by LSC. In addition, 100 µL of urine was diluted with 100 µL water (1:1 ratio), vortexed, and filtered through a 0.45 µm Whatman® nylon syringe filter. The filtered sample was transferred into an insert in a HPLC vial for HPLC-UV/Vis-radiometric analysis. Samples of urine for LC/MS analysis was from animals that were administered non-radiolabeled Bmim-Cl. These samples were prepared using the same methods as for HPLC-UV/Vis-radiometric analysis.

Following collection, feces were mixed with water to form a homogeneous mixture. To determine total [^{14}C] equivalent content, fecal samples were solubilized with Soluene-350® (Thompson and Burns, 1996). In addition, aliquots of feces (250 mg) were extracted with 1.25 mL methanol (3x) and pooled. Pooled supernatants were evaporated to dryness and reconstituted in methanol. Reconstituted samples (250 µL) were transferred into an insert in a HPLC vial for HPLC-UV/Vis-radiometric analysis.

Following solubilization and addition of Pico-Fluor (15 mL), all samples were stored in the dark for 48 h to control for chemiluminescence and were corrected for background. Total radioactivity in all samples was determined by liquid scintillation counting (LSC).

At the terminus of each study, animals were euthanized by CO_2 inhalation. Blood was collected from the posterior vena cava and the animals subjected to necropsy. All collected samples (adipose, blood, cecum, cecum contents, heart, intestine, intestinal contents, kidneys, lung, liver, muscle, spleen, stomach, stomach contents, skin, and testes) were analyzed immediately or stored at -80^{o}C until analysis. Body composition estimates of 50% for muscle, 8% for blood, 11% for adipose tissue, and 16% for skin

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were used to estimate total masses of these tissues (Birnbaum *et al*, 1980). Triplicate aliquots from collected tissues (approx. 100 mg) were solubilized using Solvable® (Thomson and Burns, 1996).

Dermal application studies

Male F-344 rats were prepared for non-occluded dermal application of Bmim-Cl as described previously (Winter and Sipes, 1993). Rats were topically dosed with [^{14}C] Bmim-Cl (5 mg/kg, 100 µCi/kg, 400 µL/kg) in water, ethanol/water (1.8:1) or DMF/water (1.8:1). Additionally, Bmim-Cl (5 mg/kg in DMF/water [1.8:1]) was applied for five consecutive days to determine the irritancy potential of repeated dermal exposure to Bmim-Cl. Feces, urine, and cage rinses were collected and analyzed as described above. Animals dosed with aqueous vehicle were euthanized by CO_2 inhalation after 72 h, while animals dosed with [^{14}C] Bmim-Cl in DMF/water or ethanol/water were euthanized at 48 h post application. Skin from the application area was treated in accordance to the Organization for the Economic Cooperation and Development guideline (OCDE No. 427) for the testing of chemicals (OECD, 2004). Briefly, the skin was swabbed 5 times using filter paper soaked with a 10% soap solution then tape stripped 5 times using clear sticky-tape to maximize recovery of dose remaining on the surface of the skin. Skin and tape strips were solubilized using Soluene as described above and were analyzed by LSC. Skin washes were analyzed directly by LSC.

Blood toxicokinetics of Bmim-Cl

Dosing regimen

[^{14}C] Bmim-Cl (5 mg/kg, 50 µCi/kg, 1 mL/kg) was administered intravenously through the jugular vein cannula in saline for determination of IV blood kinetics. Blood (100 µL) was then drawn into the cannula, returned to the circulation and the cannula

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flushed with normal saline (1 mL/kg). For oral blood kinetic studies, [^{14}C] Bmim-Cl (50 mg/kg, 100 µCi/kg, 2 mL/kg), in saline was administered by oral gavage to JVC male F-344 rats (N=4).

For toxicokinetic studies, blood samples (300 µL) were collected via the JVC at 0.125, 0.25, 0.5, 0.75, 1, 1.5, 3, 6, 9, 12, 24 and 36 h into heparinized syringes. Aliquots of blood removed via the JVC were replaced with an equal volume of saline. The strategy for blood sampling was to reduce inter-animal variability by obtaining blood time points from the same animal, instead of obtaining time points from different animals. The decision to obtain 300 µL per time point was based on recommendations made in the report by Diehl *et al* (2001) where it was demonstrated that in short term toxicokinetic studies, the removal of 20% of the blood volume over 24 h produces minimal disturbance of normal physiological function. Aliquots of blood (2 x 50 µL) were solubilized for quantification of [^{14}C] radioactivity by LSC. For HPLC-radiometric analysis of [^{14}C] radioactivity in blood, aliquots of blood (150 µL) were mixed with acetonitrile (450 µL), vortexed and centrifuged. Extractions were performed 3 times. Supernatants from each sample were collected, pooled and the solvent was evaporated to dryness under vacuum (MiVac, Genevac, Valley Cottage, NY, USA). Extracts were reconstituted in 150 µL of water: acetonitrile mixture (93:7%, v/v) and placed in HPLC vials for analysis.

Toxicokinetic analyses

The blood concentration-time data following oral or IV administration were analyzed using a one- or two-compartment toxicokinetic model, respectively. A modeling program (WinNonlin Professional, Version 5.1, Pharsight Corp; Mountain View, CA) was utilized to fit the data using non-linear regression analyses, assuming

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first-order kinetics for all processes. The parameters of the model were used to calculate values for the area under the blood concentration–time curve from time zero to infinity ($AUC_{[0-\infty]}$), distribution half-life ($t_{1/2\alpha}$), terminal elimination half-life ($t_{½\beta}$), maximum concentration of Bmim-Cl in the blood (C_{max}), rate of clearance from blood (CL_b), and volume of distribution under steady-state conditions (V_{ss}). Only samples containing quantities of compound above the limit of quantification were used in toxicokinetic analyses. For this reason data points after 6 h were not used for toxicokinetic analyses. Kinetic parameters were not adjusted for sample loss due to blood withdrawal because total loss was only 0.05 and 0.19% for the oral and IV doses, respectively.

ANALYTICAL METHODS

HPLC analysis of Bmim-Cl in blood, urine and feces

Aliquots of the reconstituted samples (100 µL) were subjected to HPLC separation (Agilent Technologies, Palo Alto, CA, USA). The samples were injected onto a C18 guard column (Phenomenex SecurityGuard, 4.0 x 3.0 mm, AJO-4287) followed by a C18 reversed phase column (Phenomenex Luna Prodigy ODS3, 5 µm, 250 x 4.6 mm). The mobile phases consisted of acidified water (0.1 M trifluoroacetic acid) and acidified acetonitrile at a flow rate of 1 mL/min with a total run time of 35 min. The mobile phase gradient was run from 93% water and 7% acetonitrile for the first 8 min, then up to 9% acetonitrile over 7 min, then to 15% acetonitrile over 10 min and finally to 95% acetonitrile for the last 10 min. Mobile phase was brought back to initial conditions and the column allowed to re-equilibrate for 10 min between injections. Using this gradient, Bmim-Cl eluted at 18 min. The eluent from the HPLC was monitored with a diode array detector for Bmim-Cl (λ_{max} = 220 nm) and a flow-through beta ram detector for [^{14}C] radioactivity (IN-US, Tampa, FL). Fractions were collected at 1 min intervals

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using an Agilent 220 fraction sampler. Data were acquired with ChemStation for LC 3D, Rev B 01.01 [164] data acquisition software (Agilent Technologies). Calibration standards and quality control samples were prepared from concentrated stock solutions. The limit of detection (LOD) using HPLC-UV/Vis detection at 220 nm was 4.83 µg/mL and the limit of quantification (LOQ) was 14.63 µg/mL. The LOD and LOQ using LSC were 0.1 and 0.35 ng, respectively, as determined using the methods described by Zhu, *et al* (2005).

LC-MS analysis Bmim-Cl in urine

HPLC separation of urine samples was performed as described above. The HPLC system was coupled to MSD-Trap SL ion trap mass spectrometer (Agilent, Palo Alto, CA). Analytes were ionized using an electrospray ionization (ESI) source in the positive ion mode over a scan range of *m/z* = 50-1000. Samples were dried with nitrogen at a flow rate of 10 mL/min, drying temperature of 350°C, nebulizer pressure of 50 psi, capillary and the end plate currents were 17 nA and 650 nA respectively. MS/MS fragmentation was performed at *m/z* = 4.0 isolation width at 1 V fragmentation amplitude.

RESULTS

TOXICOKINETICS OF BMIM-CL

Blood data following IV administration of [^{14}C] Bmim-Cl (5 mg/kg) indicated that radiolabel was rapidly removed from the systemic circulation. HPLC-radiometric analysis of blood extracts showed a single radioactive peak that coeluted with Bmim-Cl (R_t = 17.1 min; Figure 2 (A) and (B)). This peak decreased with time after dosing and new peaks were not observed. As no evidence of metabolism was obtained, the blood toxicokinetics of Bmim-Cl were determined from total radioactivity detected in whole

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blood. The effect of time on the concentration of Bmim-Cl in blood was described by a biexponential equation consistent with a two-compartment model (Figure 3A). Toxicokinetic analysis showed that Bmim-Cl was rapidly ($t_{1/2\alpha}$ = 13 min) and widely distributed to the tissues (V_{ss} = 618.2 mL) and readily eliminated ($t_{1/2\beta}$ = 85.4 min). The $AUC_{[0-\infty]}$ and CL_b values were 141.3 µg*min/mL and 7.4 mL/min, respectively.

The effect of time on the blood concentration of [^{14}C] Bmim-Cl following oral administration [^{14}C] Bmim-Cl at (50 mg/kg, 100 µCi/kg) is shown in Figure 3(B). HPLC analysis of extracts showed a single peak that co-eluted with the [^{14}C] Bmim-Cl standard (Figure 2 (A) and (C)). Toxicokinetic analysis of Bmim-Cl present in blood revealed that it was readily absorbed following oral dosing; C_{max} (3.6 µg/mL) was reached 67 min after administration. Following C_{max}, [^{14}C] Bmim-Cl disappeared from the blood with a $t_{1/2\beta}$ of 77.2 min. Oral systemic bioavailability (*F*) was based on the ratio of $AUC_{[0-\infty]}$ calculated for oral administration (733.3 µg*min/mL) and $AUC_{[0-\infty]}$ calculated for IV administration (141.3 µg*min/mL) and adjusted for dose ($dose_{oral}$ = 8756 µg/animal; $dose_{IV}$ = 1047 µg/animal). *F* was determined to be 62.1%. A summary of toxicokinetic parameters is given in Table 1.

DISPOSITION AND ELIMINATION OF BMIM-CL

IV administered Bmim-Cl

The route of elimination of Bmim-Cl was almost exclusively urinary following IV administration (Table 2). HPLC-radiometric analyses of the urine detected a single radioactive peak that coeluted with the [^{14}C] Bmim-Cl standard. This single peak accounted for 96% of the radioactivity detected in the urine. The remaining 4% was due to contaminants present in the dosing solution. Within the first 12 h, 84±10% of the administered radioactivity was eliminated in the urine. An additional 7% was eliminated

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by this route in the next 12 h. By 48 h, 92% of the dose had been recovered in urine (Figure 4).

Orally administered Bmim-Cl

Following oral administration of [^{14}C] Bmim-Cl (50, 5 or 0.5 mg/kg) as a single oral bolus dose to male F-344 rats 72-77 % of the total administered radioactivity was eliminated in the urine over the 72 h collection period. Administration of [^{14}C] Bmim-Cl to female B6C3F1 mice (50 mg/kg) resulted in a similar elimination profile to that of male F-344 rats (Table 3). The more variable recoveries of radioactivity in murine studies were attributed to the probability of the mice urinating and defecating in the feed hoppers. This presumption was based on observations of feces found in the ground feed and notable amounts of time spent in the enclosures. The peak excretion for all doses and in both species occurred between 6-12 h after administration (Figure 5). The elimination of radioactivity in feces accounted for less than 30% of the administered dose in both species. Disposition of radioactivity in tissues following oral administration of Bmim-Cl was negligible. Regardless of species or dose level, routes of elimination were the same and recovery was >80% of the administered dose.

Urine was analyzed to ascertain the chemical identity of radioactivity present in the samples over time. Figure 6 shows the HPLC-radiometric profile of rat urine samples obtained 6 and 12 h after a single oral dose of 5 or 0.5 mg/kg [^{14}C] Bmim-Cl. In all samples, one major peak was detected which co-eluted with the Bmim-Cl standard (R_t =18 min). This peak accounted for greater than 96% of [^{14}C] radioactivity detected in urine following oral administration of [^{14}C] Bmim-Cl. Two minor peaks that were observed co-eluted with impurities (R_t = 4.4 and 5.4 min) present in the [^{14}C] Bmim-Cl dosing solution. These two peaks accounted for 3% of total [^{14}C] radioactivity detected

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in urine. Similar HPLC profiles were observed from urine obtained from rats dosed with 50 mg/kg (oral) or 5 mg IV and mice orally administered 50 mg/kg (data not shown).

Urine samples from rats dosed with unlabeled Bmim-Cl (50 mg/kg) were analyzed by LC-MS to confirm the identity of the major chemical present. Mass spectrometry confirmed the presence of the molecular mass of $Bmim^+$ (*m/z* = 139.2). Collision induced fragmentation of this ion resulted in the loss of 56 mass units (butyl group) and the formation of a single ion (*m/z* = 83.2). This mass corresponded to the 3-methylimidazolium ion (Figure 7).

Analysis of fecal extracts obtained from rats administered [^{14}C] Bmim-Cl orally (50 mg/kg, 12 hr time point) detected a number of peaks that did not co-elute with [^{14}C] Bmim-Cl. These peaks accounted for 10-20% of the administered dose. Further analysis of these were not attempted. A peak that co-eluted with the [^{14}C] Bmim-Cl standard accounted for 3-7% of the dose.

Following each daily oral dose of [^{14}C] Bmim-Cl to male F-344 rats, elimination of Bmim-Cl was rapid, with the majority of administered radioactivity being eliminated via the urine in each 24 h dosing period. The effect of repeated oral administrations on the excretion of Bmim-Cl is shown in Figure 8. Elimination patterns over sequential 24 h periods were similar; with 86-92% of each dose eliminated in a 24 h period. Of this 51-68% of the dose was eliminated via the urine per 24 h. No notable differences in elimination were observed between the group dosed with a single administration of [^{14}C] Bmim-Cl and the group receiving serial administrations. Summaries of radioactivity recovered from tissues are shown in Table 4.

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Topically applied Bmim-Cl

Following dermal application of [^{14}C] Bmim-Cl, (5 mg/kg; 190 µg/cm^2), small amounts of [^{14}C] Bmim-Cl were detected in the urine. By 48 h, 12% of the applied dose was found in the urine when it was applied in DMF/water (Figure 9). When Bmim-Cl was applied in water or ethanol/water, 1.1 and 2.4% of the applied dose was recovered in the urine, respectively. Excretion in feces accounted for 0.3-0.8% of the dose (Table 5). At termination, the majority (71-85%) of the applied dose remained on the skin surface. A major portion of this was removed by washing and tape stripping. However, 14-28% of the dose that was present at the site of application was not removed by the washing/stripping procedure. At the terminus of the experiments (48 or 72 h), Bmim-Cl was not detectable in the blood, liver and kidney. No gross abnormalities were noted at the site of application or in tissues that were analyzed. The dosing vehicles were non-irritating (data not shown).

No skin irritation or discomfort was observed at any time following application of this dose (5 mg/kg) to rats in any of the above mentioned vehicles. Also, when Bmim-Cl (5 mg/kg) was topically administered for five consecutive days, no irritation or discomfort was noted at the site of application. Hair growth at the site of application precluded further applications.

DISCUSSION

Because they possess low vapor pressures, ionic liquids have been referred to as “green solvents.” They are expected to decrease the contribution of industrial solvents to air pollution, particularly indoor air pollution, when compared to conventionally used volatile organic solvents. However, less polluting does not mean that ionic liquids may be without negative biological effect or hazard. In fact, some

studies have appeared which report ecological as well as mammalian toxicity of ionic liquids. Bernot and colleagues (2005a, b) showed non-lethal concentrations of a series of imidazolium and pyridinium-based ILs negatively impacted freshwater invertebrates, indicating potential hazards to exposed ecosystems and watersheds. Landry *et al* (2005) reported that topical exposure to Bmim-Cl (2,000 mg/kg) resulted in dermal irritation in rats and systemic toxicity in mice.

The results of the studies reported here describe the absorption, distribution, metabolism and excretion of a specific 1,3-dialkyl imidazolium cation, Bmim-Cl. Following oral administration to rats, Bmim-Cl was extensively absorbed into the systemic circulation with C_{max} achieved at 67 min. Comparison of the blood $AUC_{[0-\infty]}$ values obtained after oral administration to that obtained after IV administration resulted in an oral systemic bioavailability of 62% for male F-344 rats. This value closely approximates the percent of dose eliminated in the urine at 24 h after a single oral administration of Bmim-Cl as well as at 24 h after each of 5 repeated daily doses of Bmim-Cl. The volume of distribution following IV administration exceeded 600 mL, indicating that systemically available Bmim-Cl is widely distributed outside the plasma compartment. Therefore, recoveries of Bmim-Cl in the urine at later time points most likely represent elimination of Bmim-Cl that distributed to fluids associated with extra-vascular tissues. Recoveries of [^{14}C] radioactivity in the feces following oral administration ranged from 20-30%. This most likely represents elimination of unabsorbed Bmim-Cl rather than Bmim-Cl that was extracted by the liver and eliminated in the bile. This conclusion is supported by the results of the IV study, which indicated

that systemically available Bmim-Cl was almost exclusively eliminated in the urine as unchanged parent compound.

Parallel studies that utilized female B6C3F1 mice showed that apparent rates and route of elimination of Bmim-Cl by mice were similar to that seen in male F-344 rats. Slightly higher urinary recoveries were observed at 72 h following oral administration of a single dose of Bmim-Cl to male F-344 rats than female B6C3F1 mice. However, these differences in total recovery were not reflected in differences in disposition in tissues.

Based on toxicological data presented by Landry *et al* (2005), Bmim-Cl can be absorbed following dermal exposure. However, the extent of and rate of absorption is greatly influenced by vehicle used for application. Landry *et al* (2005) reported that high doses of Bmim-Cl caused severe systemic toxicity when it was applied in DMF, but not water. The results obtained in the dermal absorption studies reported here provide an explanation for those observations. Urine levels of Bmim-Cl recovered in 48 h were 10-fold higher when applied in DMF: water as compared to application of Bmim-Cl in water. Also, skin irritation, as a result of the large dose of Bmim-Cl applied topically by Landry *et al* (2005) could have promoted additional percutaneous absorption. Urinary levels of Bmim-Cl suggest that while only 10-14% of the dose was systemically available, an additional 16-22% remained in the skin after washing and tape-stripping the site of application. This remaining dose has the potential for subsequent systemic exposure. The ethanol: water and water vehicle groups showed a similar level of Bmim-Cl remaining at the site of application after washing and stripping. Additionally, the urinary recoveries of Bmim-Cl in the ethanol: water and water vehicle groups did not appear to

plateau, suggesting that extended exposure to Bmim-Cl in water based vehicles could lead to continued systemic exposure.

As indicated above, no evidence was obtained for the metabolism of systemically available Bmim-Cl. After oral or IV administration of Bmim-Cl, greater than 97% of [^{14}C] equivalents eliminated in the urine were associated with Bmim-Cl. HPLC-radiometric analysis of extracts of fecal samples from animals that had received an oral administration of Bmim-Cl provided some evidence of biotransformation. A number of small peaks that did not co-elute with parent or known contaminants were observed. The peaks may represent products of biotransformation of Bmim-Cl mediated by gut microflora. However, it is also possible that at the 10 fold higher oral dose of Bmim-Cl, the liver may form a spectrum of metabolites that are eliminated via the bile. While these metabolites (microflora and/or mammalian) have not been characterized, theoretical metabolic schemes for Bmim-Cl have been published (Jastorff *et al*, 2003; Stepnowski and Storoniak, 2005).

A number of small molecular weight organic cations are known to be substrates for organic cation transporters (OCT). OCTs promote both the intestinal uptake of such cations into the systemic circulation as well as facilitate their secretion in the nephron. The prototypical organic cations tetraethyl ammonium (TEA) and tributylmethyl ammonium (TBuMA) are actively transported across the intestinal mucosa by a sodium-independent cation transporter (OCT) system (Bowman *et al*, 1978; Kim *et al*, 2005; Koepsell *et al*, 1998). As Bmim-Cl has structural characteristics similar to these water soluble compounds, it is likely that its uptake from the intestine into the systemic circulation is mediated by these transporters present in the intestinal mucosa.

The estimated systemic clearance rate of Bmim-Cl determined in the studies reported here suggests that Bmim-Cl may also be secreted from the nephron by OCT. The estimated clearance rates of Bmim-Cl (CL_b = 7.4-11.9 mL/min) approached reported values for renal blood flow (12 mL/min; Brown *et al*, 1997; Corley *et al*, 2005). Since glomerular filtration is approximately equivalent to 12% of renal blood flow (Karon and Kramp, 1999; Shargel and Yu, 1992), glomerular filtration of Bmim-Cl cannot explain the efficient systemic clearance and rapid urinary elimination of Bmim-Cl. Based on its structural characteristics, rapid urinary excretion as parent compound, and high systemic clearance it is suggested that Bmim-Cl is a substrate for OCT and these transporters play a major role in the renal secretion of this and other ionic liquids.

In summary, following IV, oral or dermal administration, Bmim-Cl is rapidly cleared from the systemic circulation and is excreted unchanged in the urine. Efficient oral absorption suggests this highly water soluble compound may be a substrate for intestinal OCT. Similarly, the rapid clearance by the kidney suggests it may be transported and secreted by OCT present on the basolateral membrane of proximal tubule of the nephron. Studies are underway to assess the role of OCT in the disposition of Bmim-Cl and other structurally similar ionic liquids.

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FOOTNOTES

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LEGENDS FOR FIGURES

Figure 1: Chemical structure of 1-butyl-3-methylimidazolium chloride (* = [^{14}C] radiolabel)

Figure 2: Representative radio-chromatograms of extracts from whole blood samples obtained from male F-344 rats. (A) Control blood spiked with [^{14}C] Bmim-Cl; (B) blood sampled at 7.5 min after a single IV administration of Bmim-Cl (5 mg/kg); (C) blood sampled at 90 min after a single oral administration of Bmim-Cl (50 mg/kg). A single peak that co-eluted with the [^{14}C] Bmim-Cl standard (R_t = 17.1 min) was the only peak detected in all extracts from whole blood, independent of route of administration or time.

Figure 3: Time course of Bmim-Cl in blood following administration in male F-344 rats (N=4, mean ± S.D.). (A): IV dose (5 mg/kg); (B): Oral dose (50 mg/kg). Predicted values were calculated based on best-fit-analyses using two- and one-compartment model fits, respectively. Samples containing analyte concentrations below the LOQ were not utilized in best-fit analyses.

Figure 4: Cumulative elimination of radioactivity following IV administration of Bmim-Cl (5 mg/kg) to male F-344 rats. (N=4, mean ± S.D.; (■): total, (▼): urine, (●): feces).

Figure 5: Cumulative elimination following oral administration of [^{14}C] Bmim-Cl (50 mg/kg; (■): total; (▼): urine; (●): feces). (A) Male F-344 rats (N=4); (B) female B6C3F1 mice (N=10). Data are expressed as mean ± S.D.

Figure 6: Representative radio-chromatograms of urine samples obtained from male F-344 rats after a single oral administration of 0.5 or 5 mg/kg Bmim-Cl. (A) [^{14}C] Bmim-Cl standard (R_t = 17.1 min); (B) 0.5 mg/kg; (C) 5 mg/kg at 6 h. Dashed lines: 6 h; Solid lines: 12 h.

Figure 7: Mass spectral analysis of urine following oral administration of unlabeled Bmim-Cl (50 mg/kg) from urine: (A) 6 h after oral administration and (B) following collision-induced fragmentation of the *m/z* = 139.2 peak. Collision-induced fragmentation of $Bmim^+$ resulted in formation of a 3-methylimidazolium ion (*m/z* = 83.2). The loss of 56 mass units corresponded with the subtraction of a butyl group.

Figure 8: Cumulative excretion of radioactivity following serial oral administration of Bmim-Cl (50 mg/kg) in male F-344 rats (N=4 per dosing group, mean ± S.D., open symbols: 1 administration, closed symbols: 5 administrations) (◊/♦: total; ○/●: feces; □/■: urine + cage rinse). Stepwise line shows the cumulative administered dose.

Figure 9: Cumulative elimination of radioactivity following dermal administration of Bmim-Cl (5 mg/kg) in male F-344 rats (●: water vehicle, N = 4; ▼: ethanol/water vehicle [1.8:1, v/v], N = 3; ■: DMF/water vehicle [1.8:1, v/v], N = 3). All data are expressed as mean ± S.D. Feces contained <1% of the applied dose.

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	Dose (µg)	AUC (min*µg/mL)	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (h)	CL_b (mL/min)	V_{ss} (mL)	C_{max} (µg/mL)	T_{max} (min)	*F* (%)
IV	1,047	141.3	13.0	1.4	7.4	618.2	-	-	-
Oral	8,756	733.3	30.0	1.3	11.9	-	3.6	67.0	62.1

Table 1

Toxicokinetics of Bmim-Cl following IV (5 mg/kg) or oral (50 mg/kg) administration to male F-344 rats (N = 4 per dosing route). $AUC_{[0-\infty]}$: area under the blood concentration-time curve from 0 to infinity. $t_{1/2\alpha}$: distribution half life. $t_{1/2\beta}$: terminal elimination half life. CL_b: systemic clearance from blood. V_{ss}: volume of distribution at steady-state. *F*: systemic bioavailability.

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Dose recovered (%) 5 mg/kg			
Feces	2.5	±	0.4
Urine	76.8	±	10.4
Cage rinse	15.3	±	1.8
Blood	0.0	±	0.0
Total Recovery	**94.7**	±	**10.0**

Table 2

Dose recovered in 48 h following IV administration of Bmim-Cl (5 mg/kg) to male F-344 rats (N = 4, mean ± S.D.).

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	Dose Recovered (%)			
	0.5 mg/kg*	**5 mg/kg***	**50 mg/kg***	**50 mg/kg†**
Feces	21.4 ± 1.8	19.4 ± 1.7	28.2 ± 3.1	18.1 ± 5.8
Urine	77.5 ± 6.4	77.0 ± 3.1	72.3 ± 6.2	65.0 ± 12.0
Total Recovery	**98.9 ± 4.8**	**96.4 ± 4.2**	**100.6 ± 5.6**	**83.3 ± 13.6**

Table 3

Dose recovered in 72 h following oral administration of Bmim-Cl to male F-344 rats* (N = 4) or female B6C3F1 mice† (N = 10). Data are presented as mean ± S.D.

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	Dose Recovered (%)					
	1 administration			5 administrations		
Feces	29.9	±	1.3	25.5	±	5.1
Urine*	56.3	±	3.2	61.6	±	2.4
Blood	0.1	±	0	0	±	0
Tissues	5.3	±	2.4	1.1	±	0.2
Total Recovery	91.5	±	2.3	88.2	±	3.9

Table 4

Dose recovered at 24 h post final dose following 1 or 5 serial daily oral administrations of Bmim-Cl (50 mg/kg/day) to male F-344 (N=4 per treatment group).

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	Dose Recovered (%)		
	DMF:H_2O*	**EtOH:H_2O***	**H_2O†**
Feces	0.5 ± 0.0	0.8 ± 1.1	0.3 ± 0.4
Urine‡	12.3 ± 1.8	2.4 ± 1.4	1.1 ± 1.1
Tissues	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Application Site Skin	18.9 ± 2.9	14.6 ± 3.5	28.2 ± 9.0
Application Site Skin (Washes and Strips)	54.3 ± 6.8	71.4 ± 3.5	57.3 ± 6.3
Naïve Site Skin	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
Trap	3.5 ± 2.9	8.8 ± 7.0	5.4 ± 3.3
Total Recovery	**89.5 ± 11.7**	**98.1 ± 9.7**	**92.4 ± 5.1**

Table 5

Dose recovered after dermal administration of Bmim-Cl (5 mg/kg) in a series of vehicles to male F-344 rats (N=3: [DMF/water and EtOH/water]; N=4: [water], mean ± S.D.). Tissues (blood, liver, kidneys) were collected at 48 (*) or 72 h (†) after administration.

Figure 1

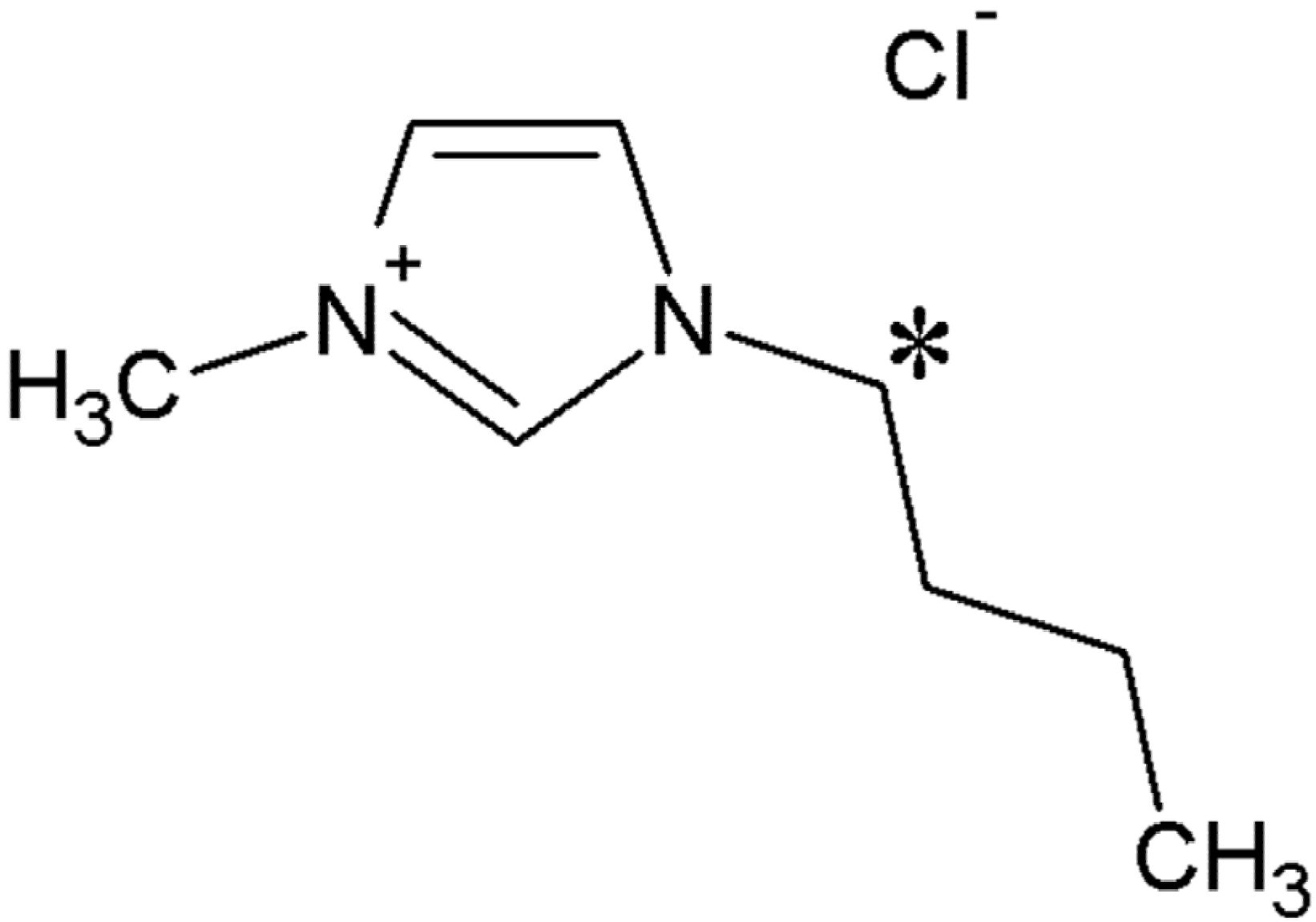


Figure 2

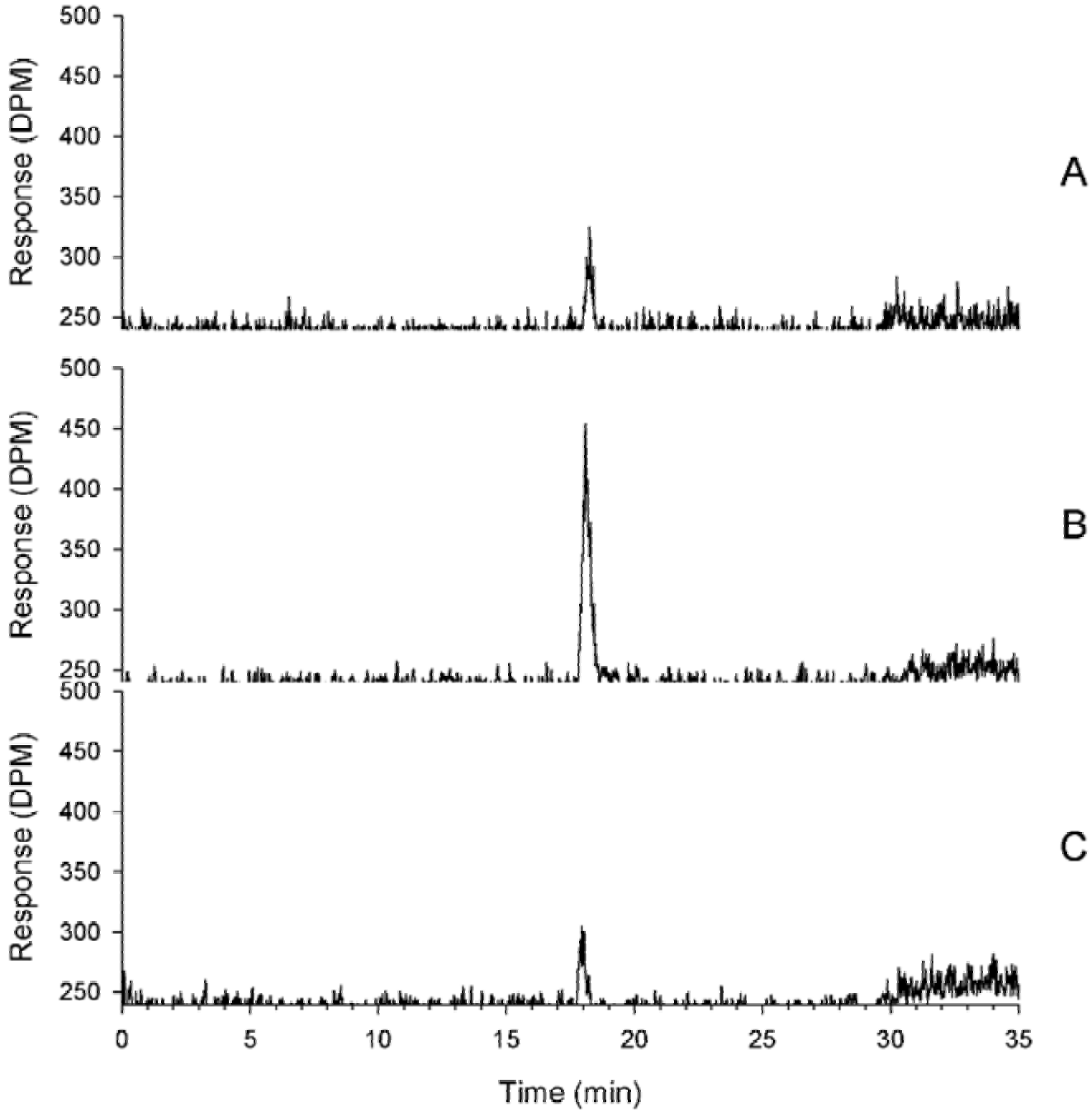


Figure 3

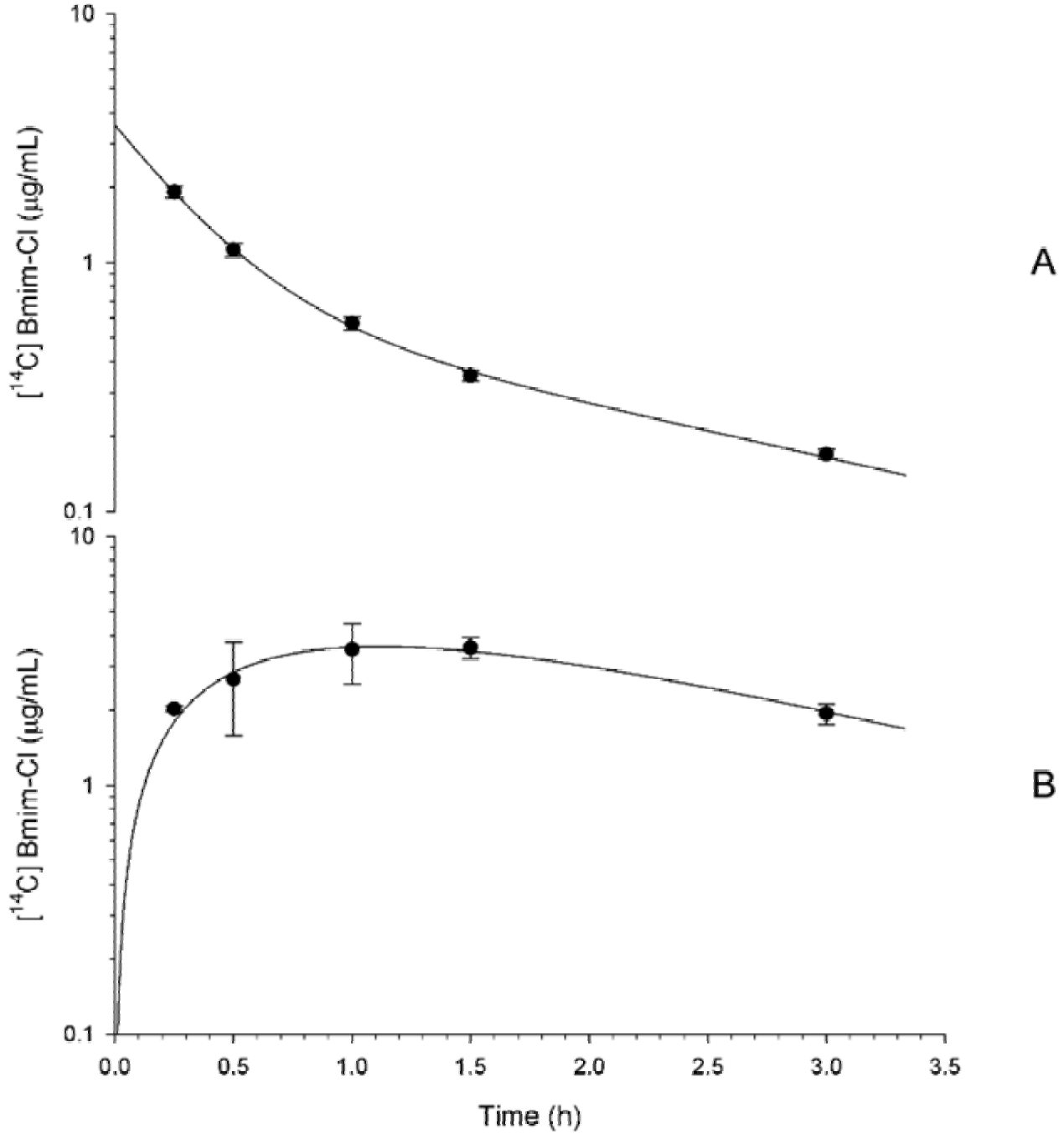


Figure 4

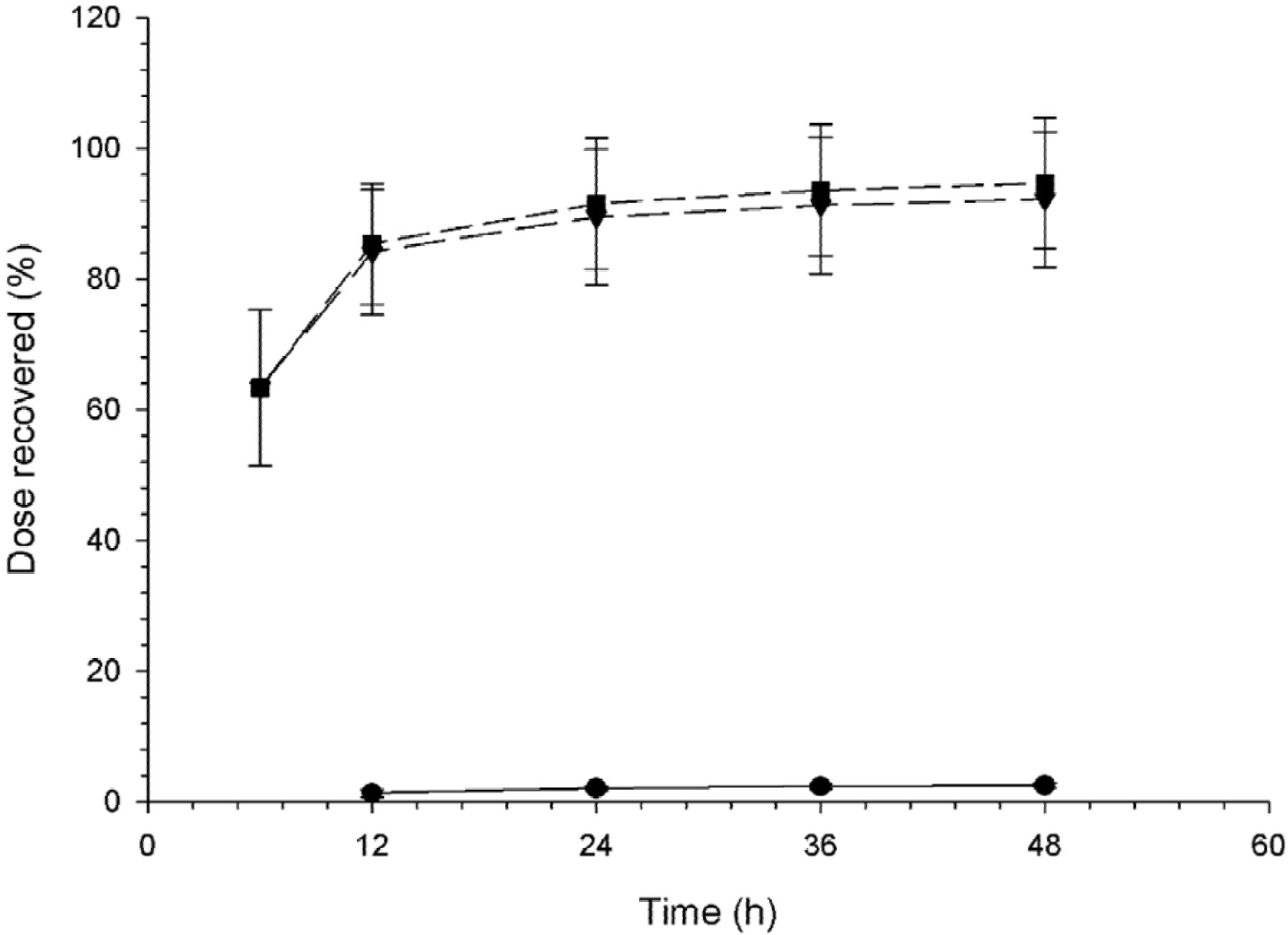


Figure 5

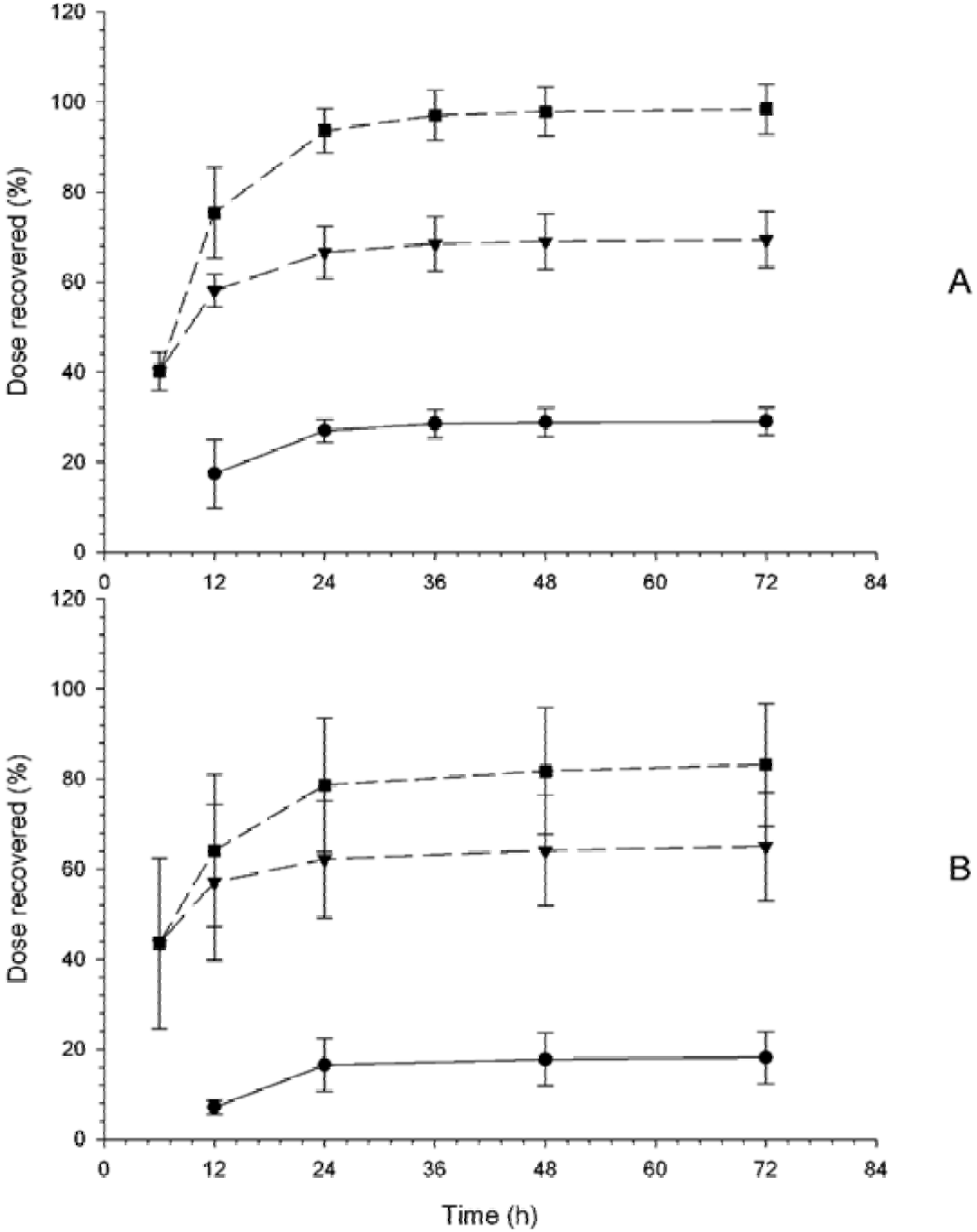
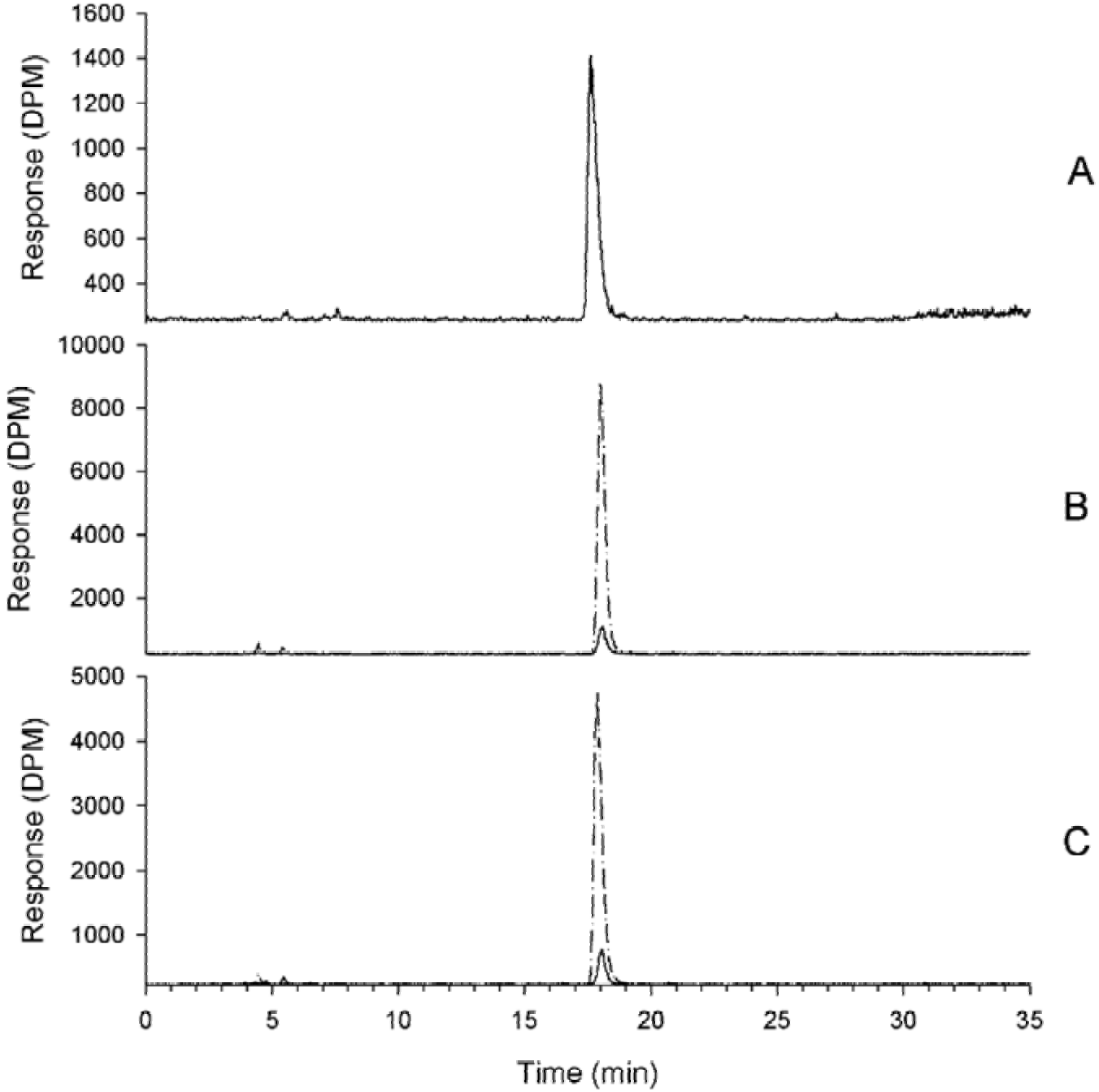


Figure 6



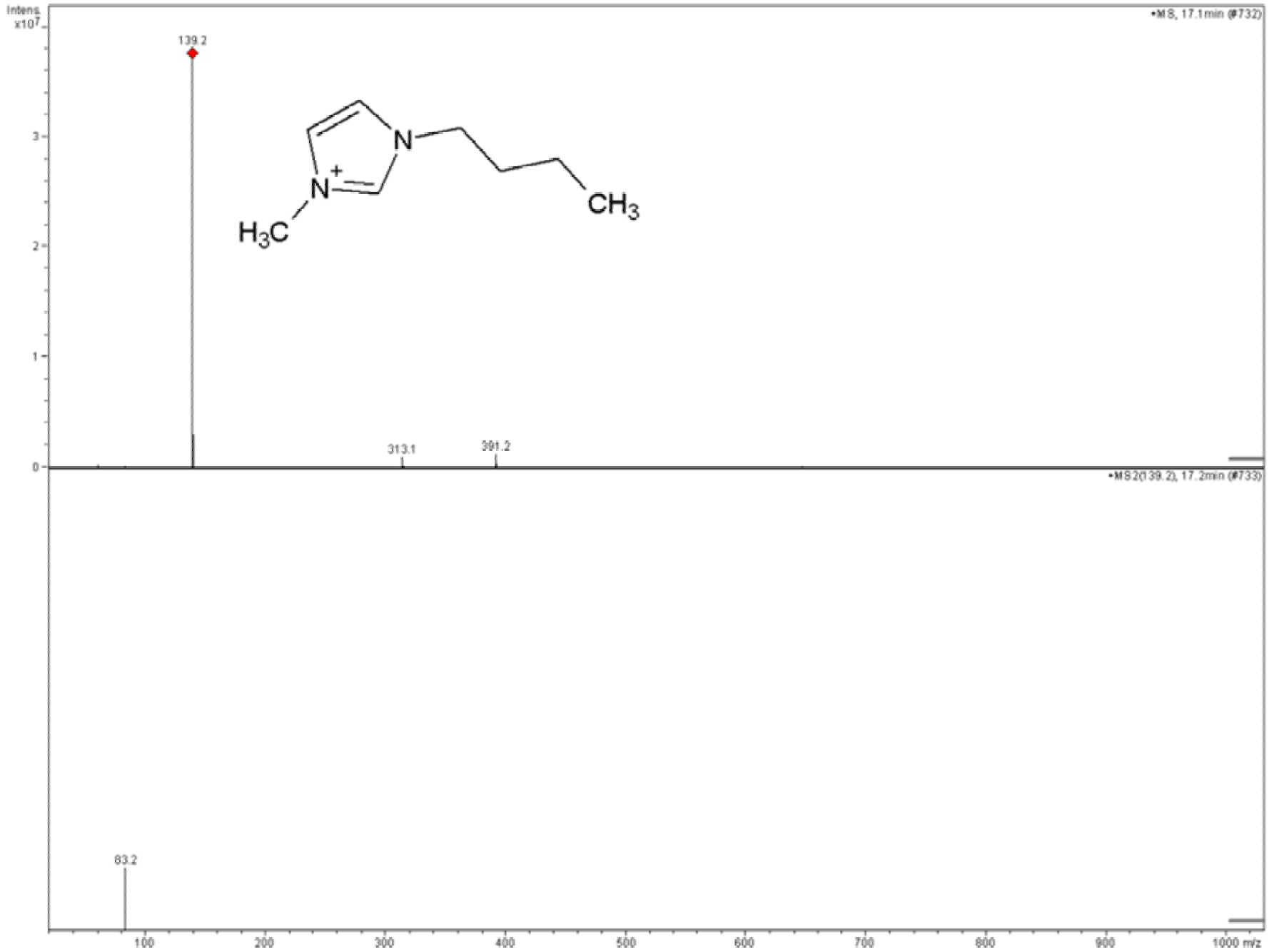


Figure 7

Figure 8

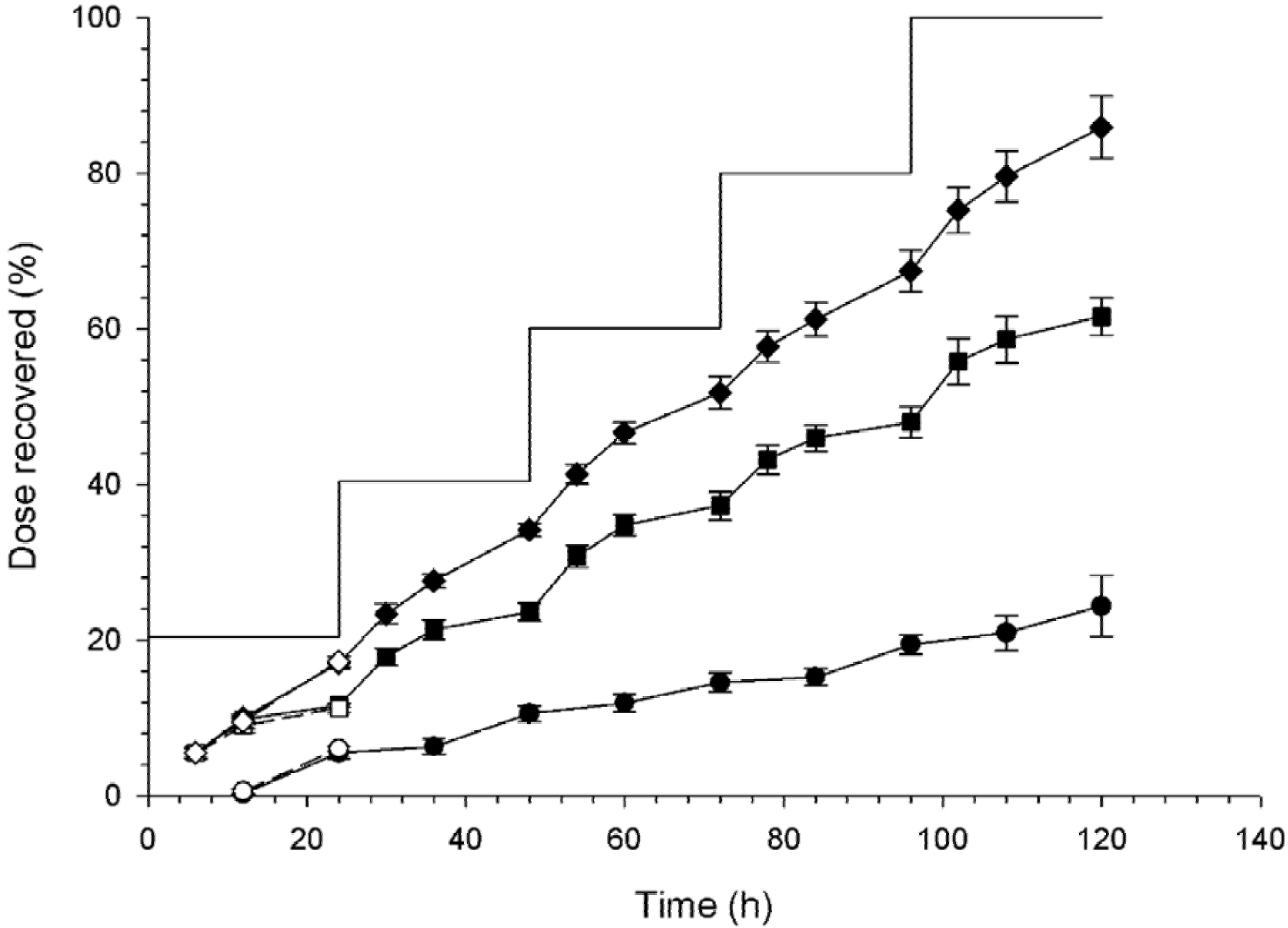


Figure 9