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Acute Toxicity Profile of 1-Butyl-3-Methylimidazolium Chloride

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Ionic liquids (IL) are salts that are liquids at low temperatures, e.g., room temperature to around 100 °C. They are commonly, but not always, composed of a cation that is an organic ammonium ion and an anion that varies widely in composition, from a simple chloride ion to complex polyatomic anions. The most commonly encountered ILs are imidazolium based materials, a few of which are commercially available. They tend to be non-volatile, thereby having a handling advantage in not being a "Volatile Organic Compound". Rogers and Simon (2003) considered whether ionic liquids were the "solvents of the future", noting that some ionic liquids are extremely toxic, but could be designed to be environmentally benign.

Anhydrous 1-butyl-3-methylimidazolium chloride (BMIC) is a hygroscopic solid and considered an "ionic liquid". It rapidly absorbs water from the air, forming an aqueous solution. Dissolution in water, a polar protic solvent, yields a fully ionized solution, where both the imidazolium cation and chloride anion are fully solvated and surrounded by a solvent "cage". Dissolution in DMF, a polar aprotic solvent, also yields an ionized solution but the cationic species is well solvated and the chloride anion is poorly solvated. Solvents with the properties of DMF are often employed in organic synthesis when it is desired to increase the reactivity and availability of the anionic species in a reaction.

There is limited information on BMIC, though Hassoun et al. (2003) assessed cytotoxicity in J774A.1 macrophage cells. The LC50 for cellular death by Trypan blue exclusion was 0.50 mg BMIC per liter of culture media at 48 hours. Ebel et. al. (2000) compiled information on methylimidazole isomers, including some unpublished information from BASF. Alkyl-substituted imidazoles were noted to increase in toxicity with increasing length of side chain. Rabbit dermal LD50s, for example, were reported to be between 400 and 640 mg/kg (1-methylimidazole) and less 200 mg/kg (1-*n*-butylimidazole).

To help provide information for safe handling, a series of acute toxicity tests were conducted with BMIC. Some degree of dermal toxicity was noted in the initial administration of test material in a mouse local lymph node assay (LLNA), which was designed to determine contact sensitization potential. The potential for

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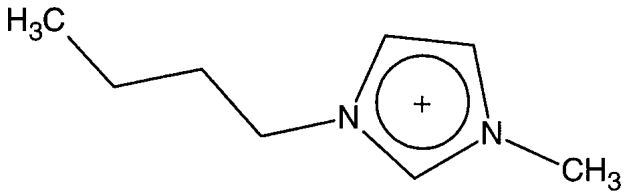

Figure 1. Structure of 1-butyl-3-methylimidazolium ion.

accentuation of dermal toxicity when BMIC was administered with a small amount of organic solvent (DMF) was addressed in this series of studies.

MATERIALS AND METHODS

Test material (CAS RN 79917-90-1) was obtained from Sachem (Austin, TX 78704) as an 85 wt% solution in water. The as-received BMIC contained colored impurities that the vendor claimed were present in very low quantities. However, the color of the BMIC could be improved by removal of these impurities by treatment with charcoal. The solution was diluted with water to about 50 wt% solids, and then shaken at room temperature for about 3 hours with activated charcoal (1 gram/50 ml solution). The charcoal was removed by suction filtration through a pad of diatomaceous earth filtering aid (Celite Corp., Santa Barbara, CA) and the nearly colorless filtrate evaporated to dryness under vacuum at 90 degrees C. The material remaining after removal of the water was typically a viscous, straw-colored syrup. Crystallization was hastened by adding a seed crystal from a previous batch of BMIC. The purified sample was examined by ^{1}H, ^{13}C, and water suppression NMR spectroscopic techniques using a 400 MHz JEOL NMR spectrometer. The ^{13}C NMR spectrum was compared with a predicted ^{13}C NMR spectrum. The measured spectra were consistent with the expected structure, and peaks at chemical shift positions inconsistent with the structure of BMIC were not detected. A quantitative value for purity was not determined. The structure is shown in Figure 1.

We conducted an acute oral toxicity study in female Fischer 344 rats utilizing the OPPTS 870.1100 Up/Down procedure (EPA, 2002). Young adult rats (9-10 weeks age), that had been obtained from Hilltop Lab Animals, Inc. (Scottdale, PA), were used in the oral and dermal toxicity evaluations. Rats were given BMIC 25 % wt/wt in water by gavage. Initially a dose of 175 mg/kg was administered. Following the up/down procedure, eight additional animals were dosed with 175, 550 or 2000 mg/kg. All animals were observed daily for mortality, gross toxicity (e.g. signs of incoordination, decreased activity, perineal soiling, tremors), and behavioral changes for up to 14 days. Body weights were recorded prior to administration, on days 7 and 14 (termination), or after death. Necropsies were performed on all animals.

Dermal toxicity was assessed in Fischer 344 rats using the OPPTS Health Effects Test Guideline 870.1200 (EPA, 1998) and OECD 402 (OECD, 1987). BMIC was evaluated in either distilled water (95% wt/wt) or in DMF (75% wt/wt). The solvents (water or DMF) allowed the BMIC to be applied as a viscous liquid from a disposable syringe (no needle). Groups of five rats/sex were evaluated, starting at 2000 mg/kg. The test solution was applied evenly over an area of approximately 2 x 3 inches on the back of each animal (approximately 10% of the animal's surface area), the area was covered with gauze and secured with tape for 24 hours. As with the oral toxicity assessment, all animals were observed for mortality, gross toxicity, and behavioral changes for up to 14 days. Body weights were recorded prior to administration, on days 7 and 14 (termination), or after death. Necropsies were performed on all animals.

Dermal irritation tests were conducted in rabbits according to Health Effects Test Guideline OPPTS 870.2500 (EPA, 1998) and OECD 404 (OECD, 2002). Young adult New Zealand White rabbits were obtained from Robinson Services Inc. (Clemmons, NC) for the dermal and eye irritation studies. The BMIC test material (0.5 g) was moistened with water or DMF, placed on a 1" x 1" gauze pad and applied to one six cm^2 test site during the four-hour application period. No vehicle control was required or evaluated. The pad was fixed in position with semi-occlusive Micropore tape and Elizabethan collars were placed on the rabbits. After four hours the pads and collars were removed, and the sites were gently cleaned. The sites were evaluated at 1, 24, 28, and 72 hours after patch removal according to the Draize system (Draize et al., 1944). Primary eye irritation was assessed in rabbits by a stepwise procedure starting with one rabbit receiving undiluted 0.1 ml of BMIC (0.09 g) in the right eye. Ocular anesthetic (Proparacaine Hydrochloride ophthalmic solution, 0.5%) was applied to both eyes after administering the test material. A total of three rabbits were evaluated, with eye irritation assessed according to Draize et al. (1944). In each case the left eye served as the control.

A mouse Local Lymph Node Assay (LLNA), consistent with OPPTS Health Effects Test Guideline 870.2600 (EPA 2003) and OECD 429 (OECD, 2003), was conducted in which BMIC was dissolved in N, N-dimethylformamide (DMF) and applied. The female BALB/c mice for the LLNA studies were obtained from Charles River Laboratories (Kingston, NY). Prior to conduct of the mouse LLNA, an initial screen was performed to evaluate primary irritation to mouse ears and dermal toxicity. Ear thickness measurements (digital micrometer) and erythema scores were recorded prior to administration of dosing solutions and approximately 24 hours after the final test material administration (n=1 mouse/dose concentration). The administration of the materials (25 µl per ear) was made for two consecutive days using an adjustable pipettor with a disposable tip. Each mouse received one concentration of either the BMIC in DMF or DMF alone on the dorsal surface of each ear. The applied concentrations of BMIC (75%, 50%, 25%, 12.5%, 6.25%, and vehicle alone) were based upon solubility using DMF. Irritation was scored on a scale of 0 to 4 representing no erythema (0)

to well-defined erythema (2), to eschar damage (4). Based on the probe study, exposure levels of 0.4%, 2%, or 10% w/v were selected for the LLNA.

The LLNA was conducted similarly to that described by Kimber and Weisenberger, 1989) and EPA (2003), using female BALB/c mice (Woolhiser et al., 2000). All mice (six mice/group) received one of three concentrations of BMIC or vehicle (DMF), for three consecutive days. On day 6 (two days rest), mice received a 250 µl intravenous (i.v.) tail injection containing 20 µCi of ^{3}H-thymidine (specific activity 2 Ci/mmol; Amersham Biosciences code TRA310) diluted in phosphate-buffered saline (PBS). Approximately five hours later, the mice were euthanized and the draining auricular lymph nodes located at each bifurcation of the jugular veins were excised and pooled for each mouse. A single cell suspension of lymph node cells was prepared by gentle mechanical disaggregation using a tissue homogenizer (Stomacher® 80 Lab System, Seward Medical Unlimited, London, U.K.). The cells were washed twice using PBS and were suspended in 3 ml of 5% trichloroacetic acid (TCA) overnight. The suspended precipitates were centrifuged (200 x g for 10 minutes) and the supernatant removed. The pellets were reconstituted in 1 ml of 5% TCA and subsequently transferred to a scintillation vial containing 10 ml of Aquasol-2 scintillation cocktail (Packard Instrument Company, Meridan, Connecticut) along with two, 2-ml aliquots of water used to rinse the tubes. The radioactivity in each precipitate was measured using a β-scintillation counter and reported as disintegration per minute (dpm) per mouse. SI was calculated using the absolute dpm value for each mouse as the numerator, and the mean dpm value from the vehicle control mice as the denominator.

The Effective Concentration estimated to cause a 3-fold stimulation (SI = Stimulation Index) in the auricular lymph node (EC_3) was calculated according to Basketeer, et al. (2000). A three-fold increase in stimulation has been used as a threshold for categorization as a skin sensitizer. A concentration that causes a three-fold increase is used as an indication of potency. Data from this study (absolute and body weight gains and dpm) were analyzed by a one-way analysis of variance (Steele and Torrie, 1960). Comparisons of treated vs. control groups were done by Dunnett's t-test (Steele and Torrie, 1960) when ANOVA results suggested differences, alpha = 0.05.

The acute toxicity studies were conducted at Product Safety Laboratories, Dayton, NJ, under the direction of G.E. Moore. The LLNA was conducted by P.K. Anderson at the Toxicology and Environmental Consulting Lab, The Dow Chemical Company, Midland, MI.

RESULTS AND DISCUSSION

The two rats administered 175 mg/kg BMIC by oral gavage survived, gained body weight, and appeared active and healthy. There were no signs of gross toxicity, adverse clinical signs, or abnormal behavior. No gross abnormalities

were noted for either of the animals when necropsied at the conclusion of the 14-day observation period. One of four animals given 550 mg/kg died within one day of test substance administration. There were no adverse clinical signs noted prior to death. The three remaining animals survived, gained body weight, and appeared active and healthy. There were no signs of gross toxicity, adverse clinical signs, or abnormal behavior in these three animals. Gross necropsy of the decedent revealed discoloration of the intestines. No gross abnormalities were noted for any of the surviving animals when necropsied at the conclusion of the 14-day observation period. All three animals died within one day of administering 2,000 mg/kg. Adverse clinical signs noted prior to death included hypoactivity and abnormal posture. Gross necropsy of the decedents revealed discoloration of the intestines. Under the conditions of this study, the acute oral LD50 of 1-butyl-3-methylimidazolium chloride was estimated to be 550 mg/kg of body weight in female rats with a PL2 Confidence interval of 380.9 mg/kg (lower) to 1,710 mg/kg (upper).

Following dermal administration of 2,000 mg/kg (as 95 % BMIC in water), all male and female rats survived, gained weight and appeared active and healthy. Apart from dermal irritation (erythema, edema) noted at the dose site of all animals between Days 1 and 3, there were no signs of gross toxicity, adverse clinical signs, or abnormal behavior. No gross abnormalities were noted for the animals when necropsied at the conclusion of the 14-day observation period.

Following dermal administration of 2,000 mg/kg in DMF (75% BMIC), two of five males died and five of five females died. Toxic signs noted prior to death included hypoactivity, prone posture, and reduced fecal volume. Surviving males also exhibited hypoactivity and reduced fecal volume after BMIC administration. Apart from dermal irritation, which persisted up to day 3, surviving males appeared active and healthy from day 2 to study termination, though one male lost weight through day seven. Gross observations of decedents revealed discolored intestines and/or with injected blood vessels. A black / red semi solid mass was observed in the intestines of four females. No gross pathology observations were seen at fourteen days in any surviving rats. Groups of five females were subsequently administered 200 and 800 mg/kg, with all rats surviving. Only dermal irritation (erythema and edema) was noted in rats given 200 mg/kg. All rats given 800 mg/kg survived, with red ocular discharge noted in two rats. Apart from dermal irritation (up to day five), all rats appeared active and healthy by day 2 and for the remainder of the study. The LD50 for BMIC in water as greater than 2,000 mg/kg (both sexes). The LD50 for BMIC (75%) in DMF was greater than 2,000 mg/kg (males), and between 800 and 2,000 mg/kg (females).

In the acute dermal irritation study, the water moistened BMIC was classified as slightly irritating to the skin (Draize score of 1.5). One hour after patch removal, all three treated sites exhibited well-defined erythema and slight edema. The overall incidence and severity of irritation decreased with time. All animals were free of all dermal irritation within 48 hours.

In the eye irritation study, undiluted BMIC was considered mildly irritating to the eye. One hour after test substance instillation, all three treated eyes exhibited conjunctivitis. No iritis was observed at any time. By 24 hours, one animal also developed corneal opacity. The overall incidence and severity of irritation decreased thereafter. The first animal placed on test was free of ocular irritation by 72 hours and the remaining two animals were free of ocular irritation by Day 7 (study termination).

During the LLNA probe study, two mice given 75% BMIC in DMF died by the following morning. The mouse given 50% BMIC for two consecutive days was also found dead by the third morning. Appreciable ear swelling was noted in mice given 12.5% or 25% BMIC. Although not intended as a lethality determination, 75% BMIC (2 x 25 µl; approximately 20 gram mouse) corresponds to approximately 1,900 mg/kg. This was not inconsistent with the rat dermal lethality findings. Based upon these results, BMIC was subsequently tested in the LLNA at 0.4, 2, and 10% w/v BMIC (10 mg/kg – 250 mg/kg). In the LLNA phase of the study, topical applications of 2% (w/v) BMIC elicited barely perceptible erythema (scores = 1) in 4/6 mice on day 3 which recovered to no erythema (score = 0) on day 6. Topical applications of 10% (w/v) BMIC in the LLNA study phase elicited moderate to severe erythema (scores =3) in 3/5 mice and well-defined erythema (scores = 2) in 2/5, mice all of which were barely perceptible on day 6. While body weight data were unremarkable, one mouse treated with 10% w/v BMIC (approximately 250 mg/kg/day) was found dead prior to dosing on day 3.

BMIC demonstrated LLNA results consistent with some dermal sensitization potential as the lymph nodes draining the area of topical application demonstrated a proliferative response 3-fold greater than vehicle controls. BMIC applications induced proliferation that increased dose responsively as mice treated with 2% or 10% BMIC elicited proliferative responses that were respectively 2.1 +/- 0.5- and 4.3 +/- 1.3 fold greater than vehicle controls. Only one mouse (given 10%) exhibited erythema on the site of application. A BMIC concentration of 5.2% was calculated to cause a 3-fold increase in proliferation (EC3). Particularly in consideration of the modest response at near lethal levels, BMIC exhibited minimal sensitization potential.

The transdermal toxicity of BMIC was influenced by the vehicle of administration. Use the organic solvent, DMF, accentuated the acute toxicity. Very high concentrations of BMIC (up to 95% BMIC in water) applied to the rat skin were markedly less acutely toxic. This may have a practical significance where ionic liquids are handled in pure form, or with water as a cosolvent, in contrast to handling ionic liquids in an organic solvent.

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