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A Comparison of the Effects of Prenatal Exposure of CD-1 Mice to Three Imidazolium-Based Ionic Liquids

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Abstract

BACKGROUND—Ionic liquids (ILs; salts with melting points below 100 °C) exhibit wide liquid ranges, non-flammability, and thermal stability among other properties. These unique salts are best known as ‘green’ alternatives to traditional volatile organic solvents, which are utilized in both academia and industry. Our current study compares the developmental toxicity potential of three representative ionic liquids, with various chain lengths: 1-ethyl-3-methylimidazolium chloride ([C₂mim]Cl), 1-butyl-3-methylimidazolium chloride ([C₄mim]Cl), and 1-decyl-3-methylimidazolium chloride ([C₁₀mim]Cl).

METHODS—From gestation days (GD) 6–16, mated CD-1 mice were orally dosed with one of the following: 1000, 2000, or 3000 mg/kg/day [C₂mim]Cl; 113, 169, or 225 mg/kg/day [C₄mim]Cl; 50, 75, or 100 mg/kg/day [C₁₀mim]Cl; or the vehicle only. Dams were sacrificed on GD 17, and their litters were examined for adverse effects.

RESULTS—Fetal weight was significantly decreased in the two highest dosage groups exposed to [C₄mim]Cl and [C₁₀mim]Cl in comparison with their controls, but the [C₂mim]Cl treated groups were not affected. An apparent teratogenic effect was associated with both [C₄mim]Cl and [C₁₀mim]Cl, as the offspring exhibited certain uncommon morphological defects. However, the incidences of malformations were low and no correlation between incidence and dosage could be made. No morphological defects were observed in any of the [C₂mim]Cl-treated groups, despite maternal morbidity at the highest dosage level.

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CONCLUSIONS—This study indicates that [C₄mim]Cl and [C₁₀mim]Cl may have adverse effects on development at high maternal exposures and strongly supports the supposition that the toxicity of imidazolium-based ILs is influenced by alkyl chain length.

Keywords

ionic liquids; imidazolium; developmental toxicity; mouse; mice

INTRODUCTION

Ionic liquids (ILs; salts with melting points below 100 °C) can exhibit wide liquid ranges, non-flammability, and thermal stability among other useful properties (Walden, 1914; Welton, 1999; Olivier-Bourbigou and Magna, 2002; Rogers and Seddon, 2003). The potential properties have attracted attention in the search for ‘green’ alternatives to traditional volatile organic solvents, which are utilized in both academia and industry (Wilkes et al., 1982; Jessop and Heldebrant, 2005). Even though ILs are promoted as ‘green’, few studies have been published regarding the safety of ILs to developing offspring, although general toxicity data has shown that some ILs can be significantly toxic to adult species (Bernot et al., 2005a,b; Pretti et al., 2006; Samori et al., 2007).

One of the most popular cation classes used to form ILs, includes the 1-alkyl-3-methylimidazolium [C_nmim]⁺ ions. The alkyl chain length at the 1-position varies from short (e.g. methyl) to long (e.g. decyl), however 1-butyl-3-methylimidazolium chloride ([C₄mim]Cl) is one of the most widely used and studied ILs in this class. This is a hygroscopic, water soluble salt which, when dissolved in water, yields fully hydrated ions (Pernak et al., 2004; Landry et al., 2005). [C_nmim]⁺-containing ILs, especially [C₄mim]Cl, have numerous applications in solvent replacement, coatings, refractive index, metal plating, fuel cells, catalysis, chromatography columns, biomass processing, and many more (Joglekar et al., 2007; Plechkova and Seddon, 2008).

Because of its widespread use and the corresponding potential for human exposure, the majority of toxicity studies have focused on [C₄mim]Cl in various biological models. The models employed have ranged from simpler test systems, such as bacteria, algae, human (HeLa, CaCo-2, HT-29, and MCF7) and rat (ICP-81 and C₆ glioma) cell lines, to complex organisms, such as snails, zebrafish, and Fisher 344 rats (Matsumoto et al, 2004; Stepnowski et al., 2004; Bernot et al., 2005a,b; Landry et al., 2005; Latala et al., 2005; Lee et al., 2005; Ganske and Bornscheuer, 2006; Pretti et al., 2006; Chefson and Auclair, 2007; Cho et al., 2007; Frade et al., 2007; Matzke et al., 2007; Salminen et al., 2007; Samori et al., 2007; Stolte et al., 2007; Wang et al., 2007). The results from these studies have indicated that the cation, [C₄mim]⁺, is the primary influence on the toxicity of the IL. This assumption seems reasonable because the cations have typically been paired with simple inorganic anions, such as chloride and bromide (Lee et al., 2005; Salminen et al., 2007; Plechkova and Seddon, 2008). The toxicity of an IL is believed to be partly a consequence of the cation’s ability to intercalate with the cell’s bilayer membrane, thereby increasing membrane permeability and causing cell death (Ranke et al., 2004). This ability increases with increasing alkyl chain

length, as the IL becomes more lipophilic and more surfactant-like (Matsumoto et al., 2004; Ranke et al., 2004; Samori et al., 2007).

Sipes *et al* (2008) have determined the toxicokinetics of orally, intravenously, and dermally administered [C₄mim]Cl, *N*-butylpyridinium chloride, and 1-butyl-1-methylpyrrolidinium chloride in mice and rats as these ILs represent the most common cation classes (Cheng et al., 2009; Knudsen et al., 2009). Regardless of the administration route, all of the ILs were almost completely, > 97%, eliminated unchanged from the animal through the urine. No metabolism by the liver or any other mammalian enzymes of the ILs were observed for any of the tested compounds. The authors attribute this efficient clearance to the water solubility and structural features of the IL, as it may be excreted from the body via a sodium-independent cation transporter. These results are interesting as little information is currently available regarding the toxicity, disposition, or metabolism of ILs in mammalian systems.

While the toxicological effects of increasing alkyl chain length in an IL are known, no studies have examined the effects of this length increase of an imidazolium-based IL on development, despite the potential human exposure through groundwater contamination from industrial spills or wastewater. That is the case even though our previous study indicated a dosage-related increase in adverse effects, such as reduced fetal weight and certain morphological defects, as a result of exposure to [C₄mim]Cl during development in mice (Bailey et al., 2008). Other studies conducted with zebrafish and frogs have also indicated adverse effects of exposure to ILs during development (Pretti et al., 2006; Lu et al., 2009). To address this issue, we compared the developmental toxicity potential in CD-1 mice of three ILs of various chain lengths: 1-ethyl-3-methylimidazolium chloride ([C₂mim]Cl), 1-butyl-3-methylimidazolium chloride ([C₄mim]Cl), and 1-decyl-3-methylimidazolium chloride ([C₁₀mim]Cl). The comparison data for the intermediate chain length IL were derived from Bailey et al. (2008).

MATERIALS AND METHODS

Animals and Husbandry

Male and female CD-1 mice, obtained from Charles River Breeding Laboratories International (Wilmington, MA), were maintained at 22±2 °C, with 40-60% humidity and a 12 h photoperiod. The mice were bred naturally, two females with one male. Observation of a copulation plug designated gestation day 0 (GD 0). Mated females were individually housed in shoebox type cages with hardwood bedding and were given Teklad LM-485 rodent diet (Harlan Teklad, Madison, WI) and tap water *ad libitum*. All procedures performed on these animals were in accordance with established guidelines and were reviewed and approved by The University of Alabama's or Emporia State University's Institutional Animal Care and Use Committee.

Test Chemicals

All chemicals were obtained from Sigma-Aldrich (St. Louis, MO) unless otherwise stated and used as received. Each of the ILs, [C₂mim]Cl, [C₄mim]Cl, and [C₁₀mim]Cl were prepared via alkylation of methylimidazole with the corresponding chloroalkanes using

previously described methods (Huddleston et al., 1998,2001). Before the toxicity studies were carried out, the ILs were rigorously dried by heating to 70 °C under reduced pressure. The water contents were determined using a volumetric Aquastar Karl Fischer titrator (EM Science, Gibbstown, NJ). Triplicate measurements were performed on each sample, and agreed to within 100 ppm. To ensure purity (*i.e.*, complete reaction), the ILs were analyzed using NMR (^1H and ^{13}C at 360 MHz), and the analyses indicated no residual reactants. The $[\text{C}_2\text{mim}]\text{Cl}$ and $[\text{C}_4\text{mim}]\text{Cl}$ were off-white crystalline solids at room temperature, and the $[\text{C}_{10}\text{mim}]\text{Cl}$ was a colorless viscous liquid at room temperature. Stock solutions were prepared by dissolving the ionic liquids in sterile water at the following final concentrations: $[\text{C}_2\text{mim}]\text{Cl}$ 500.35 mg mL $^{-1}$, $[\text{C}_4\text{mim}]\text{Cl}$ 103.0 mg mL $^{-1}$, and $[\text{C}_{10}\text{mim}]\text{Cl}$ 52.5 mg mL $^{-1}$.

Treatments

Prior to the definitive study, a preliminary range-finding study was conducted at a $[\text{C}_2\text{mim}]\text{Cl}$ dosage of 4000 mg/kg/day. Treatments were given by gavage once daily to a total of 9 mated females, commencing on GD 6 and ending on GD 16 (or sooner if morbidity or mortality occurred). A preliminary study was conducted similarly with $[\text{C}_4\text{mim}]\text{Cl}$ or $[\text{C}_{10}\text{mim}]\text{Cl}$ gavage dosages of 0, 250, 500, or 1000 mg/kg/day, with five females in each dosage group. Six of the nine female mice in the 4000 mg/kg/day dosage group died after an average of 6 doses and one female died after a non-IL related dosing error. Of the two surviving females, one was not pregnant. Fetuses from the surviving female were not found to have gross malformations.

All mice in the 500 and 1000 mg/kg/day $[\text{C}_4\text{mim}]\text{Cl}$ or $[\text{C}_{10}\text{mim}]\text{Cl}$ treatment groups died immediately or became moribund and were subsequently euthanized by CO $_2$ overdose approximately 8 h after the initial dose. The moribund mice displayed ataxia, hypoactivity, and labored breathing. Three of the five mice in the 250 mg/kg/day $[\text{C}_{10}\text{mim}]\text{Cl}$ treatment group died or became moribund and were subsequently euthanized within 8 hours of the initial dose. Two of the five mice in the 250 mg/kg/day $[\text{C}_4\text{mim}]\text{Cl}$ treatment group appeared sluggish after the initial dose, but they recovered within 24 h and survived multiple doses.

For the definitive study, mated female CD-1 mice were randomly assigned to one of the following treatment groups: (1) DI H $_2\text{O}$ (vehicle control), (2) 1000, 2000, or 3000 mg/kg/day $[\text{C}_2\text{mim}]\text{Cl}$, (3) 113, 169, or 225 mg/kg/day $[\text{C}_4\text{mim}]\text{Cl}$, or (4) 50, 75, or 100 mg/kg/day $[\text{C}_{10}\text{mim}]\text{Cl}$. Initially, dosages of 113, 169, or 225 mg/kg/day $[\text{C}_{10}\text{mim}]\text{Cl}$ were employed; however these dosages were lowered to the definitive dosages because of significant maternal morbidity and mortality. Eleven out of 11 dams died after an average of 3 doses of 225 mg/kg/d (3 were moribund and subsequently sacrificed), 5/11 dams in the 169 mg/kg/d died after an average of 4 doses, and 1 dam (out of 11) was moribund and subsequently sacrificed in the 113 mg/kg/d dosage group after 6 doses. Animals were dosed once daily from GD 6 – 16, with an average of 25 mated females per treatment group. The dosage volume was 0.01 mL/g body weight. Clinical signs were recorded daily and females were weighed on GD 0, as well as prior to each dosing.

Data Collection

On GD 17, mated females were euthanized by CO₂ overdose, their uteri were exposed, and the numbers of resorptions and dead or live fetuses were recorded. Live fetuses were removed from the uterus, weighed individually, and examined for gross malformations. Maternal body weight, minus the gravid uterine weight, was then obtained. Fetuses were initially fixed in 70% ethanol and then cleared and stained by the double-staining technique described by Webb and Byrd (1994). All fetuses were subsequently examined for skeletal abnormalities.

Data Analysis

The litter was used as the experimental unit for statistical analysis. This study was performed in multiple replicates. The data from each study replicate were calculated independently, tested for homogeneity of variance by means of the Levene statistic, using SPSS (SPSS, Inc., Chicago, IL), and then pooled and analyzed to give the results reported. All tabular data are presented as the mean $\pm$ standard error (SEM). Data were analyzed by one-way analysis of variance (ANOVA) followed by a least significant difference (LSD) post-hoc test to determine specific significant differences ($P \leq 0.05$) or by a Pearson chi-square test ($P \leq 0.05$).

RESULTS

Maternal Data

Maternal weight gain was not significantly affected by exposure to any of the ILs (Tables 1-3). There was an apparent treatment effect on maternal weight gain in the highest [C₂mim]Cl and [C₄mim]Cl dosage groups; however, the differences from their respective control values were not statistically significant. There was evidence of dosage-dependent maternal morbidity/mortality in the [C₂mim]Cl and [C₄mim]Cl exposed mice (Tables 1 and 2). The percentage of animals dying or becoming moribund and necessitating euthanasia prior to the completion of dosing were significantly greater in the 3000 mg/kg/day [C₂mim]Cl and 225 mg/kg/day [C₄mim]Cl treatment groups as compared to both the controls and the lower IL doses. There was an apparent treatment effect on maternal morbidity/mortality in the [C₁₀mim]Cl exposed animals; however this effect was not statistically significant at the revised dosage levels (Table 3).

Fetal Data

The numbers of implantations and the percentages of resorbed or dead fetuses did not differ significantly among treatment groups (Tables 1-3). In the [C₄mim]Cl and [C₁₀mim]Cl treatment groups, fetal weight was significantly affected (Fig. 1). Fetuses exposed to either 169 or 225 mg/kg/day [C₄mim]Cl weighed significantly less than either the control or 113 mg/kg/day [C₄mim]Cl exposed fetuses. Fetuses in the 75 and 100 mg/kg/day [C₁₀mim]Cl treatment group weighed significantly less than fetuses in the control group. There was no effect on fetal weight among the [C₂mim]Cl exposed fetuses.

Several of the gross malformations observed in the [C₄mim]Cl exposed fetuses were reported in a previous publication and are included in Table 4 for comparison purposes

(Bailey et al., 2008). Malformations were also observed in fetuses exposed to the higher dosages of [C₁₀mim]Cl, including ectopia cordis, exencephaly, and ablepharia. Two fetuses in the 100 mg/kg/day [C₁₀mim]Cl dosage group displayed malformations, with one fetus exhibiting gastroschisis and another exhibiting exencephaly (Table 4). No gross malformations were observed in fetuses exposed to [C₂mim]Cl.

In both the [C₄mim]Cl and [C₁₀mim]Cl treatment groups, the mean litter percentages of fetuses and the per litter incidences of any specific malformation were not statistically significantly greater than the incidences in the fetuses of control dams. Both the fetal and litter incidences of total malformations increased in an apparently dosage-dependent manner in the [C₄mim]Cl and [C₁₀mim]Cl treatment groups, although the increase was not statistically significant (Tables 2 and 3).

When fetal skeletons were examined for malformations and variations, it appeared that exposure to [C₂mim]Cl increased the litter incidence of supernumerary ribs. However, this increase was not statistically significant (Table 1). A statistically significant decrease in the incidence of fetuses with rudimentary ribs, as well as affected litters, was found in fetuses exposed to [C₄mim]Cl, which we reported previously (Bailey et al., 2008)(Table 2), and a similar effect was also observed at the highest dosage of [C₁₀mim]Cl (Table 3).

The present study did not find significant differences in the numbers of implantations, viable fetuses, or resorbed/dead fetuses among any of the treatment groups. Fetal weight was significantly reduced in the two highest dosage groups for both [C₄mim]Cl and [C₁₀mim]Cl, while no such reduction was observed among [C₂mim]Cl exposed fetuses (Figure 1). An apparent teratogenic effect was also seen in the [C₄mim]Cl and [C₁₀mim]Cl treatment groups, with fetuses exhibiting a variety of uncommon morphological defects, such as ectopia cordis, craniorachischisis, and hemimelia. There were no morphological aberrations in fetuses exposed to [C₂mim]Cl. Historical control data from more than 180 litters/2300 fetuses contained 2 incidences of bent tail and 2 incidences of ablepharia, but in the current study there were no incidences of any of the other malformations observed in fetuses from IL treated dams .

DISCUSSION

Although the number of malformed fetuses observed was relatively modest, it must be noted that the multiple-dose regimen employed is relatively inefficient at producing malformed offspring, even if the test compound is capable of doing so. It should also be mentioned that the positive fetal findings all occurred at maternal dosages associated with at least some maternal morbidity or mortality. In the dose ranges tested, the apparent No Observed Adverse Effect Level (NOAEL) for fetal effects in mice exposed to [C₂mim]Cl was greater than 3000 mg/kg/day, as no adverse effects were seen. However, the [C₄mim]Cl NOAEL for fetal effects was 113 mg/kg/day, while the Lowest Observed Adverse Effect Level (LOAEL) was 169 mg/kg/day, based on effects on fetal weight. The NOAEL for [C₁₀mim]Cl was 50 mg/kg/day, while the LOAEL was 75 mg/kg/day. Nonetheless, these values may have been lower if visceral defects had been evaluated, and although the low

dose fetal weights for [C₁₀mim]Cl did not differ significantly from the control value, they appeared to have been affected.

Solvents such as benzene, toluene, acetonitrile, xylene, and commercial polychlorinated biphenyl mixtures tend to produce effects similar to those observed with the tested ILs, including reduced fetal weight and skeletal anomalies, particularly at high maternal exposures (Stepnowski et al., 2004; Bernot et al., 2005b). However, comparison of the tested IL's toxicities to those of traditional solvents is difficult, primarily because of the different routes of administration i.e. inhalation for volatile traditional solvents or animal species utilized in other studies.

The results of the present study strongly indicate that the toxicity of ILs increases with increasing alkyl chain length. The dosages employed for the [C₂mim]Cl treatment groups were some 10-30 times greater than the highest dosage tolerated by the [C₁₀mim]Cl treated dams. In the preliminary study, a [C₁₀mim]Cl dosage of 1000 mg/kg/day was immediately lethal, while multiple administrations of the same dosage of [C₂mim]Cl were well tolerated and caused no obvious maternally toxic or embryo/fetotoxic effects. Indications of developmental toxicity, such as reductions in fetal weight and the occurrence of morphological defects, were observed primarily in the [C₄mim]Cl and [C₁₀mim]Cl treatment groups, despite using dosages of [C₂mim]Cl that were an order of magnitude higher.

The mechanism for the developmental toxicity of ILs remains unknown. It is known that imidazolium-based ILs can act as cationic surfactants, which have the potential to be incorporated into biological membranes, disrupting the lipid bilayer and increasing membrane permeability (Jastorff et al., 2003; Kumar et al., 2008). The hydrophobicity and surfactant quality of ILs increase with alkyl chain length, therefore, increasing the toxicity (Zhao et al., 2007). Enzyme inhibition is another possible means of toxicity, as several imidazolium- and pyridinium-based ILs, including [C₄mim]Cl, have been shown to inhibit enzymes, such as acetylcholinesterase, *in vitro* (Stock et al., 2006). ILs have also been shown to inhibit the P450 enzyme CYP3A4 to a degree comparable to the effects of organic co-solvents such as acetonitrile (Chefson and Auclair, 2007). Any of these mechanisms might negatively affect embryonic development and viability as well as contribute to maternal toxicity; however, further investigation to elucidate the mechanisms of developmental toxicity of ILs would be needed to address such questions, as the current data are only observational.

CONCLUSIONS

The present comparison study conducted with pregnant mice gavaged with imidazolium-based ionic liquids supports the contention that toxic effects of the ILs increase with increasing alkyl chain length. That relationship was manifested by maternal morbidity and mortality, and by effects on fetal weight. The same may be true of malformations, as they were seen only in fetuses from dams exposed to the two longer chain ILs, although differences in dosage levels among the three test compounds make direct comparison more difficult. Although the term 'green solvent' is typically misused as a synonym for 'safe', it is

important that all users of ILs understand the fate, transport, and toxicity of these chemicals and take appropriate measures to avoid unnecessary exposure.

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Figure 1.

Fetal Weights of Offspring Exposed to Imidazolium-Based Ionic Liquids

^a Significantly differs from control and 113 mg/kg/day groups ($P < 0.05$).

^b Significantly differs from control group ($P < 0.05$).

Table 1Maternal weight gain and litter parameters of CD-1 mice exposed to [C₂mim]Cl

	Treatment and dose (mg/kg/day)			
	Vehicle Control	[C ₂ mim]Cl (1000)	[C ₂ mim]Cl (2000)	[C ₂ mim]Cl (3000)
Maternal weight gain (g ± SEM)	12.46 ± 0.68	13.51 ± 0.80	13.72 ± 0.74	11.31 ± 0.90
Maternal				
Morbidity/Mortality (No./% affected dams)	0	0	0	8/33.3 ^a
Fetuses/litters examined	243/20	192/17	206/17	72/6
Implantations (mean ± SEM)	12.30 ± 0.53	12.12 ± 0.54	11.70 ± 0.68	12.33 ± 0.84
Resorbed or dead fetuses (No. ± SEM)	0.90 ± 0.64	0.47 ± 0.47	2.51 ± 1.22	2.67 ± 1.69
Litters with resorbed or dead fetuses (No./%)	2/10.0	1/5.9	4/22.2	2/33.3
Malformed fetuses (% ± SEM) ^{b,c}	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
Malformed fetuses (% affected litters) ^b	0.0	0.0	0.0	0.0
Supernumerary Ribs (% ± SEM)	13.64 ± 3.47	15.41 ± 3.66	19.36 ± 5.82	23.00 ± 3.47
Rudimentary Ribs (% ± SEM)	11.12 ± 3.84	15.91 ± 3.42	10.43 ± 2.59	10.33 ± 2.72

^aSignificantly different from all other mean values ($P < 0.01$)^bFetuses displaying any gross malformation.^cGrand mean of litter mean percentages.

Table 2Maternal weight gain and litter parameters of CD-1 mice exposed to [C₄mim]Cl

	Treatment and dose (mg/kg/day)			
	Vehicle Control	[C ₄ mim]Cl (113)	[C ₄ mim]Cl (169)	[C ₄ mim]Cl (225)
Maternal weight gain (g ± SEM)	10.67 ± 0.39	10.29 ± 0.44	10.38 ± 0.42	9.28 ± 0.64
Maternal				
Morbidity/Mortality (No./% affected dams)	0	1/3.3	2/6.7	10/28.3 ^a
Fetuses/litters examined	489/36	288/23	319/26	314/23
Implantations (mean ± SEM)	13.97 ± 0.36	12.78 ± 0.55	13.40 ± 0.36	13.91 ± 0.44
Resorbed or dead fetuses (No. ± SEM)	2.77 ± 0.84	2.24 ± 0.88	6.20 ± 2.82	3.24 ± 0.98
Litters with resorbed or dead fetuses (No./%)	10/27.8	6/26.1	8/30.8	9/39.1
Malformed fetuses (% ± SEM) ^{b,c}	0.91 ± 0.44	1.09 ± 0.60	1.56 ± 0.76	2.47 ± 0.93
Malformed fetuses (% affected litters) ^b	11.1	13.0	15.4	26.1
Supernumerary Ribs (% ± SEM)	24.53 ± 4.16	16.49 ± 4.42	16.97 ± 4.17	18.10 ± 5.28
Rudimentary Ribs (% ± SEM)	18.85 ± 2.70	21.47 ± 4.50	12.72 ± 2.16 ^a	7.92 ± 2.35 ^a

^aSignificantly different from all other mean values (*P* = 0.01)^bFetuses displaying any gross malformation.^cGrand mean of litter mean percentages.

Table 3Maternal weight gain and litter parameters of CD-1 mice exposed to [C₁₀mim]Cl

	Treatment and dose (mg/kg/day)			
	Vehicle Control	[C ₁₀ mim]Cl (50)	[C ₁₀ mim]Cl (75)	[C ₁₀ mim]Cl (100)
Maternal weight gain (g ± SEM)	11.42 ± 0.74	12.11 ± 0.70	11.42 ± 0.77	12.01 ± 0.85
Maternal Morbidity/Mortality (No./% affected dams)	0	3/12.5	2/9.5	3/12.5
Fetuses/litters examined	271/23	241/21	233/19	234/21
Implantations (mean ± SEM)	12.28 ± 0.62	12.22 ± 0.60	12.80 ± 0.49	11.39 ± 0.67
Resorbed or dead fetuses (No. ± SEM)	2.17 ± 0.93	1.49 ± 0.90	2.41 ± 1.44	4.16 ± 2.01
Litters with resorbed or dead fetuses (No./%)	5/21.7	3/14.3	3/15.8	5/23.8
Malformed fetuses (% ± SEM) ^{a,b}	0 ± 0.0	0.62 ± 0.62	0.74 ± 0.51	0.95 ± 0.66
Malformed fetuses (% affected litters) ^a	0.0	5.0	10.5	10.0
Supernumerary Ribs (% ± SEM)	13.57 ± 3.91	14.70 ± 5.04	10.48 ± 3.14	9.40 ± 2.91
Rudimentary Ribs (% ± SEM)	10.38 ± 2.78	11.71 ± 3.56	11.61 ± 2.82	4.43 ± 2.23

^aFetuses displaying any gross malformation.^bGrand mean of litter mean percentages.

Gross malformations in CD-1 mouse fetuses exposed prenatally to [C₄mim]Cl or [C₁₀mim]Cl^{a,b}

Table 4

Malformation (No./%)	Treatment and Dose (mg/kg/day)											
	[C ₄ mim]Cl ^a				[C ₁₀ mim]Cl				[C ₁₀ mim]Cl			
	0	113	169	225	0	113	169	225	0	50	75	100
Fetuses	489	288	319	314	126	170	117	35	273	267	251	239
Examined (No.)												
Bent Tail	2	3	4	3	0	0	0	0	0	0	2	0
Talipes	0	0	1	0	0	0	0	0	0	0	0	0
Hemimelia	0	0	0	2	0	0	0	0	0	0	0	0
Ablepharia	2	0	1	1	0	0	1	0	0	0	0	0
Exencephaly	0	0	1	1	0	1	1	1	0	0	0	1
Craniorachischis	0	0	0	1	0	0	0	0	0	0	0	0
Ectopia Cordis	0	0	0	0	0	1	0	0	0	0	0	0
Cleft Palate	0	0	2	0	0	0	0	0	0	0	0	0
Gastroschisis	0	0	0	0	0	0	0	0	0	0	0	1
Total (No./%)	4	3	9	8	0	2	2	1	0	0	2	2

^a [C₄mim]Cl data previously reported in Bailey et al., 2008.

^b No malformations seen in any [C₂mim]Cl dosage group.