NTP
National Toxicology Program
U.S. Department of Health and Human Services

NTP Technical Report on the Toxicity Studies of

Select Ionic Liquids (1-Ethyl-3-Methylimidazolium Chloride, 1-Butyl-3-Methylimidazolium Chloride, 1-Butyl-1-Methylpyrrolidinium Chloride, and N-Butylpyridinium Chloride) Administered in Drinking Water to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats and B6C3F1/N Mice

NTP TOX 103

MAY 2022

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Toxicity Report 103

May 2022

National Toxicology Program
Public Health Service
U.S. Department of Health and Human Services
ISSN: 2378-8992

Research Triangle Park, North Carolina, USA

Foreword

The National Toxicology Program (NTP), established in 1978, is an interagency program within the Public Health Service of the U.S. Department of Health and Human Services. Its activities are executed through a partnership of the National Institute for Occupational Safety and Health (part of the Centers for Disease Control and Prevention), the Food and Drug Administration (primarily at the National Center for Toxicological Research), and the National Institute of Environmental Health Sciences (part of the National Institutes of Health), where the program is administratively located. NTP offers a unique venue for the testing, research, and analysis of agents of concern to identify toxic and biological effects, provide information that strengthens the science base, and inform decisions by health regulatory and research agencies to safeguard public health. NTP also works to develop and apply new and improved methods and approaches that advance toxicology and better assess health effects from environmental exposures.

The Toxicity Report series began in 1991. The studies described in the NTP Toxicity Report series are designed and conducted to characterize and evaluate the toxicological potential of selected substances in laboratory animals (usually two species, rats and mice). Substances (e.g., chemicals, physical agents, and mixtures) selected for NTP toxicity studies are chosen primarily on the basis of human exposure, level of commercial production, and chemical structure. The interpretive conclusions presented in the toxicity reports are derived solely from the results of these NTP studies, and extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection for study per se is not an indicator of a substance's toxic potential.

NTP conducts its studies in compliance with its laboratory health and safety guidelines and Food and Drug Administration Good Laboratory Practice Regulations and meets or exceeds all applicable federal, state, and local health and safety regulations. Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals. Studies are subjected to retrospective quality assurance audits before they are presented for public review. Draft reports undergo external peer review before they are finalized and published.

The NTP toxicity reports are available free of charge on the NTP website and cataloged in PubMed, a free resource developed and maintained by the National Library of Medicine (part of the National Institutes of Health). Data for these studies are included in NTP's Chemical Effects in Biological Systems database.

For questions about the reports and studies, please email NTP or call 984-287-3211.

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National Toxicology Program[1]

[1]Division of the National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA

Collaborators

R.A. Dee, K.R. Ryan, S.A. Elmore, K.L. Aillon, J.W. Algaier, T.P. Arndt, B. Astroff, C.R. Blystone, M.A. Buccellato, B.L. Burback, M.F. Cesta, K.Y. Cimon, A.P. Clark, P.S. Clemons, P.S. Coder, D.A. Contos, J. Cookinham, M.C. Cora, H.C. Cunny, R.H. Elbekai, J.M. Fostel, R.P. Frawley, D.R. Germolec, C.A. Granville, S.W. Graves, S.J. Harbo, M.R. Hejtmancik, M.J. Hooth, A. Kazerooni, A.P. King-Herbert, K.A.B. Knostman, A.J. Mainey, D.E. Malarkey, B.S. McIntyre, D. Messer, E. Mutlu, E. Mylchreest, B. O'Brien, M. Paranjpe, K.M. Patton, J.S. Richey, G.K. Roberts, M. Roth, M.J. Ryan, K.E. Schnare, K.E. Setty, S.A. Shah, K.A. Shipkowski, K.R. Shockley, L. Siemann, A.J. Skowronek, N.L. South, B.R. Sparrow, M.D. Stout, G.S. Travlos, S. Waidyanatha, N.J. Walker, M.L. Wenk, K.L. Witt, A.M. Zmarowski

Division of the National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA

Designed studies, evaluated and interpreted results, and reported findings

R.A. Dee, Ph.D., Co-Study Scientist
K.R. Ryan, Ph.D., Co-Study Scientist
S.A. Elmore, D.V.M., M.S., Study Pathologist
C.R. Blystone, Ph.D.
M.F. Cesta, D.V.M., Ph.D.
M.C. Cora, D.V.M.
H.C. Cunny, Ph.D.
R.P. Frawley, M.S.
D.R. Germolec, Ph.D.
M.J. Hooth, Ph.D.
A.P. King-Herbert, D.V.M.
D.E. Malarkey, D.V.M., Ph.D. (Retired)
B.S. McIntyre, Ph.D.
E. Mutlu, Ph.D.
G.K. Roberts, Ph.D.
K.A. Shipkowski, Ph.D.
K.R. Shockley, Ph.D.
M.D. Stout, Ph.D.
G.S. Travlos, D.V.M.
S. Waidyanatha, Ph.D.
N.J. Walker, Ph.D.
K.L. Witt, M.S.

Provided oversight for data management
J.M. Fostel, Ph.D.

Battelle, Columbus, Ohio, USA
Conducted 3-month studies and evaluated pathology findings
M.R. Hejtmancik, Ph.D., Principal Investigator
B.R. Sparrow, Ph.D., Principal Investigator
T.P. Arndt, Ph.D.
M.A. Buccellato, D.V.M., Ph.D.
P.S. Coder, Ph.D.
C.A. Granville, Ph.D.
S.J. Harbo, D.V.M.
K.A.B. Knostman, D.V.M., Ph.D.
E. Mylchreest, Ph.D.
K.M. Patton, D.V.M., Ph.D.
M.J. Ryan, D.V.M., Ph.D.
A.J. Skowronek, D.V.M., Ph.D.
A.M. Zmarowski, Ph.D.

Conducted prestart chemistry activities and dose formulations
B.L. Burback, Ph.D.
P.S. Clemons, Ph.D.
D.A. Contos, M.S.
S.W. Graves, B.S.
J.S. Richey, M.S.
N.L. South, B.S.

BioReliance Corporation, Rockville, Maryland, USA
Conducted 2-week studies
M.L. Wenk, Ph.D., Principal Investigator
R.H. Elbekai, Ph.D.
M. Paranjpe, D.V.M., Ph.D.
S.A. Shah, Ph.D.

MRIGlobal, Kansas City, Missouri, USA
Conducted preliminary chemistry activities
J.W. Algaier, Ph.D., Principal Investigator
A.P. Clark, B.S.E., Co-Principal Investigator
K.L. Aillon, Ph.D.
B. Astroff, Ph.D.
J. Cookinham, B.S.
A. Kazerooni, M.S.
A.J. Mainey, B.S.
D. Messer, Ph.D.
B. O'Brien, M.S.
M. Roth, B.S.
K.E. Schnare, B.S.

L. Siemann, B.S.

Experimental Pathology Laboratories, Inc., Research Triangle Park, North Carolina, USA
Provided pathology review
K.Y. Cimon, D.V.M.

Coordinated NTP Pathology Working Group on 3-month mice (July 30, 2019)
K.Y. Cimon, D.V.M.

ICF, Fairfax, Virginia, USA
Contributed to technical writing and data integration and ensured report quality
K.E. Setty, Ph.D.

Contributors

Division of the National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA
Provided oversight of external peer review
S.L. Scruggs, Ph.D.
M.S. Wolfe, Ph.D.

Kelly Government Services, Research Triangle Park, North Carolina, USA
Supported external peer review
E.A. Maull, Ph.D. (retired from NIEHS, Research Triangle Park, North Carolina, USA)

Experimental Pathology Laboratories, Inc., Research Triangle Park, North Carolina, USA
Supervised pathology review
G.A. Willson, B.V.M.S., Principal Investigator

NTP Pathology Working Group, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA
Participated in NTP Pathology Working Group on 3-month mice (July 30, 2019)
R. Adler, D.V.M., Ph.D., GlaxoSmithKline
A.E. Brix, D.V.M., Ph.D., Experimental Pathology Laboratories, Inc.
M.F. Cesta, D.V.M., Ph.D., National Institute of Environmental Health Sciences
S.A. Elmore, D.V.M., M.S., National Institute of Environmental Health Sciences
G. Krane, D.V.M., National Institute of Environmental Health Sciences
C.J. Willson, D.V.M., Ph.D., Integrated Laboratory Systems, LLC

Integrated Laboratory Systems, LLC, Research Triangle Park, North Carolina, USA
Conducted micronucleus and bacterial mutagenicity assays
L. Recio, Ph.D., Principal Investigator
C.A. Hobbs, Ph.D.
C.D. Swartz, D.V.M., Ph.D.

CSS Corporation, Research Triangle Park, North Carolina, USA
Prepared quality assessment audits
S. Brecher, Ph.D., Principal Investigator
S. Iyer, B.S.
V.S. Tharakan, D.V.M.

Social & Scientific Systems, a DLH Company, Research Triangle Park, North Carolina, USA
Provided statistical analyses
S.J. McBride, Ph.D., Principal Investigator
L.J. Betz, M.S.
S.F. Harris, M.S.
G. Larson, Ph.D.

ASRC Federal, Research Triangle Park, North Carolina, USA
Prepared data for report
J. Berke, B.S.
P. Brown, B.S.
H. Gong, M.S.
C. Martini, B.S.
C. Myers, M.S.
N. Sayers, B.S.

ICF, Fairfax, Virginia, USA
Provided contract oversight
D. Burch, M.E.M., Principal Investigator
J.A. Wignall, M.S.P.H.

Prepared and edited report
K.A. Addo, Ph.D.
S.K. Colley, M.S.P.H.
K.S. Duke, Ph.D.
S.R. Gunnels, M.A.
T. Hamilton, M.S.
R.C. McGill, B.S.
K.L. McKinley, M.E.M.
M.E. McVey, Ph.D.
K. O'Donovan, B.A.
J.I. Powers, M.A.P.
L.M. Prince, Ph.D.
J.R. Rochester, Ph.D.
S.J. Snow, Ph.D.

Supported external peer review
M.C. Rooney, B.A.
S.K. Whately, B.A.

Peer Review

The National Toxicology Program (NTP) conducted a peer review of the draft *NTP Technical Report on the Toxicity Studies of Select Ionic Liquids (1-Ethyl-3-Methylimidazolium Chloride, 1-Butyl-3-Methylimidazolium Chloride, 1-Butyl-1-Methylpyrrolidinium Chloride, and N-Butylpyridinium Chloride) Administered in Drinking Water to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats and B6C3F1/N Mice* by letter in October 2021 by the experts listed below. Reviewer selection and document review followed established NTP practices. The reviewers were charged to:

1. Peer review the draft *NTP Technical Report on the Toxicity Studies of Select Ionic Liquids (1-Ethyl-3-Methylimidazolium Chloride, 1-Butyl-3-Methylimidazolium Chloride, 1-Butyl-1-Methylpyrrolidinium Chloride, and N-Butylpyridinium Chloride)*.
2. Comment on NTP's interpretations of the data.

NTP carefully considered reviewer comments in finalizing this report.

Peer Reviewers

Sarah Blossom, Ph.D.
Professor of Pharmacology, Department of Pharmaceutical Sciences
The University of New Mexico, College of Pharmacy
Albuquerque, New Mexico, USA

Lawrence Lash, Ph.D.
Professor and Associate Department Chair, Department of Pharmacology
Wayne State University, School of Medicine
Detroit, Michigan, USA

Publication Details

Publisher: National Toxicology Program

Publishing Location: Research Triangle Park, NC

ISSN: 2378-8992

DOI: https://doi.org/10.22427/NTP-TOX-103

Report Series: NTP Toxicity Report Series

Report Series Number: 103

Official citation: National Toxicology Program (NTP). 2022. NTP technical report on the toxicity studies of select ionic liquids (1-ethyl-3-methylimidazolium chloride, 1-butyl-3-methylimidazolium chloride, 1-butyl-1-methylpyrrolidinium chloride, and n-butylpyridinium chloride) administered in drinking water to Sprague Dawley (Hsd:Sprague Dawley® SD®) rats and B6C3F1/N mice. Research Triangle Park, NC: National Toxicology Program. Toxicity Report 103.

Acknowledgments

This work was supported by the Intramural Research Program (ES103316, ES103318, and ES103319) at the National Institute of Environmental Health Sciences, National Institutes of Health and performed for the National Toxicology Program, Public Health Service, U.S. Department of Health and Human Services under contracts HHSN271201800012I, HHSN273201800006C, HHSN273201600011C, GS00Q14OADU417 (Order No. HHSN273201600015U), HHSN273201600020C, HHSN273201500006C, HHSN273201500014C, HHSN273201400001C, HHSN273201400015C, HHSN273201400020C, HHSN273201300009C, HHSN316201200054W, HHSN273201100001C, N01-ES-45517, N01-ES-55536, N01-ES-55552, and N01-ES-75408.

Abstract

Ionic liquids (ILs) are synthetic solvents with applications in a variety of industrial and chemical industries. Human exposure to this diverse chemical class is primarily through dermal or oral routes. Research suggests toxicity may be associated with IL structural characteristics, including the type of cation base or alkyl chain substitutions associated with the cation. To further investigate this hypothesis, the National Toxicology Program (NTP) conducted 3-month toxicity studies in male and female Sprague Dawley (Hsd:Sprague Dawley® SD®) rats and B6C3F1/N mice (n = 10/sex/exposure group; 3 exposure concentrations per IL) to compare the relative toxicities of four ILs administered via drinking water—1-ethyl-3-methylimidazolium chloride (Emim-Cl),1-butyl-3-methylimidazolium chloride (Bmim-Cl), 1-butyl-1-methylpyrrolidinium chloride (Bmpy-Cl), and n-butylpyridinium chloride (NBuPy-Cl).

To select exposure concentrations for the 3-month studies, 2-week drinking water studies in rats and mice were conducted to assess palatability and toxicity of each IL. Informed by the literature and preliminary palatability studies, exposure concentrations in the 2-week studies ranged from 0 to 100 mg/mL. Clinical observations (e.g., thinness and ruffled fur), lower water consumption, and lower mean body weights were associated with higher IL exposure concentrations. At the end of the 2-week exposure period, a range of organ weight changes and histological lesions was observed in rats and mice exposed to ILs. These observations were considered secondary to body weight changes and/or stress, rather than a direct toxic effect from 2-week IL exposure.

Exposure concentrations (ranging from 0 to 30 mg/mL) were selected for the 3-month studies because of the observed relative decreases in mean body weight (≤10%) and water consumption (<25%) in the 2-week studies. Rats were exposed to lower concentrations than mice for Emim-Cl, Bmim-Cl, and NBuPy-Cl. Bmpy-Cl exposure concentrations differed by sex and species. Like the 2-week studies, a general pattern of lower water consumption at higher IL exposure concentrations was observed among rats and mice. Estimated average IL compound consumption indicated that mice consumed more IL per unit of body weight than rats at comparable drinking water concentrations for all ILs.

Rats exposed to Emim-Cl and Bmim-Cl for 3 months had similar terminal mean body weights compared to the control group at all exposure concentrations. Rats exposed to Bmpy-Cl and NBuPy-Cl, however, had significantly decreased terminal mean body weights at high exposure concentrations. Mice exposed to all ILs had significantly decreased terminal mean body weights, and male mice typically showed greater body weight differences than female mice at comparable concentrations. Several organ weight changes occurred in male and female mice, but most of these changes were considered secondary effects of decreased body weight and/or water consumption.

Rats exposed to ILs did not display an increase in histological changes; however, mice exposed to ILs displayed a significant increase in the incidences of nonneoplastic kidney and adrenal gland lesions. Incidences of chronic progressive nephropathy were significantly increased in male mice at the highest exposure concentrations of Emim-Cl and NBuPy-Cl. A positive trend for this lesion was observed in male mice exposed to Emim-Cl, Bmim-Cl, and NBuPy-Cl, as well as in female mice exposed to Bmim-Cl and NBuPy-Cl. Additionally, significantly increased incidences of renal tubule cytoplasmic alteration occurred in male mice exposed to the highest concentrations of Emim-Cl, Bmpy-Cl, or NBuPy-Cl. In female mice, the incidences of a

persistent X-zone in the adrenal cortex were significantly increased for all four ILs at the highest exposure concentrations.

Additional data collected from this study suggest IL exposure has minimal effects on hematological or clinical chemistry parameters, does not influence overall survival or the female reproductive cycle, and is generally not genotoxic.

Overall, the results from this comparative study suggest that Emim-Cl, Bmim-Cl, Bmpy-Cl, and NBuPy-Cl in drinking water have minimal effects on rats and mice at low exposure concentrations (<3 mg/mL). Exposure to higher IL concentrations (≥3 mg/mL) results in lower body weights and nonneoplastic lesions in the kidneys of male and female mice and in the adrenal glands of female mice. The lowest-observed-effect levels (LOELs) were determined using these data. The LOELs were assigned as 10 mg/mL for Emim-Cl-exposed male mice, 3 mg/mL for Bmim-Cl-exposed male mice, 10 mg/mL for Bmpy-Cl-exposed male mice, and 6 mg/mL for NBuPy-Cl-exposed male mice. The LOELs for female mice differed slightly. The LOELs were 30 mg/mL for Emim-Cl-exposed female mice, 3 mg/mL for Bmim-Cl-exposed female mice (the same as for Bmim-Cl-exposed male mice), 3 mg/mL for Bmpy-Cl-exposed female mice, and 3 mg/mL for NBuPy-Cl-exposed female mice. These studies indicate that IL-induced toxicity may be attributable to alkyl chain length and cation type. ILs with longer alkyl chains typically exhibit increased toxicity compared to ILs with shorter alkyl chains. In this report, mice exposed to Bmim-Cl (an IL with a 4-carbon alkyl chain) had a lower LOEL than did mice exposed to Emim-Cl (an IL with a 2-carbon alkyl chain). The difference in toxicity among cations (imidiazolium vs. pyrrolidinium vs. pyridinium), however, is less clear and is both sex- and endpoint-dependent.

Synonyms:

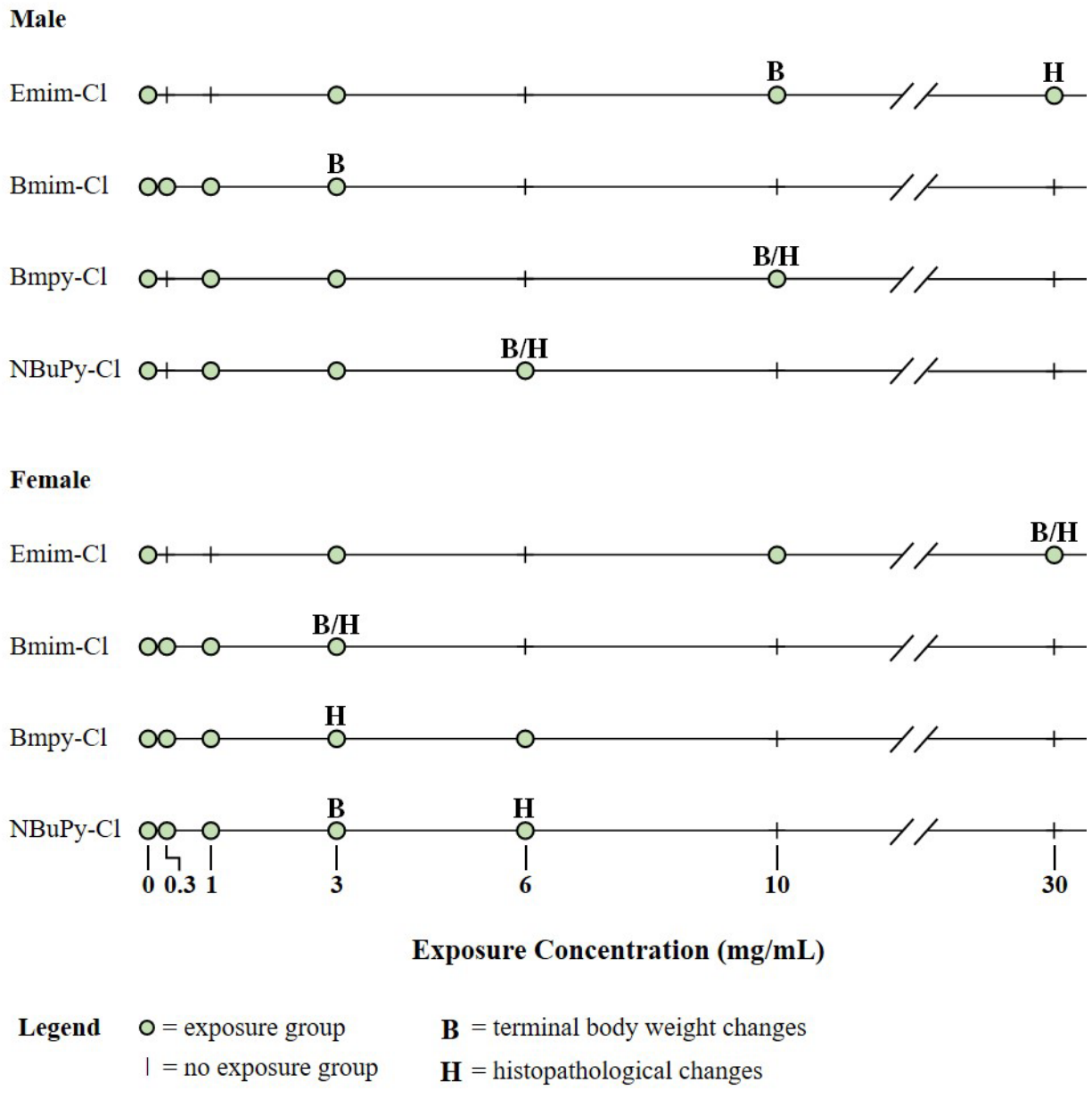
- 1-ethyl-3-methylimidazolium chloride: 1-ethyl-3-methyl-1H-imidazol-3-ium chloride; 1H-imidazolium, 1-ethyl-3-methyl-, chloride (1:1)
- 1-butyl-3-methylimidazolium chloride: 1-butyl-3-methyl-1H-imidazol-3-ium chloride; 1H-imidazolium, 3-butyl-1-methyl-, chloride (1:1); 1-n-butyl-3-methylimidazolium chloride; 3-butyl-1-methyl-1H-imidazol-3-ium chloride
- 1-butyl-1-methylpyrrolidinium chloride: 1-butyl-1-methylpyrrolidin-1-ium chloride; n-butyl-N-methylpyrrolidinium chloride; pyrrolidinium, 1-butyl-1-methyl-, chloride (1:1)
- N-butylpyridinium chloride: 1-butylpyridin-1-ium chloride

Summary of Findings Considered Toxicologically Relevant in Male and Female Rats and Mice Exposed to Ionic Liquids in Drinking Water for Three Months

	Male Sprague Dawley Rats	Female Sprague Dawley Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Concentrations in Water (mg/mL)				
Emim-Cl	0, 1, 3, or 10	0, 1, 3, or 10	0, 3, 10, or 30	0, 3, 10, or 30
Bmim-Cl	0, 0.1, 0.3, or 1	0, 0.1, 0.3, or 1	0, 0.3, 1, or 3	0, 0.3, 1, or 3
Bmpy-Cl	0, 0.3, 1, or 3	0, 1, 3, or 6	0, 1, 3, or 10	0, 1, 3, or 6
NBuPy-Cl	0, 0.3, 1, or 3	0, 0.3, 1, or 3	0, 1, 3, or 6	0, 1, 3, or 6
Survival Rates[a]	No effect[b,c]	No effect	No effect	No effect
Terminal Mean Body Weights[d]				
Emim-Cl	No effect	No effect	↓	↓
Bmim-Cl	No effect	No effect	↓	↓
Bmpy-Cl	↓	↓	↓	No effect
NBuPy-Cl	↓	↓	↓	↓
Clinical Findings[a]	No effect	No effect	No effect	No effect
Water Consumption[d]	↓	↓	↓	↓
Organ Weights[d]				
Emim-Cl	No effect	↑ Relative kidney weight	No effect	No effect
Bmim-Cl	↓ Absolute heart weight	↓ Absolute lung weight	No effect	No effect
Bmpy-Cl	No effect	↓ Absolute and relative liver weight	↑ Relative liver weight	↑ Relative kidney weight
NBuPy-Cl	No effect	No effect	↓ Absolute and ↑ relative kidney weight ↓ Absolute and ↑ relative liver weight	No effect
Nonneoplastic Findings				
Emim-Cl	No effect	No effect	Kidney: infarct, (0/10, 0/10, 0/10, 2/10); chronic progressive nephropathy (1/10, 1/10, 2/10, 8/10); renal tubule, cytoplasmic alteration (0/10, 0/10, 0/10, 9/10)	Adrenal gland: adrenal cortex, X-zone, persistent (0/10, 1/10, 0/10, 8/10)
Bmim-Cl	No effect	No effect	Kidney: chronic progressive nephropathy (1/10, 3/10, 0/10, 5/10)	Kidney: chronic progressive nephropathy (0/10, 0/10, 1/10, 3/10) Adrenal gland: adrenal cortex, X-zone, persistent (0/10, 1/10, 0/10, 5/10)

	Male Sprague Dawley Rats	Female Sprague Dawley Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Bmpy-Cl	No effect	No effect	Kidney: renal tubule, cytoplasmic alteration (0/10, 0/10, 0/10, 5/10)	Adrenal gland: adrenal cortex, X-zone, persistent (0/10, 1/10, 4/10, 4/10)
NBuPy-Cl	No effect	No effect	Kidney: chronic progressive nephropathy (2/10, 0/10, 4/10, 8/10); renal tubule, cytoplasmic alteration (0/10, 0/10, 0/10, 10/10)	Kidney: chronic progressive nephropathy (2/10, 0/10, 2/10, 5/10) Adrenal gland: adrenal cortex, X-zone, persistent (0/10, 2/10, 3/10, 9/10)
Clinical Pathology[a]	No effect	No effect	No effect	No effect
Reproductive Findings[a]	No effect	No effect	No effect	No effect
Genetic Toxicology				
Bacterial Mutagenicity[a]		Negative in *Salmonella typhimurium* strains TA98 and TA100 and *Escherichia coli* strain WP2 *uvrA* (pKM101), with and without S9		
Micronucleated Erythrocytes (In Vivo)				
Rat peripheral blood		Emim-Cl and Bmim-Cl: negative in males and females Bmpy-Cl: positive in males and negative in females NBuPy-Cl: equivocal in males and negative in females		
Mouse peripheral blood		Emim-Cl, Bmim-Cl, Bmpy-Cl, and NBuPy-Cl: negative in males and females		

Emim-Cl = 1-ethyl-3-methylimidazolium chloride; Bmim-Cl = 1-butyl-3-methylimidazolium chloride; Bmpy-Cl = 1-butyl-1-methylpyrrolidinium chloride; NBuPy-Cl = n-butylpyridinium chloride.
[a]Results were consistent across all four ionic liquids evaluated.
[b]One male rat exposed to 3 mg/mL NBuPy-Cl died during the study; however, this death was not considered related to exposure.
[c]No effect = no toxicologically relevant effects for this endpoint.
[d]Results reported for the highest exposed group.



Lowest-observed-effect Levels Related to Significant Decreases in Terminal Mean Body Weights and/or Histopathological Changes in Male and Female Mice

Emim-Cl = 1-ethyl-3-methylimidazolium chloride; Bmim-Cl = 1-butyl-3-methylimidazolium chloride; Bmpy-Cl = 1-butyl-1-methylpyrrolidinium chloride; NBuPy-Cl = n-butylpyridinium chloride.

Overview

Ionic liquids (ILs) are synthetic solvents composed of organic cations and organic or inorganic anions. Because the cations can have numerous alkyl chain substitutions, the number of potential IL combinations is estimated to exceed 10 trillion.[1] Chemicals from this class have replaced volatile organic compounds (VOCs) in the pharmaceutical, chemical, and energy industries, among others, because they are potentially less toxic and more environmentally friendly than VOCs.[2; 3]

Although research has shown that some ILs are ecotoxic,[4] few studies characterize the toxicity of these compounds in humans. In a recent metanalysis of ionic liquid literature and toxicology, in vivo mammalian toxicity studies constituted only 8% of all tests conducted with these compounds.[5] Four ionic liquids—1-ethyl-3-methylimidazolium chloride (Emim-Cl), 1-butyl-3-methylimidazolium chloride (Bmim-Cl), 1-butyl-1-methylpyrrolidinium chloride (Bmpy-Cl), and n-butylpyridinium chloride (NBuPy-Cl)—were nominated to the National Toxicology Program (NTP) for testing due to widespread interest in using them as solvent alternatives to VOCs. These four compounds were recommended for toxicity testing because, at the time of nomination, they represented the three most common cation classes (i.e., imidazolium, pyridinium, and pyrrolidinium) used in various industrial applications. In addition to investigating whether one cation is more toxic than another, two imidazolium compounds with different chain lengths (2-carbon Emim-Cl and 4-carbon Bmim-Cl) were nominated to determine whether alkyl chain length can contribute significantly to toxicity.

The structure of an IL determines its stability, bioavailability, and potential toxicity. Thus, NTP aimed to determine the general and relative toxicity of members of this chemical class and to test the hypothesis that specific structural variations (e.g., alkyl chain length) alter relative toxicity. Because of the potential for human exposure, NTP designed a series of studies to investigate effects of these four ILs on skin sensitization, genotoxicity, and short-term and subchronic toxicity and to learn more about their absorption, distribution, metabolism, and excretion. This toxicity report presents results from the 2-week (short-term) and 3-month (subchronic) studies of adult male and female rats and mice following IL exposure via drinking water.

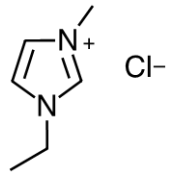
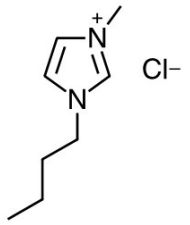
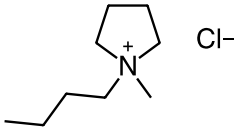
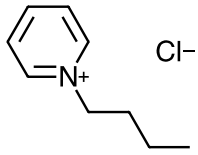
Introduction

Chemical and Physical Properties

Structurally, ionic liquids (ILs) consist of a cation with an alkyl side chain and an anion. ILs' chemical and physical properties are widely documented in published manuscripts, review articles, and book chapters. Most ILs are liquid at room temperature (although some are solid) and have low vapor pressure.[1; 6-8] Other properties can be diverse, but most have low volatility and flammability, a melting point below 100°C, high thermal stability, and the ability to act as solvents for a range of polar and nonpolar compounds. Their solubility and other physical-chemical properties allow for formation of application-specific ILs; therefore, this chemical class is often referred to as "designer solvents."[1; 7; 9-11] Some "designer" ILs improve industrial processes by reducing wastes and increasing the efficiency of product extraction.[12]

The ILs assessed in this report are chlorides of 1-ethyl-3-methylimidazolium (Emim-Cl), 1-butyl-3-methylimidazolium (Bmim-Cl), 1-butyl-1-methylpyrrolidinium (Bmpy-Cl), and n-butylpyridinium (NBuPy-Cl) cations. All are solid at room temperature with relatively low melting points (predicted averages from 56.7ºC to 107ºC) and a pH ranging from 7 to 8.[13-16] Table 1 provides basic information for the ILs evaluated in the present studies.

Table 1. Ionic Liquids Evaluated

Chemical Name[a]	Chemical Formula	CASRN	Molecular Weight (g/mol)	Structure
1-ethyl-3-methylimidazolium chloride (Emim-Cl) 1-ethyl-3-methyl-1H-imidazol-3-ium chloride[b] 1H-imidazolium, 1-ethyl-3-methyl-, chloride (1:1)[b]	$C_6H_{11}N_2Cl$	65039-09-0	146.62	
1-butyl-3-methylimidazolium chloride (Bmim-Cl) 1-butyl-3-methyl-1H-imidazol-3-ium chloride[b] 1H-imidazolium, 3-butyl-1-methyl-, chloride (1:1)[b,c] 1-n-butyl-3-methylimidazolium chloride[b,c] 3-butyl-1-methyl-1H-imidazol-3-ium chloride[c]	$C_8H_{15}N_2Cl$	79917-90-1	174.67	
1-butyl-1-methylpyrrolidinium chloride (Bmpy-Cl) 1-butyl-1-methylpyrrolidin-1-ium chloride[b,c] n-butyl-N-methylpyrrolidinium chloride[c] pyrrolidinium, 1-butyl-1-methyl-, chloride (1:1)[b,c]	$C_9H_{20}NCl$	479500-35-1	177.72	
n-butylpyridinium chloride (NBuPy-Cl) 1-butylpyridin-1-ium chloride[b,c]	$C_9H_{14}NCl$	1124-64-7	171.67	

[a]The four ionic liquid chemical names (in bold) and common synonyms are listed.
[b]Common synonym listed in EPA CompTox Chemicals Dashboard.[17]
[c]Common synonym listed in PubChem.[18]

Production, Use, and Human Exposure

The history of IL use and production is well documented.[5; 11; 19-21] A market analysis published in 2016 noted, "the global ionic liquids market was valued at USD 20.36 million in 2015," and "North America contributed to over 30% of the total global ionic liquids market."[22] IL production volumes in the United States are not readily available, but several U.S.-based companies (e.g., Ionic Liquid Chemicals LLC; NanoTechLabs, Inc.; Strem Chemicals, Inc.; and TCI America) are currently producing and/or selling these compounds in bulk or smaller quantities. In 2020 PubChem vendor lists, Emim-Cl, Bmim-Cl, Bmpy-Cl, and NBuPy-Cl were available from many suppliers.[23-26]

ILs are synthetic solvents with applications in the chemical, pharmaceutical, and energy industries. Certain physiochemical properties make these compounds favorable for use in catalysis, synthesis, separation, and polymerization processes.[8] ILs may also be effective in medical applications such as drug delivery systems[27; 28] or antimicrobial agents.[3; 29] Some identified uses of Emim-Cl, Bmpy-Cl, and NBuPy-Cl include catalysis and synthesis reactions, battery electrolysis, and other electrochemical uses. Bmim-Cl uses include biomass conversion stabilization[30] and biocatalysis processes,[31; 32] as well as extraction methods for biomolecules[33; 34] due to its nondenaturing properties.

Human exposure to ILs, including the four assessed in this report, is expected to occur primarily through the dermal and oral routes.[35] Occupational handling of ILs for a variety of applications can result in dermal exposure.[36-38] Handling (e.g., drumming and bagging) the powdered materials also can result in ocular and inhalation exposures. For the general population, direct exposure could result from use and disposal of consumer products, and indirect exposure could occur via drinking water (i.e., where source waters are affected by industrial discharges). Consumer products such as batteries, electrochromic devices, fuel cells, plastics, and surgical implants may contain ILs.[4; 7; 10; 39] Some ILs, including Bmim-Cl, have been used in cosmetics, fragrances, hair care products, and household cleaning products.[35; 39; 40] Insufficient data exist to define the potential for human exposure and environmental contamination from these compounds. Bubalo and colleagues have proposed, however, that more hydrophobic ILs could become persistent contaminants in the environment, while less adsorbent, more mobile ILs could be transported to surface and groundwater, possibly contaminating aquatic ecosystems or drinking water supplies.[8] Oral exposures through drinking water are anticipated because many ILs are soluble in water and challenging to remove from wastewater.[41; 42]

Regulatory Status

No federal or state regulations have been established to protect human health or the environment from Emim-Cl, Bmim-Cl, Bmpy-Cl, NBuPy-Cl, or any other IL.

Absorption, Distribution, Metabolism, and Excretion

Experimental Animals

The National Toxicology Program (NTP) investigated the absorption, distribution, metabolism, and excretion (ADME) and toxicokinetic properties of Bmim-Cl and NBuPy-Cl in male Fischer 344 (F344) rats and female B6C3F1 mice[43; 44] and of Bmpy-Cl in male F344 rats[45] following

single or repeated gavage, intravenous administration, or dermal application. The doses selected in these three studies were 0.5, 5, or 50 mg/kg, representing approximately 0.1%, 1%, or 10%, respectively, of the reported oral median lethal dose (LD_{50}) of 550 mg/kg in female F344 rats.[46] In general, the disposition pattern of the three ILs were similar, with moderate to high oral absorption, low to moderate dermal absorption, rapid excretion, and low tissue retention. The ILs were poorly metabolized, despite the expectation that ILs with a butyl substitution (e.g., Bmim-Cl) would undergo side chain oxidation.[47] No other studies investigating the ADME of ILs were found in the literature.

Cheng et al.[43] administered [^{14}C]NBuPy-Cl by gavage, intravenous, and dermal application. Following a single gavage dose of 50 mg/kg in male F344 rats, [^{14}C]NBuPy-Cl was rapidly absorbed with a maximum blood concentration occurring at 1.3 hours. For all three doses administered (0.5, 5, or 50 mg/kg), excretion was rapid, with 55%–66% and 20%–25% of the dose recovered in urine and feces, respectively, after 24 hours. Urine profiles showed a single peak corresponding to the parent chemical, suggesting poor metabolism. The elimination half-life of [^{14}C]NBuPy-Cl in blood was 2.2 hours after a single intravenous dose of 5 mg/kg, and the estimated oral bioavailability was 47% following administration of a single oral dose of 50 mg/kg [^{14}C]NBuPy-Cl in male rats. After 72 hours, the dose recovered in tissues of male rats orally administered 50 mg/kg was low (approximately 0.01%, based on selected organs). There was no dose-related difference in the disposition of [^{14}C]NBuPy-Cl in male rats following a single dose versus daily gavage administration for 5 days.[43] Following a single gavage dose of 50 mg/kg [^{14}C]NBuPy-Cl in female B6C3F1 mice, the excretion pattern was similar to male rats (72% in urine and 10% in feces after 96 hours). Dermal absorption (estimated based on excreta and dose-site skin) of 5 mg/kg [^{14}C]NBuPy-Cl 96 hours after application to a covered dose site was higher (35% of dose) when formulated in dimethylformamide (DMF)/water (55:45, v/v). The same dermal dose applied in other vehicles (ethanol/water [55:45 v/v] or water alone) was less well absorbed (16% or 10% of the dose, respectively). The pattern of excretion following dermal application was similar to that following oral administration.

Sipes et al.[44] examined oral and dermal ADME of Bmim-Cl in male F344 rats and female B6C3F1 mice. In male rats following a single gavage administration of 0.5, 5, or 50 mg/kg [^{14}C]Bmim-Cl, absorption was rapid with the maximum blood concentration occurring at 67 minutes. Of the administered dose, 72%–78% was excreted in urine and 19%–28% in feces by 72 hours, with the majority eliminated in urine by 24 hours (55%–74%). The blood elimination half-life of [^{14}C]Bmim-Cl was 77.2 minutes (approximately 1.3 hours), and the estimated oral bioavailability was 62.1% (for 50 mg/kg). After 72 hours, retention of radioactivity in tissue was negligible. The chemical analysis of urine showed only a single peak corresponding to the parent compound, although fecal profiles showed several peaks that did not coelute with the parent. No dose-related difference was found in the disposition of [^{14}C]Bmim-Cl following a single dose. The disposition of [^{14}C]Bmim-Cl between single and multiple (5 daily doses) gavage dosing was similar. The excretion pattern following a single oral administration of 50 mg/kg [^{14}C]Bmim-Cl in female mice was similar to male rats, with 65% and 18% of the administered dose recovered in urine and feces, respectively, by 72 hours. Dermal absorption of 5 mg/kg [^{14}C]Bmim-Cl 48 hours after a single application to a covered dose site was low to moderate, with 31.7%, 17.8%, or 29.6% (estimated based on excreta and dose-site skin) of the dose absorbed when formulated in DMF/water (1.8:1, v/v), ethanol/water (1.8:1, v/v), and water, respectively.

Knudsen et al.[45] examined oral and dermal ADME of Bmpy-Cl in F344 rats. Following a single gavage dose of 0.5, 5, or 50 mg/kg [^{14}C]Bmpy-Cl in male rats, 29%–38% of the administered dose was eliminated in the urine and 51%–69% was excreted in feces by 72 hours. Maximum blood concentration of [^{14}C]Bmpy-Cl occurred at 1.5 hours, with an elimination half-life of 5.6 hours and an estimated oral bioavailability of 43.4% following oral administration of a single 50 mg/kg dose. The urine profiles showed only a single peak corresponding to the parent chemical, suggesting poor metabolism. The pattern of excretion following repeated gavage with 50 mg/kg/day for 5 consecutive days was similar to that following a single gavage administration. Following a single intravenous administration, 86% of the dose was recovered in urine after 72 hours with approximately 8% in feces. The difference between fecal elimination following intravenous versus oral administration (8% vs. 51%–69%) suggests most of the dose excreted in feces was unabsorbed. Dermal absorption of 5 mg/kg [^{14}C]Bmpy-Cl 96 hours after application to a covered dose site was low to moderate, with higher absorption observed when formulated in DMF/water (63:37, v/v) (34% of dose) compared to when formulated in ethanol/water (63:37, v/v) or water (≤22% of dose). The pattern of excretion following dermal application was similar to that following oral administration.

In vitro studies with these ILs highlight their function as substrates for transport-mediated secretion via human organic cation transporter 2 (hOCT2), rat OCT 1 and 2 (rOCT1/2), as well as multidrug and toxic extrusion transporters (MATEs).[43; 45; 48] These data are consistent with properties of other small molecular weight cation compounds including 1-paraquat, methyl-4-phenylpyridinium, tetraethylammonium, and tributylmethylammonium, which are also substrates for OCTs.[49-52] In addition, NBuPy-Cl, Bmpy-Cl, and Bmim-Cl were determined to be potent inhibitors of rOCT1/2 and hOCT2, much like other structurally similar compounds that potently inhibit hOCT2.[53] While many ILs behave in a similar manner, structure-activity relationship (SAR) studies suggest that slight differences in IL structure can influence their ADME, kinetics, and drug-chemical interactions. For example, Cheng and colleagues determined the OCT inhibitory effects of pyridinium-based ILs are influenced by the length of the alkyl side chain; an increase in the number of carbons on the alkyl chain significantly decreased median inhibitory concentration (IC_{50}).[48] This SAR has been observed for other chemical classes[53; 54]; therefore, additional research is needed to understand the relationship between IL structure and physiochemical and biological properties.

Humans

The literature contains no studies on the ADME of ILs in humans.

Toxicity

In Vitro

The structure of an IL is likely to influence its toxicity; the most significant factors are the associated anion and the alkyl chain length.[8; 55; 56] In cases in which the anion and alkyl chain length are the same, aromatic cations (e.g., pyridinium or imidazolium) tend to be more toxic than nonaromatic cations (e.g., pyrrolidinium) in inhibition respiration assays[57] and plant cytotoxicity models,[4; 58] possibly due to greater hydrophobicity of the cation.[57] When only alkyl chain length differs among ILs, longer carbon chains are associated with increased lipophilicity of the IL, disruption of cellular membranes, and induction of cell death.[8; 59; 60]

For example, assessment of IL toxicity in a Langmuir monolayer cell membrane model, rat IPC-81 leukemia cells, and bacterial bioassays confirmed ILs with alkyl chain lengths of 2–5 carbons exhibit limited toxicity, ILs with chain lengths of 6–10 carbons exhibit linear increases in toxicity with increasing chain length, and ILs with chain lengths >10 carbons exhibit plateaued toxicity.[59; 61-63] Bmim-Cl (4 carbons), 1-octyl-3-methylimidazolium chloride (Omim-Cl, 8 carbons), and 1-dodecyl-3-methylimidazolium chloride (Ddmim-Cl, 12 carbons) inhibited cell growth and viability in mouse J774A.1 macrophage cells,[35] rat pheochromocytoma PC12 cells,[64] and human hepatocellular carcinoma HepG2 cells,[65] respectively. Omim-Cl and Ddmim-Cl also induced DNA damage, oxidative stress, apoptosis, and altered membrane permeability in PC12 and HepG2 cells. In addition, Omim-Cl induced adenosine triphosphate depletion in PC12 cells, and Ddmim-Cl altered expression of apoptosis-related genes in HepG2 cells.[64; 65]

These effects may be due to the surfactant-like activity observed at ≥6 carbons[61] or the very low octanol:water partition coefficient (log K_{ow} = 1) measured at 8–10 carbons.[63] Molecular dynamic simulations indicated that 14-carbon alkyl chains were of similar length to the fatty acids in the lipids, resulting in straight line conformation of the alkyl chain, greater stability of the lipids, and IL aggregates in the membrane.[66] Although the anion component of an IL is not a major contributor to its toxicity, hydrophobic anions (e.g., bis(trifluoromethanesulfonyl)imide [Tf2N]) have been shown to be more toxic than hydrophilic anions (e.g., Cl, tetrafluoroborate [BF4]). This difference in toxicity is evidenced by the lower median effective concentration (EC_{50}) for Emim-Cl-Tf2N (1.69 mM) compared to Emim-Cl (4.19 mM) in inhibition respiration assays[57] and by the increased disruption of the lipid bilayer by hydrophobic anions, relative to hydrophilic anions, in a biomimetic membrane model.[67]

Experimental Animals

High-dose oral exposure to ILs is reported to induce acute toxicity in rodents. Morbidity and mortality increased in adult F344 rats exposed to Bmim-Cl (≥550 mg/kg)[46] and in pregnant CD-1 mice exposed to Emim-Cl (3,000 mg/kg body weight/day; mg/kg/day) and Bmim-Cl (225 mg/kg/day).[68] Gestational exposure of CD-1 mice to Bmim-Cl (≥169 mg/kg/day) and 1-decyl-3-methylimidazolium chloride (Dmim-Cl; ≥75 mg/kg/day) resulted in decreased fetal body weight.[68]

Rodent dermal exposure studies with ILs have shown toxicity to be concentration- and vehicle-dependent. In brief, dermal studies of Bmim-Cl exposure in F344 rats confirmed compound absorption was more effective when the IL was dissolved in DMF:water compared to ethanol:water or water alone.[43; 44] When dissolved in DMF:water, lower concentrations of Bmim-Cl (5 mg/kg) were not well absorbed through the skin. When the concentration increased to 200–800 mg/kg, Bmim-Cl induced dermal irritation. At higher concentrations (2,000 mg/kg), Bmim-Cl in DMF:water resulted in mortality of male and female rats, likely due to an increase in the amount of Bmim-Cl absorbed.[46] Similar effects were observed in female Balb/c mice exposed dermally to Bmim-Cl in DMF. Two percent (approximately 50 mg/kg) Bmim-Cl was nontoxic. When the concentrations increased to 10%–75% Bmim-Cl (≥250 mg/kg), a dose-related effect on dermal irritation and mortality was observed following dermal application.[46]

Skin sensitization potential of Emim-Cl, Bmim-Cl, Bmpy-Cl, and NBuPy-Cl in female Balb/c mice was previously evaluated by NTP using a combined local lymph node (LLNA)/primary

irritancy assay.[69] In addition, these four ILs were evaluated in a suite of in chemico and in vitro assays validated by the OECD (Organisation for Economic Co-operation and Development): Direct Peptide Reactivity Assay (DRPA) (OECD 442C), KeratinoSens™ Assay (OECD 442D), and human Cell Line Activation Test (hClat) (OECD 442E).[70-72] Overall, Bmim-Cl, Bmpy-Cl, and NBuPy-Cl were more potent than Emim-Cl, which was negative in all in vivo, in chemico, and in vitro assessments.[69] NBuPy-Cl was found to be a dermal irritant, but not a sensitizer, when applied to the skin at concentrations of 3.12%–12.5%. NBuPy-Cl was classified as a sensitizer in vitro; however, false positives in these assays have been reported when a compound is also a skin irritant.[73; 74] In vivo, Bmim-Cl and Bmpy-Cl were found to be skin sensitizers at lower applied concentrations of 12.5% and 6.25%, respectively, and were acutely toxic at higher concentrations of 25% and 12.5%, respectively.[69] This result for Bmim-Cl matches that reported by Landry and colleagues.[46] Although other studies suggest that aromatic cations (e.g., Bmim-Cl) are more toxic than nonaromatic cations (e.g., Bmpy-Cl).[57; 58; 75] Frawley et al.[69] found that Bmim-Cl and Bmpy-Cl have similar toxicity in mammalian and in vitro models of hypersensitivity.

Humans

There has been little to no research evaluating the toxicity of ILs to humans. However, the Globally Harmonized System of Classification and Labeling of Chemicals (GHS)-aggregated information for Bmim-Cl, one of the most studied ILs, suggests that human exposure can cause irritation to the eye, skin, and respiratory system (specifically, the mucous membranes in the mouth, pharynx, esophagus, and gastrointestinal tract following swallowing). Inhalation of the compound may result in irritation of the mucous membranes, coughing, and dyspnea.[35; 76]

Genetic Toxicity

The literature contains no studies on the genetic toxicity of ILs in animals or humans.

Study Rationale

ILs were nominated to NTP by the Center for Green Manufacturing, University of Alabama, for toxicological testing because of widespread interest in their use as alternatives to volatile organic solvents and the limited toxicity, ADME, and environmental fate data available for this class of compounds. At the time of nomination, the compounds recommended for evaluation represented the three most common cation classes (e.g., imidazolium, pyridinium, and pyrrolidinium cations) being investigated for use in various industrial applications. NTP characterized both the general and relative toxicity of four ILs to begin evaluating this chemical class and to test the hypothesis that specific structural variations (e.g., cation and/or alkyl chain length) affect the toxicity of ILs.

Materials and Methods

Procurement and Characterization of Ionic Liquids

1-Ethyl-3-methylimidazolium chloride (Emim-Cl) was obtained from Sigma-Aldrich (St. Louis, MO) in three lots (S37784, S46787, and STBB3624); 1-butyl-3-methylimidazolium chloride (Bmim-Cl) was obtained from Solvent Innovations (Koln, Germany) in a single lot (99/787) and from Sigma-Aldrich (St. Louis, MO) in a single lot (STBB3444); 1-butyl-1-methylpyrrolidinium chloride (Bmpy-Cl) was obtained from Solvent Innovations (Koln, Germany) in a single lot (99/831) and from Promy Chemical, Inc. (El Sobrante, CA) in a single lot (20100610); and n-butylpyridinium chloride (NBuPy-Cl) was obtained from Solvent Innovations (Koln, Germany) in a single lot (99/830) and from Promy Chemical, Inc. (El Sobrante, CA) in a single lot (20100610). Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at MRIGlobal (Kansas City, MO) (Appendix A). Reports on analyses performed in support of the ionic liquid (IL) studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

Lot S37784 (Emim-Cl), a faint yellow solid; lot S46787 (Emim-Cl), a brown solid; lot STBB3624 (Emim-Cl), a yellow-brown solid; lot 99/787 (Bmim-Cl), a pale amber solid; lot STBB3444 (Bmim-Cl), a solid white mass with small orange streaks; lot 99/831 (Bmpy-Cl), a white chunky powder; lot 20100610 (Bmpy-Cl), a pale-yellow crystalline powder; lot 99/830 (NBuPy-Cl), an off-white solid with a peach hue; and lot 20100610 (NBuPy-Cl), a pale-yellow crystalline powder, were identified using infrared (IR) spectroscopy, ^{1}H nuclear magnetic resonance (NMR) spectroscopy, and mass spectrometry (MS). In addition, elemental analysis was performed by Prevalere Life Sciences, Inc. (Whitesboro, NY) to confirm the identity of each compound.

The purity of each IL was determined using high-performance liquid chromatography (HPLC) with ultraviolet (UV), evaporative light scattering detection (ELSD), or charged aerosol detection (CAD) (Table A-1). Residual solvent content was determined by weight loss on drying or headspace gas chromatography (GC/headspace) with flame ionization detection (FID), and any impurities were identified using GC with MS detection. The chloride content of each IL was quantified using ion chromatography (IC). Moisture content was measured for each IL using Karl Fischer titration; however, the calculated results are estimates due to the hygroscopic nature of these compounds. The analytical methods and systems used for each respective lot are described in detail in Appendix A. Purity analysis results for each lot are listed in Table 2.

Table 2. Purity of Chemicals in the Two-week and Three-month Drinking Water Studies of Ionic Liquids

Chemical	Lot Number	Water (%)[a]	Elemental Analysis (%)[b]				Cl (% w/w)[c]	Overall Purity (%)	Impurities (%)[d]
			C	H	N	Cl			
Emim-Cl	S37784	0.2	49.01	7.46	18.60	24.17	23.80 ± 0.14	>99	<0.05
	S46787	0.6	49.17	7.82	18.81	24.37	23.50 ± 0.08	>99	<0.05
	STBB3624	1.1	47.27	7.86	17.66	22.19	22.26 ± 0.33	>99	<0.05
Bmim-Cl	99/787	0.5	54.64	8.91	15.83	19.91	20.01 ± 0.02	>99	<0.05
	STBB3444	0.7	53.72	8.71	14.98	18.92	19.28 ± 0.43	>99	<0.05
Bmpy-Cl	99/831	0.5	60.29	11.66	7.78	19.97	19.71 ± 0.27	>99	<0.05
	20100610	1.2	60.43	11.26	7.50	20.67	19.16 ± 0.65	>98	<0.05
NBuPy-Cl	99/830	0.3	62.75	8.31	7.99	20.57	20.47 ± 0.13	>99	0.61[e]
	20100610	0.5	62.66	8.21	7.72	20.49	20.25 ± 0.11	>98	0.73[e]

Emim-Cl = 1-ethyl-3-methylimidazolium chloride; Bmim-Cl = 1-butyl-3-methylimidazolium chloride; Bmpy-Cl = 1-butyl-1-methylpyrrolidinium chloride; NBuPy-Cl = n-butylpyridinium chloride.
[a]Estimates determined by Karl Fischer titration.
[b]Theoretical values (%): Emim-Cl: C, 49.2; H, 7.6; N, 19.1; Cl, 24.2; Bmim-Cl: C, 55.0; H 8.7; N 16.0; Cl 20.3; Bmpy-Cl: C, 60.8; H, 11.3; N 7.9; Cl, 19.9; NBuPy-Cl: C, 63.0; H 8.2; N 8.2; Cl 20.7
[c]Determined by ion chromatography; mean ± standard deviation.
[d]Determined by high-performance liquid chromatography with evaporative light scattering detection (HPLC/ELSD); limit of detection ≥0.05%.
[e]Determined by high-performance liquid chromatography with ultraviolet detection (HPLC/UV).

Accelerated stability studies were conducted on samples of lots STBB3624 (Emim-Cl), STBB3444 (Bmim-Cl), 20100610 (Bmpy-Cl), and 20100610 (NBuPy-Cl). Samples were stored for 2 weeks under an inert headspace in amber glass vials sealed with Teflon®-lined screw-top lids at frozen (−20°C), refrigerated (5°C), room (25°C), and elevated (60°C) temperatures. Stability was confirmed for all analyzed lots at all four temperatures. The bulk chemicals (all lots) were stored at room temperature in sealed amber glass containers under inert gas headspace. Reanalysis of the bulk chemicals on all lots except S46787 (Emim-Cl) were performed, and no degradation was detected.

Preparation and Analysis of Dose Formulations

The dose formulations for the 2-week study were prepared once at MRIGlobal (Kansas City, MO) by mixing Emim-Cl, Bmim-Cl, Bmpy-Cl, or NBuPy-Cl with tap water to give the required concentrations (Table 3). Dose formulations of Emim-Cl were prepared with lot S37784, except for the 100 mg/mL formulation, which was a combination of lots S37784 and S46787. Dose formulations of Bmim-Cl, Bmpy-Cl, and NBuPy-Cl were prepared with lots 99/787, 99/831, and 99/830, respectively (Table A-3).

Homogeneity was confirmed for the highest and lowest dose formulations of each IL. Stability studies were completed for representative formulations of each IL and are described in Appendix A. The storage stability of Emim-Cl, Bmim-C, Bmpy-Cl, and NBuPy-Cl was confirmed at refrigerated temperatures when protected from light for up to 21 days, 42 days, 42 days, and 35 days, respectively. All dose formulations were stored protected from light at

refrigerated temperatures (2°C–8°C) for ≤7 days until shipment to the study laboratory (BioReliance, Rockville, MD). At the study laboratory, all dose formulations were stored protected from light in a plastic carboy at refrigerated temperatures (2°C–8°C) and used within 42 days.

Analyses of preadministration and postadministration dose formulations for the 2-week studies of Emim-Cl, Bmim-Cl, and NBuPy-Cl were conducted once for each study by the MRIGlobal analytical chemistry laboratory using HPLC/UV. HPLC/ELSD was used to conduct analysis of preadministration and postadministration dose formulations of Bmpy-Cl. Postadministration dose formulation analysis results are averages from animal room drinking water bottles and bulk containers. All dose formulations were within 10% of the target concentrations for all four chemicals (Emim-Cl [Table A-5], Bmim-Cl [Table A-8], Bmpy-Cl [Table A-11], and NBuPy-Cl [Table A-14]). The pH of the Emim-Cl and Bmpy-Cl dose formulations was measured postadministration and is reported in Table A-17 and Table A-18, respectively. pH ranged from 7.1 to 9.1 across all samples.

The dose formulations for the 3-month studies were prepared at the Battelle study laboratory (Columbus, OH) by mixing Emim-Cl, Bmim-Cl, Bmpy-Cl, or NBuPy-Cl with tap water to give the required concentrations for each species (Table 4). Dose formulations were prepared with lots STBB3624 (Emim-Cl), STBB3444 (Bmim-Cl), 20100610 (Bmpy-Cl), and 20100610 (NBuPy-Cl), respectively (Table A-4). Stability and homogeneity studies were completed for representative formulations of each IL and described in Appendix A. All four IL dose formulations were considered stable for ≤42 days when stored refrigerated or at room temperature, protected from light. The formulations were stored protected from light in a plastic carboy at refrigerated temperatures (2°C–8°C). Emim-Cl and NBuPy-Cl formulations were used within 35 days of preparation, and Bmim-Cl and Bmpy-Cl formulations were used within 42 days of preparation.

Analyses of preadministration and postadministration dose formulations for the 3-month studies of Emim-Cl, Bmim-Cl, and NBuPy-Cl were conducted once after each new formulation was prepared at the Battelle study laboratory using HPLC/UV. HPLC/CAD was used to conduct similar analyses of the dose formulations for the 3-month study of Bmpy-Cl. All preadministration dose formulations for all four chemicals were within 10% of the target concentrations. All animal room samples for Emim-Cl (Table A-6, Table A-7) and Bmim-Cl (Table A-9, Table A-10) were within 10% of the target concentrations. All animal room samples for Bmpy-Cl (Table A-12, Table A-13) were within 10% of the target concentrations, apart from three samples for the rat study and five samples for the mouse study (10.7%–15.8%). All animal room samples for NBuPy-Cl (Table A-15, Table A-16) were within 10% of the target concentrations, apart from one carboy sample for the rat study, which was 13.1% above the target concentration.

Animal Source

Male and female Sprague Dawley (Hsd:Sprague Dawley® SD®) rats were obtained from Envigo (formerly Harlan Laboratories, Inc., Indianapolis, IN). Male and female B6C3F1/N mice were obtained from the National Toxicology Program (NTP) colony maintained by Taconic Biosciences, Inc. (Germantown, NY).

Animal Welfare

Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. All animal studies were conducted in an animal facility accredited by AAALAC International. Studies were approved by the BioReliance (Rockville, MD) and Battelle (Columbus, OH) Animal Care and Use Committees and conducted in accordance with all relevant National Institutes of Health and NTP animal care and use policies and applicable federal, state, and local regulations and guidelines.

Two-week Studies

The 2-week dose range-finding studies were designed to run concurrently. Thus, all four ILs evaluated for toxicity were compared to the same set of control animals. Details of the experimental design are shown in Table 3. Concentrations for the 2-week studies were informed by the water solubility of each IL and preliminary palatability studies (e.g., changes in mortality, clinical signs of toxicity, body weight decreases, and feed/water consumption) in rats and mice (data not shown). Additional information in the literature suggested that Emim-Cl was less toxic than Bmim-Cl. Therefore, a high exposure concentration of 100 mg/mL was selected for Emim-Cl on the basis of a 10% drinking water concentration, and three lower concentrations were selected to provide overlapping concentrations with the other compounds. Data from the 2-week studies were evaluated for selection of concentrations to be used in the 3-month exposure studies.

Rats and mice were approximately 4 weeks old on receipt. Animals were quarantined for 11 days (male rats), 12 days (female rats), 14 days (male mice), or 15 days (female mice) and were approximately 6 weeks old on the first day of the studies. Rats and mice were randomly assigned to either the control group or one of three (Bmim-Cl, Bmpy-Cl, and NBuPy-Cl) or four (Emim-Cl) exposed groups. Randomization was stratified by body weight that produced similar group mean weights using Provantis^{TM}, Version 6.5.0.1 software (Instem, Philadelphia, PA).

Due to the short duration of the study, disease screening was not conducted at the study laboratory; however, both rats and mice were obtained from commercial vendors with extensive health surveillance programs. Rats were obtained from a colony free of the following rat pathogens: Sendai virus, pneumonia virus of mice, sialodacryoadenitis virus, Kilham rat virus, Toolan's H1 virus, rat minute virus, reovirus, rat theilovirus, lymphocytic choriomeningitis virus, hantavirus, mouse adenovirus, rat parvovirus, *Mycoplasma pulmonis*, and *Pneumocystis carinii*. Mice were obtained from a colony free of the following mouse pathogens: ectromelia virus, epizootic diarrhea of infant mice (EDIM), lymphocytic choriomeningitis virus, *Mycoplasma pulmonis*, mouse hepatitis virus, mouse norovirus, mouse parvovirus, minute virus of mice, pneumonia virus of mice, reovirus, Sendai, and Theiler's murine encephalomyelitis virus.

Groups of five male and five female rats and mice were provided Emim-Cl, Bmim-Cl, Bmpy-Cl, or NBuPy-Cl in drinking water for 15 days at the following exposure concentrations: Emim-Cl (0, 3, 10, 30, or 100 mg/mL), Bmim-Cl (0, 0.3, 1, or 3 mg/mL for rats; 0, 1, 3, or 6 mg/mL for mice), Bmpy-Cl (0, 1, 3, or 6 mg/mL for rats; 0, 3, 6, or 10 mg/mL for mice), or NBuPy-Cl (0, 1, 3, or 6 mg/mL). Groups of 10 male and 10 female rats and mice were provided tap water as the vehicle control.

Feed and water were available ad libitum. Rats and mice were housed individually (male mice) or five per cage by sex (male and female rats, female mice). Rats and mice were observed twice daily for signs of mortality or moribundity. Clinical observations and body weights were recorded initially, on study day 8, and at the end of the 2-week exposure period. Water consumption data were recorded on study days 1, 4, 8, 11, 14, and 15 for rats and study days 1, 5, 8, 12, and 15 for mice. Details of the study design and animal maintenance are summarized in Table 3. Information on feed composition and contaminants is provided in Appendix B.

Necropsies were performed on all rats and mice. Organ weights were determined for heart, right kidney, liver, lungs, right testis, and thymus. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (except eyes and Harderian gland, which were first fixed in Davidson's solution), processed, and trimmed, embedded in paraffin, sectioned to a thickness of approximately 5 μm, and stained with hematoxylin and eosin (H&E). Complete histopathological examinations were performed by the study laboratory pathologist on all control animals and all animals in the highest exposed group for each IL. Table 3 lists the tissues and organs examined.

Table 3. Experimental Design and Materials and Methods in the Two-week Drinking Water Studies of Ionic Liquids

Rats	Mice
Study Laboratory	
BioReliance (Rockville, MD)	Same as in rats
Strain and Species	
Sprague Dawley (Hsd:Sprague Dawley® SD®)	B6C3F1/N
Animal Source	
Envigo (formerly Harlan Laboratories, Inc., Indianapolis, IN)	Taconic Biosciences, Inc. (Germantown, NY)
Time Held Before Studies	
Males: 11 days	Males: 14 days
Females: 12 days	Females: 15 days
Average Age When Studies Began	
6 weeks	Same as in rats
Date of First Exposure	
March 9 (males) or 10 (females), 2009	March 12 (males) or 13 (females), 2009
Duration of Exposure	
2 weeks	Same as in rats
Date of Last Exposure	
March 23 (males) or 24 (females), 2009	March 26 (males) or 27 (females), 2009
Necropsy Dates	
March 23 (males) or 24 (females), 2009	March 26 (males) or 27 (females), 2009

Rats	Mice
Average Age at Necropsy	
8 weeks	Same as in rats
Size of Study Groups	
10 males and 10 females for control groups 5 males and 5 females for exposed groups	Same as in rats
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as in rats
Animals per Cage	
5	1 (male) or 5 (female)
Method of Animal Identification	
Tail tattoo	Same as in rats
Diet	
Irradiated NTP-2000 pelleted feed (Zeigler Brothers, Inc., Gardners, PA), available ad libitum, changed weekly	Same as in rats
Water	
Tap water (Potomac, MD municipal supply), either untreated or containing a formulation of Emim-Cl, Bmim-Cl, Bmpy-Cl, or NBuPy-Cl via amber glass bottles, available ad libitum, changed twice weekly	Same as in rats
Cages	
Solid polycarbonate (Lab Products, Inc., Seaford, DE), changed at least twice weekly	Same as in rats
Cage Filters	
Omnishield paper, Reemay® 2024 (DuPont), changed every 2 weeks	Same as in rats
Bedding	
Irradiated Sani-Chips® (P.J. Murphy Forest Products Corporation, Montville, NJ), changed with cage changes	Same as in rats
Racks	
Stainless steel (Lab Products, Inc., Seaford, DE), changed every 2 weeks	Same as in rats
Animal Room Environment	
Temperature: 72°F ± 3°F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour	Same as in rats
Exposure Concentrations	
Emim-Cl: 0, 3, 10, 30, or 100 mg/mL	Emim-Cl: Same as in rats
Bmim-Cl: 0, 0.3, 1, or 3 mg/mL	Bmim-Cl: 0, 1, 3, or 6 mg/mL

Rats	Mice
Bmpy-Cl: 0, 1, 3, or 6 mg/mL	Bmpy-Cl: 0, 3, 6, or 10 mg/mL
NBuPy-Cl: 0, 1, 3, or 6 mg/mL	NBuPy-Cl: Same as in rats
Type and Frequency of Observation	
Observed twice daily; animals were weighed, and clinical observations were recorded initially, on study day 8, and at the end of the 2-week exposure period. Water consumption was recorded on study days 1, 4, 8, 11, 14, and 15.	Observed twice daily; animals were weighed, and clinical observations were recorded initially, on study day 8, and at the end of the 2-week exposure period. Water consumption was recorded on study days 1, 5, 8, 12, and 15.
Method of Euthanasia	
100% carbon dioxide	Same as in rats
Necropsy	
Necropsies were performed on all animals. Organs weighed were heart, right kidney, liver, lungs, right testis, and thymus.	Same as in rats
Histopathology	
Complete histopathology was performed on all control animals and all animals in the highest exposed groups. In addition to gross lesions and tissue masses, the following tissues were examined: brain, heart, kidney, liver, lung, ovary, stomach (forestomach and glandular), small intestine, spleen, testes, and thymus.	Same as in rats

Emim-Cl = 1-ethyl-3-methylimidazolium chloride; Bmim-Cl = 1-butyl-3-methylimidazolium chloride; Bmpy-Cl = 1-butyl-1-methylpyrrolidinium chloride; NBuPy-Cl = n-butylpyridinium chloride.

Three-month Studies

Study Design for Rats

Rats were approximately 3 to 5 weeks old on receipt. Animals were quarantined for 12 days and were approximately 5 to 7 weeks old on the first day of the studies. Rats were randomly assigned to one of four exposure groups for each IL. Randomization was stratified by body weight that produced similar group mean weights using PATH/TOX SYSTEM software (Xybion Corporation, Lawrenceville, NJ).

Before the studies began, five male and five female rats were randomly selected for parasite evaluation and gross observation of disease. Additionally, the health of the animals was monitored during the study according to the protocols of the NTP Sentinel Animal Program (Appendix C). All test results were negative.

Groups of 10 male and 10 female rats were exposed to Emim-Cl, Bmim-Cl, Bmpy-Cl, or NBuPy-Cl in drinking water for 3 months at the following exposure concentrations: Emim-Cl (0, 1, 3, or 10 mg/mL), Bmim-Cl (0, 0.1, 0.3, or 1 mg/mL), Bmpy-Cl (0, 0.3 [males only], 1, 3, or 6 [females only] mg/mL), or NBuPy-Cl (0, 0.3, 1, or 3 mg/mL); tap water served as the control.

Feed and water were available ad libitum. Rats were housed up to three (males) or five (females) per cage. Details of the study design and animal maintenance are summarized in Table 4. Information on feed composition and contaminants is provided in Appendix B.

Study Design for Mice

Mice were approximately 3 to 5 weeks old on receipt. Animals were quarantined for 12 days and were approximately 5 to 6 weeks old on the first day of the studies. Mice were randomly assigned to one of four exposure groups for each IL. Randomization was stratified by body weight that produced similar group mean weights using PATH/TOX SYSTEM software (Xybion Corporation, Lawrenceville, NJ).

Before the studies began, five male and five female mice were randomly selected for parasite evaluation and gross observation of disease. Additionally, the health of the animals was monitored during the study according to the protocols of the NTP Sentinel Animal Program (Appendix C). All test results were negative.

Groups of 10 male and 10 female mice were exposed to Emim-Cl, Bmim-Cl, Bmpy-Cl, or NBuPy-Cl in drinking water for 3 months at the following exposure concentrations: Emim-Cl (0, 3, 10, or 30 mg/mL), Bmim-Cl (0, 0.3, 1, or 3 mg/mL), Bmpy-Cl (0, 1, 3, 6 [females only], or 10 [males only] mg/mL), or NBuPy-Cl (0, 1, 3 or 6 mg/mL); tap water served as the control.

Feed and water were available ad libitum. Mice were housed individually (males) or up to four (females) per cage. Details of the study design and animal maintenance are summarized in Table 4. Information on feed composition and contaminants is provided in Appendix B.

Clinical Examination and Pathology

During the 3-month studies, rats and mice were observed twice daily for signs of mortality or moribundity. Clinical observations and body weights were recorded initially, weekly, and at the end of the 3-month exposure period. Water consumption was measured weekly for 3 months.

Blood was collected from the retroorbital plexus (rats) or retroorbital sinus (mice) of all animals at the end of the 3-month exposure period for hematology, clinical chemistry (rats only), and erythrocyte micronuclei analyses. Animals were anesthetized with a carbon dioxide/oxygen mixture and bled in a random order. Blood was collected into tubes containing ethylenediaminetetraacetic acid (EDTA) for hematology or into serum separator tubes for clinical chemistry. Hematology parameters were analyzed using the Advia® 120 (Siemens Healthcare GmbH, Erlangen, Germany). Clinical chemistry parameters were analyzed using the Roche cobas® c501 Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN). The parameters measured are listed in Table 4.

At the end of the 3-month exposure period, samples were collected for sperm motility and vaginal cytology evaluations on all rats and mice. The parameters evaluated are listed in Table 4. For 16 consecutive days before the end of the 3-month exposure period, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were collected and subsequently stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Measured parameters of cycle length, number of

cycles, and time spent in any specific stage of the estrous cycle of female rats and mice are reported in Appendix E. Male animals were evaluated for sperm count and motility. The right testis was used for organ weights and histopathology evaluation. The cauda portion of the left epididymis was used for sperm motility and concentration evaluation; the remainder of the left epididymis was fixed and processed for potential histopathological evaluation. The left testis was placed in a labeled plastic vial and then quick-frozen in dry ice for spermatid evaluation. Frozen testes were stored at approximately −70°C. However, due to artifacts identified during analysis (e.g., clumping of cells, too many sperm in sample), meaningful interpretation of motility and concentration data was not possible. Therefore, sperm motility and sperm/spermatid concentration data were not analyzed or reported.

Necropsies were performed on all animals. Organ weights were determined for the heart, right kidney, liver, lungs, right testis, and thymus from all rats and mice. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (except eyes, which were first fixed in Davidson's solution, and testes, vaginal tunics, and epididymides, which were first fixed in modified Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of approximately 5 μm, and stained with H&E. Complete histopathological examinations were performed by the study laboratory pathologist on all control animals and all animals in the highest exposed group for each IL. Table 4 lists the tissues and organs examined.

The laboratory report and select histopathology slides were reviewed by a quality assessment (QA) pathologist, who also served as the coordinator of the Pathology Working Group (PWG). The QA report and the reviewed slides were submitted to the NTP pathologist, who reviewed and addressed any inconsistencies in the diagnoses made by the study laboratory and QA pathologist. The QA/PWG pathologist presented representative histopathology slides containing examples of lesions related to test agent administration, examples of disagreements in diagnoses between the laboratory and QA pathologist, or lesions of general interest to the PWG for review. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, QA pathologist, and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman[77] and Boorman et al.[78]

Table 4. Experimental Design and Materials and Methods in the Three-month Drinking Water Studies of Ionic Liquids

Rats	Mice
Study Laboratory	
Battelle (Columbus, OH)	Same as in rats
Strain and Species	
Sprague Dawley (Hsd:Sprague Dawley® SD®)	B6C3F1/N
Animal Source	
Envigo (formerly Harlan Laboratories, Inc., Indianapolis, IN)	Taconic Biosciences, Inc. (Germantown, NY)
Time Held Before Studies	
12 days	Same as in rats
Average Age When Studies Began	
5–7 weeks	Same as in rats

Rats	Mice
Date of First Exposure	
Emim-Cl: May 9 (males) or 10 (females), 2013	Emim-Cl: May 8 (males) or 7 (females), 2013
Bmim-Cl: April 25 (males) or 26 (females), 2013	Bmim-Cl: April 24 (males) or 23 (females), 2013
Bmpy-Cl: August 22 (males) or 23 (females), 2013	Bmpy-Cl: August 21 (males) or 20 (females), 2013
NBuPy-Cl: September 19 (males) or 20 (females), 2013	NBuPy-Cl: September 18 (males) or 17 (females), 2013
Duration of Exposure	
3 months	Same as in rats
Date of Last Exposure	
Emim-Cl: August 8 (males) or 9 (females), 2013	Emim-Cl: August 7 (males) or 6 (females), 2013
Bmim-Cl: July 25 (males), or 26 (females), 2013	Bmim-Cl: July 24 (males) or 23 (females), 2013
Bmpy-Cl: November 21 (males) or 22 (females), 2013	Bmpy-Cl: November 20 (males) or 19 (females), 2013
NBuPy-Cl: December 19 (males) or 20 (females), 2013	NBuPy-Cl: December 18 (males) or 17 (females), 2013
Necropsy Dates	
Emim-Cl: August 8 (males) or 9 (females), 2013	Emim-Cl: August 7 (males) or 6 (females), 2013
Bmim-Cl: July 25 (males) or 26 (females), 2013	Bmim-Cl: July 24 (males) or 23 (females), 2013
Bmpy-Cl: November 21 (males) or 22 (females), 2013	Bmpy-Cl: November 20 (males) or 19 (females), 2013
NBuPy-Cl: December 19 (males) or 20 (females), 2013	NBuPy-Cl: December 18 (males) or 17 (females), 2013
Average Age at Necropsy	
18–20 weeks	Same as in rats
Size of Study Groups	
10 males and 10 females	Same as in rats
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as in rats
Animals per Cage	
Up to 3 (male), up to 5 (female)	1 (male), up to 4 (female)
Method of Animal Identification	
Tail tattoo	Same as in rats
Diet	
Irradiated NTP-2000 wafer feed (Zeigler Brothers, Gardners, PA), available ad libitum, changed weekly	Same as in rats
Water	
Tap water (Columbus, OH municipal supply), either untreated or containing a formulation of Emim-Cl, Bmim-Cl, Bmpy-Cl, or NBuPy-Cl via glass bottles (Thermo Fisher Scientific, Waltham, MA), available ad libitum, changed twice weekly	Tap water (Columbus, OH municipal supply), either untreated or containing a formulation of Emim-Cl, Bmim-Cl, Bmpy-Cl, or NBuPy-Cl via glass bottles (Sigma-Aldrich Corp., St. Louis, MO [male mice] or Qorpak, Clinton, PA [female mice]), available ad libitum, changed once (males) or twice (females) weekly.
Cages	
Solid polycarbonate (Lab Products, Inc., Seaford, DE), changed at least twice weekly	Solid polycarbonate (Lab Products, Inc., Seaford, DE), changed at least once (males) or twice (females) weekly

Rats	Mice
Bedding	
Irradiated Sani-Chips® (P.J. Murphy Forest Products Corporation, Montville, NJ), changed with cage changes	Same as in rats
Racks	
Stainless steel (Lab Products, Inc., Seaford, DE), changed and rotated at least every 2 weeks	Same as in rats
Rack Filters	
Spun-bonded polyester (National Filter Media Corporation, Salt Lake City, UT), changed at least every 2 weeks	Same as in rats
Animal Room Environment	
Temperature: 72°F ± 3°F Relative humidity: 43%−74% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour	Temperature: 72°F ± 3°F Relative humidity: 43%−67% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour
Exposure Concentrations	
Emim-Cl: 0, 1, 3, or 10 mg/mL	Emim-Cl: 0, 3, 10, or 30 mg/mL
Bmim-Cl: 0, 0.1, 0.3, or 1 mg/mL	Bmim-Cl: 0, 0.3, 1, or 3 mg/mL
Bmpy-Cl: 0, 0.3 (males only), 1, 3, or 6 (females only) mg/mL	Bmpy-Cl: 0, 1, 3, 6 (females only), or 10 (males only) mg/mL
NBuPy-Cl: 0, 0.3, 1, or 3 mg/mL	NBuPy-Cl: 0, 1, 3, or 6 mg/mL
Type and Frequency of Observation	
Observed twice daily; animals were weighed, and clinical observations were recorded initially, weekly thereafter, and at the end of the 3-month exposure period. Water consumption was recorded at least weekly for the duration of the study.	Same as in rats
Method of Euthanasia	
100% carbon dioxide	Same as in rats
Necropsy	
Necropsies were performed on all animals. Organs weighed were the heart, right kidney, liver, lungs, right testis, and thymus.	Same as in rats
Clinical Pathology	
At the end of the 3-month exposure period, blood was collected from the retroorbital plexus of rats for clinical chemistry, hematology, and erythrocyte micronuclei determination.	At the end of the 3-month exposure period, blood was collected from the retroorbital sinus of mice for hematology and erythrocyte micronuclei determination.
Hematology: erythrocyte count, mean corpuscular volume, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white blood cell count and differential, reticulocyte count, platelet count, and qualitative evaluation of morphological features in all cellular components.	Same as in rats

Rats	Mice
Clinical chemistry: alkaline phosphatase, alanine aminotransferase, sorbitol dehydrogenase, total protein, albumin, globulin, A/G ratio, glucose, urea nitrogen, creatinine, creatine kinase, triglyceride, cholesterol, and total bile acids.	None
Histopathology	
Complete histopathology was performed on all control animals and all animals in the highest exposed groups. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal glands, brain (olfactory bulbs, fronto-parietal cortex and basal ganglia, mid-parietal cortex and thalamus, mid-brain with substantia nigra and red nucleus, posterior colliculi, mid-cerebellum including cranial nerve VIII, and posterior medulla), clitoral glands, esophagus, eyes, femur (including diaphysis with marrow cavity and epiphysis [femoral condyle with epiphyseal cartilage plate, articular cartilage, and articular surface]), Harderian glands, heart and aorta, large intestine (cecum, colon, and rectum), small intestine (duodenum, jejunum, and ileum), kidneys, liver (two sections including left lateral lobe and median lobe), lungs and mainstem bronchi, lymph nodes (mandibular and mesenteric), mammary gland with adjacent (inguinal) skin, nasal cavity and nasal turbinates (three sections), ovaries, pancreas, parathyroid glands, pituitary gland, preputial glands, prostate, salivary glands, seminal vesicles, spleen, stomach (forestomach and glandular), right testis with epididymis, thymus, thyroid gland, trachea, urinary bladder, and uterus.	Complete histopathology was performed on all control animals and all animals in the highest exposed groups. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal glands, brain (olfactory bulbs, fronto-parietal cortex and basal ganglia, mid-parietal cortex and thalamus, mid-brain with substantia nigra and red nucleus, posterior colliculi, mid-cerebellum including cranial nerve VIII, and posterior medulla), clitoral glands, esophagus, eyes, femur (including diaphysis with marrow cavity and epiphysis [femoral condyle with epiphyseal cartilage plate, articular cartilage, and articular surface]), gallbladder, Harderian glands, heart and aorta, large intestine (cecum, colon, and rectum), small intestine (duodenum, jejunum, and ileum), kidneys, liver (two sections including left lateral lobe and median lobe), lungs and mainstem bronchi, lymph nodes (mandibular and mesenteric), mammary gland with adjacent (inguinal) skin, nasal cavity and nasal turbinates (three sections), ovaries, pancreas, parathyroid glands, pituitary gland, preputial glands, prostate, salivary glands, seminal vesicles, spleen, stomach (forestomach and glandular), right testis with epididymis, thymus, thyroid gland, trachea, urinary bladder, and uterus. Because exposure-related observations were noted in the kidneys of male and female mice and in the adrenal glands of female mice at the highest concentrations for Emim-Cl, Bmim-Cl, Bmpy-Cl, and NBuPy-Cl, the kidneys were examined for male and female mice and the adrenal glands were examined for all female mice in all exposure groups for all four ILs.
Sperm Motility and Vaginal Cytology	
At the end of the 3-month exposure period, sperm samples were collected from all male rats for sperm motility evaluations. Sperm motility and sperm/spermatid concentration data were not analyzed or reported due to artifacts identified during analysis. The right testis was weighed. Vaginal samples were collected for up to 16 consecutive days before the end of the 3-month exposure period from all female rats for vaginal cytology evaluations.	Same as in rats

Emim-Cl = 1-eethyl-3-methylimidazolium chloride; Bmim-Cl = 1-butyl-3-methylimidazolium chloride; Bmpy-Cl = 1-butyl-1-methylpyrrolidinium chloride; NBuPy-Cl = n-butylpyridinium chloride.

Statistical Methods

Calculation and Analysis of Nonneoplastic Lesion Incidences

The incidences of nonneoplastic lesions are presented as numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. Fisher's exact test,[79] a procedure that uses the overall proportion of affected animals, was used to determine statistical significance between exposed and control animals, and the Cochran-Armitage trend test was used to test for significant trends.[80]

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett[81] and Williams.[82; 83] Hematology and clinical chemistry data, which typically have skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley[84] (as modified by Williams[85] and Dunn[86]). The Jonckheere test[87] was used to assess the significance of the exposure concentration-related trends and to determine whether a trend-sensitive test (the Williams or Shirley test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic exposure concentration-related trend (the Dunnett or Dunn test). Before statistical analysis, outliers identified by the Dixon and Massey[88] test were examined by NTP personnel, and biologically implausible values (likely due to experimental error) were eliminated from the analysis.

Analysis of Vaginal Cytology Data

Vaginal cytology data consist of daily observations of estrous cycle stages over a 16-day period. Differences from the control group for cycle length and the number of cycles were analyzed using the Shirley and Dunn tests, as described above.

To identify disruptions in estrous cyclicity, a continuous-time Markov chain model (multistate model) was fit using a maximum likelihood approach,[89] producing estimates of stage lengths for each exposure group. Confidence intervals for these estimates were obtained from bootstrap sampling of the individual animal cycle sequences. Stage lengths that were significantly different from the control animals were identified using permutation testing.

Quality Assurance Methods

The 2-week and 3-month studies were conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice Regulations.[90] In addition, the 2-week and 3-month study reports were audited retrospectively by an independent QA contractor against study records submitted to the NTP Archives. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP toxicity report. Audit procedures and findings are presented in the reports and are on file at the NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this toxicity report.

Genetic Toxicology

The genetic toxicities of Emim-Cl, Bmim-Cl, Bmpy-Cl, and NBuPy-Cl were assessed by testing whether each chemical induced mutations in various strains of *Salmonella typhimurium* and *Escherichia coli* or increased the frequency of micronucleated erythrocytes in rat and mouse peripheral blood. The protocol for these studies and the results are given in Appendix D.

The genetic toxicity studies have evolved from an earlier effort by NTP to develop a comprehensive database that permits critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the relationship between the molecular structure of the chemical and its observed effects in short-term in vitro and in vivo genetic toxicity tests (structure-activity relationships). The short-term tests were developed originally to clarify proposed mechanisms of chemical-induced DNA damage, given the relationship between electrophilicity and mutagenicity,[91] and the somatic mutation theory of cancer.[92; 93] Not all cancers, however, arise through genotoxic mechanisms.

Bacterial Mutagenicity

DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites.[94] A positive response in the *Salmonella* test was shown to be the most predictive in vitro indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens).[95; 96] Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. Other tests, however, can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

Peripheral Blood Micronucleus Test

Micronuclei (literally "small nuclei" or Howell-Jolly bodies) are biomarkers of induced structural or numerical chromosomal alterations and are formed when acentric fragments or whole chromosomes fail to incorporate into either of two daughter nuclei during cell division.[97; 98] Acute in vivo bone marrow chromosome aberration and micronucleus tests appear to be less predictive of carcinogenicity than the *Salmonella* test.[99; 100] However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity; a weak response in one sex only or negative results in both sexes in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies.[101] Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, determination of in vivo genetic effects is important to overall understanding of risks associated with exposure to a particular chemical.

Results

Data Availability

The National Toxicology Program (NTP) evaluated all study data. Data relevant for evaluating toxicological findings are presented here. All study data are available in the NTP Chemical Effects in Biological Systems (CEBS) database: https://doi.org/10.22427/NTP-DATA-TOX-103.[102]

Two-week Studies

Rats and Mice

In the 2-week studies, survival of rats and mice was not affected by exposure to 1-ethyl-3-methylimidazolium chloride (Emim-Cl), 1-butyl-3-methylimidazolium chloride (Bmim-Cl), 1-butyl-1-methylpyrrolidinium chloride (Bmpy-Cl), or n-butylpyridinium chloride (NBuPy-Cl). Clinical observations, including thinness and ruffled fur, were common in rats and mice exposed to higher concentrations of each ionic liquid (IL), except for Bmim-Cl- and Bmpy-Cl-exposed rats, which did not have any exposure-related clinical findings (Appendix E).

The clinical findings may have been related to lower body weights. An exposure concentration-dependent decrease in body weight was observed in most groups of male and female rats and mice exposed to ILs; terminal mean body weights of the highest exposed groups of rats and mice were often lower than those of the control groups (Table 5). Terminal mean body weights following Emim-Cl exposure were 32%–38% of the control groups in male and female rats and approximately 54% of the control groups in male and female mice at the highest concentration (100 mg/mL). In Bmim-Cl-exposed male and female animals, terminal mean body weights were 85%–86% and 81%–87% of the control groups in rats and mice, respectively, at the highest concentrations (3 mg/mL rats, 6 mg/mL mice). Terminal mean body weights of Bmpy-Cl-exposed male and female rats were approximately 83%–91% of the control groups and 86% of the control group in female mice at the highest concentrations (6 mg/mL rats, 10 mg/mL mice). Terminal mean body weights of male mice were unaffected by Bmpy-Cl administration. In NBuPy-Cl-exposed male and female rats, terminal mean body weights were approximately 67%–68% of the control groups at the highest concentration (6 mg/mL). Terminal mean body weights of male and female mice were approximately 89% of the control groups at the highest concentration (6 mg/mL).

Lower mean body weights were associated with lower water consumption. Test article palatability likely directly affected mean water consumption and indirectly affected mean body weights. In general, mean water consumption by exposed rats was lower compared to control animals than consumption by exposed mice; this difference between rats and mice was most evident at the highest exposure concentrations for each IL (Table 6). Compared to the respective control groups, mean water consumption at the highest exposure concentration was 45%–46% by Bmim-Cl-exposed rats (3 mg/mL), 46%–57% by Bmim-Cl-exposed mice (6 mg/mL), 50%–55% by Bmpy-Cl-exposed rats (6 mg/mL), 25%–73% by Bmpy-Cl-exposed mice (10 mg/mL), 36%–38% by NBuPy-Cl-exposed rats (6 mg/mL), and 49%–71% by NBuPy-Cl-exposed mice (6 mg/mL). Water consumption values were much lower for Emim-Cl-exposed rats and mice,

which were exposed to higher concentrations of the test article in drinking water. In male and female rats exposed to 30 or 100 mg/mL Emim-Cl, mean water consumption values were approximately 40% or 5%, respectively, of those of the control groups. In mice exposed to the same concentrations of Emim-Cl, mean water consumption values were 45%–69% (males) and 21%–57% (females) of the control groups. Due to lower water consumption by the higher exposed groups, compound consumption did not increase proportionally with higher exposure concentrations of each IL in water. Compound consumption values for each IL are presented as supplemental data in Appendix E.

Table 5. Summary of Terminal Mean Body Weights of Male and Female Rats and Mice in the Two-week Drinking Water Study of Ionic Liquids

	0 mg/mL	0.3 mg/mL		1 mg/mL		3 mg/mL		6 mg/mL		10 mg/mL		30 mg/mL		100 mg/mL	
	Av. Wt. (g)	Av. Wt. (g)	Wt. (% of Controls)	Av. Wt. (g)	Wt. (% of Controls)	Av. Wt. (g)	Wt. (% of Controls)	Av. Wt. (g)	Wt. (% of Controls)	Av. Wt. (g)	Wt. (% of Controls)	Av. Wt. (g)	Wt. (% of Controls)	Av. Wt. (g)	Wt. (% of Controls)
Rats[a]															
Male															
Emim-Cl	278.3	–[b]	–	–	–	276.9	99.5	–	–	260.7	93.7	170.3	61.2	90.3	32.4
Bmim-Cl	278.3	280.5	100.8	272.0	97.7	235.9	84.7	–	–	–	–	–	–	–	–
Bmpy-Cl	278.3	–	–	281.0	101.0	268.8	96.6	229.8	82.6	–	–	–	–	–	–
NBuPy-Cl	278.3	–	–	282.6	101.5	257.8	92.6	189.9	68.2	–	–	–	–	–	–
Female															
Emim-Cl	183.1	–	–	–	–	189.7	103.6	–	–	175.8	96.0	128.0	69.9	69.6	38.0
Bmim-Cl	183.1	181.2	98.9	179.5	98.0	157.2	85.9	–	–	–	–	–	–	–	–
Bmpy-Cl	183.1	–	–	184.5	100.7	179.2	97.9	166.8	91.1	–	–	–	–	–	–
NBuPy-Cl	183.1	–	–	176.5	96.4	173.7	94.9	122.8	67.0	–	–	–	–	–	–
Mice															
Male															
Emim-Cl	26.3	–	–	–	–	26.5	100.8	–	–	25.6	97.6	24.5	93.3	14.3	54.3
Bmim-Cl	26.3	–	–	26.8	102.1	25.4	96.7	21.4	81.3	–	–	–	–	–	–
Bmpy-Cl	26.3	–	–	–	–	26.1	99.5	26.6	101.3	25.2	96.0	–	–	–	–
NBuPy-Cl	26.3	–	–	26.0	98.9	25.5	97.1	23.4	89.3	–	–	–	–	–	–
Female															
Emim-Cl	20.5	–	–	–	–	20.0	97.8	–	–	20.5	100.4	18.5	90.2	11.0	53.9
Bmim-Cl	20.5	–	–	19.5	95.1	19.1	93.4	17.9	87.3	–	–	–	–	–	–
Bmpy-Cl	20.5	–	–	–	–	19.9	97.5	18.5	90.3	17.6	86.1	–	–	–	–
NBuPy-Cl	20.5	–	–	18.1	88.7	19.6	95.8	18.3	89.2	–	–	–	–	–	–

Emim-Cl = 1-ethyl-3-methylimidazolium chloride; Bmim-Cl = 1-butyl-3-methylimidazolium chloride; Bmpy-Cl = 1-butyl-1-methylpyrrolidinium chloride; NBuPy-Cl = n-butylpyridinium chloride.

[a]N = 10 animals/vehicle control group; n = 5 animals/exposed group.

[b]Exposures at this concentration were not conducted for this group.

Table 6. Summary of Water Consumption of Male and Female Rats and Mice in the Two-week Drinking Water Study of Ionic Liquids

	0 mg/mL	0.3 mg/mL	1 mg/mL	3 mg/mL	6 mg/mL	10 mg/mL	30 mg/mL	100 mg/mL
Rats (g/animal/day)[a]								
Male								
Emim-Cl	36.0	–[b]	–	29.1	–	21.8	14.5	1.1
Bmim-Cl	36.0	27.7	27.0	16.3	–	–	–	–
Bmpy-Cl	36.0	–	29.3	25.8	18.1	–	–	–
NBuPy-Cl	36.0	–	25.5	19.4	12.9	–	–	–
Female								
Emim-Cl	22.6	–	–	21.5	–	13.4	9.7	1.8
Bmim-Cl	22.6	23.5	17.5	10.4	–	–	–	–
Bmpy-Cl	22.6	–	20.0	16.6	12.4	–	–	–
NBuPy-Cl	22.6	–	16.8	12.5	8.5	–	–	–
Mice (g/animal/day)								
Male								
Emim-Cl	4.9	–	–	4.8	–	4.4	3.4	2.2
Bmim-Cl	4.9	–	4.6	3.8	2.8	–	–	–
Bmpy-Cl	4.9	–	–	4.5	4.5	3.6	–	–
NBuPy-Cl	4.9	–	4.3	4.5	2.4	–	–	–
Female								
Emim-Cl	2.8	–	–	2.6	–	2.7	1.6	0.6
Bmim-Cl	2.8	–	2.0	1.5	1.3	–	–	–
Bmpy-Cl	2.8	–	–	2.1	1.7	0.7	–	–
NBuPy-Cl	2.8	–	0.9	2.2[c]	2.0[c]	–	–	–

Emim-Cl = 1-ethyl-3-methylimidazolium chloride; Bmim-Cl = 1-butyl-3-methylimidazolium chloride; Bmpy-Cl = 1-butyl-1-methylpyrrolidinium chloride; NBuPy-Cl = n-butylpyridinium chloride.

[a]Mean water consumption data from the last day of the study; n = 5 animals/group.

[b]Exposures at this concentration were not conducted for this group.

[c]Data missing for last day of study; mean for week 2 was used.

Several organ weight fluctuations were observed in rats and mice exposed to ILs, most of which occurred in animals exposed at the highest IL exposure concentrations. Compared to control animals, changes in mean organ weights were often characterized by lower absolute weight and higher relative weight, which corresponded to lower mean body weights. The largest organ weight differences from the control group were recorded in the Emim-Cl and NBuPy-Cl studies, which also had the greatest reduction in mean water consumption and terminal mean body weights compared to control animals. Animals exposed to Bmim-Cl or Bmpy-Cl exhibited limited organ weight differences from the control group, which were chemical- and sex-specific. Organ weight data for each IL can be found in Appendix E.

Although a formal NTP review of histopathological findings was not conducted for the 2-week studies, several lesions were found to be common in animals exposed to higher IL concentrations in both sexes and species. Lesions such as glycogen depletion of the liver, thymic atrophy, cellular depletion of the spleen, and degeneration of the testes were most common across the ILs. It is difficult to determine whether these changes were related to chronic stress and/or lower water (or hypothesized feed) consumption and lower body weights rather than a direct toxic effect from IL exposure for 2 weeks.[103] Histopathological findings for each IL can be found in Appendix E.

Exposure Concentration Selection Rationale for the Three-month Studies

Lower mean body weights and water consumption compared to control animals were the two primary toxicity parameters that guided exposure concentration selection for the 3-month IL studies. Rats appeared to be more sensitive to IL exposure in drinking water (i.e., lower water consumption and mean body weights), which was also taken into consideration for exposure concentration selection. In general, the top exposure concentrations selected for the 3-month studies demonstrated mean body weights within 10% of control animals and mean water consumption rates within 25% of control animals following the 2-week exposure period. The exposure concentrations selected for the 3-month studies are shown in Table 4. Exposure concentrations selected for Emim-Cl were higher than those for other ILs due to its apparently lower toxicity. Due to sex differences observed in the 2-week studies, different exposure concentrations were selected for males and females in the Bmpy-Cl studies.

Three-month Studies

Rats

1-Ethyl-3-Methylimidazolium Chloride (Emim-Cl)

Male and female rats were exposed to Emim-Cl at concentrations of 0, 1, 3, or 10 mg/mL in drinking water. All rats survived to the end of the 3-month exposure period (Table 7), and no clinical observations related to Emim-Cl exposure were noted (Appendix E). Male and female mean body weights of all exposed groups remained within 10% of the mean body weights of the control groups throughout the course of study and were not significantly different at the end of the 3-month exposure period (Table 7; Figure 1; Appendix E).

Table 7. Summary of Survival and Mean Body Weights of Male and Female Rats in the Three-month Drinking Water Study of 1-Ethyl-3-Methylimidazolium Chloride

Study Day[a]	0 mg/mL		1 mg/mL			3 mg/mL			10 mg/mL		
	Av. Wt. (g)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N
Male											
1	147.0	10	147.3	100.2	10	146.5	99.6	10	147.7	100.4	10
8	196.9	10	196.7	99.9	10	198.1	100.6	10	189.0	96.0	10
15	242.8	10	243.6	100.3	10	243.9	100.5	10	232.6	95.8	10
22	262.7	10	263.1	100.1	10	247.6	94.2	10	249.7	95.0	10
29	304.0	10	302.1	99.4	10	302.9	99.6	10	292.3	96.2	10
36	328.4	10	327.6	99.8	10	328.9	100.1	10	316.8	96.5	10
43	344.0	10	345.3	100.4	10	346.0	100.6	10	334.3	97.2	10
50	361.8	10	363.3	100.4	10	363.8	100.6	10	353.2	97.6	10
57	373.1	10	376.1	100.8	10	376.6	100.9	10	366.0	98.1	10
64	384.8	10	388.6	101.0	10	389.2	101.1	10	375.0	97.4	10
71	393.1	10	399.9	101.7	10	401.1	102.0	10	383.3	97.5	10
78	403.5	10	410.6	101.8	10	410.6	101.8	10	391.9	97.1	10
85	415.3	10	423.0	101.9	10	424.0	102.1	10	402.2	96.8	10
EOS	424.6	10	434.5	102.3	10	436.1	102.7	10	411.9	97.0	10
Female											
1	109.7	10	111.1	101.3	10	108.1	98.6	10	108.6	99.0	10
8	139.6	10	140.1	100.4	10	136.2	97.6	10	129.3	92.6	10
15	161.7	10	164.9	102.0	10	157.0	97.1	10	150.0	92.8	10
22	178.8	10	182.5	102.0	10	172.4	96.4	10	163.5	91.4	10
29	190.2	10	195.0	102.5	10	182.8	96.1	10	176.4	92.8	10
36	205.0	10	211.2	103.0	10	198.4	96.7	10	190.0	92.6	10
43	214.4	10	221.3	103.2	10	208.8	97.4	10	200.4	93.5	10
50	221.4	10	227.9	102.9	10	214.3	96.8	10	205.2	92.7	10
57	225.9	10	234.6	103.9	10	223.3	98.9	10	212.8	94.2	10
64	232.5	10	242.2	104.2	10	224.9	96.7	10	219.7	94.5	10
71	240.7	10	243.9	101.3	10	231.6	96.2	10	225.6	93.8	10
78	245.0	10	250.1	102.1	10	235.4	96.1	10	228.9	93.4	10
85	247.4	10	252.8	102.2	10	240.7	97.3	10	234.3	94.7	10
EOS	254.5	10	256.3	100.7	10	246.9	97.0	10	241.1	94.7	10

EOS = end of study.
[a]Study day 1 is the day animals were placed on study.

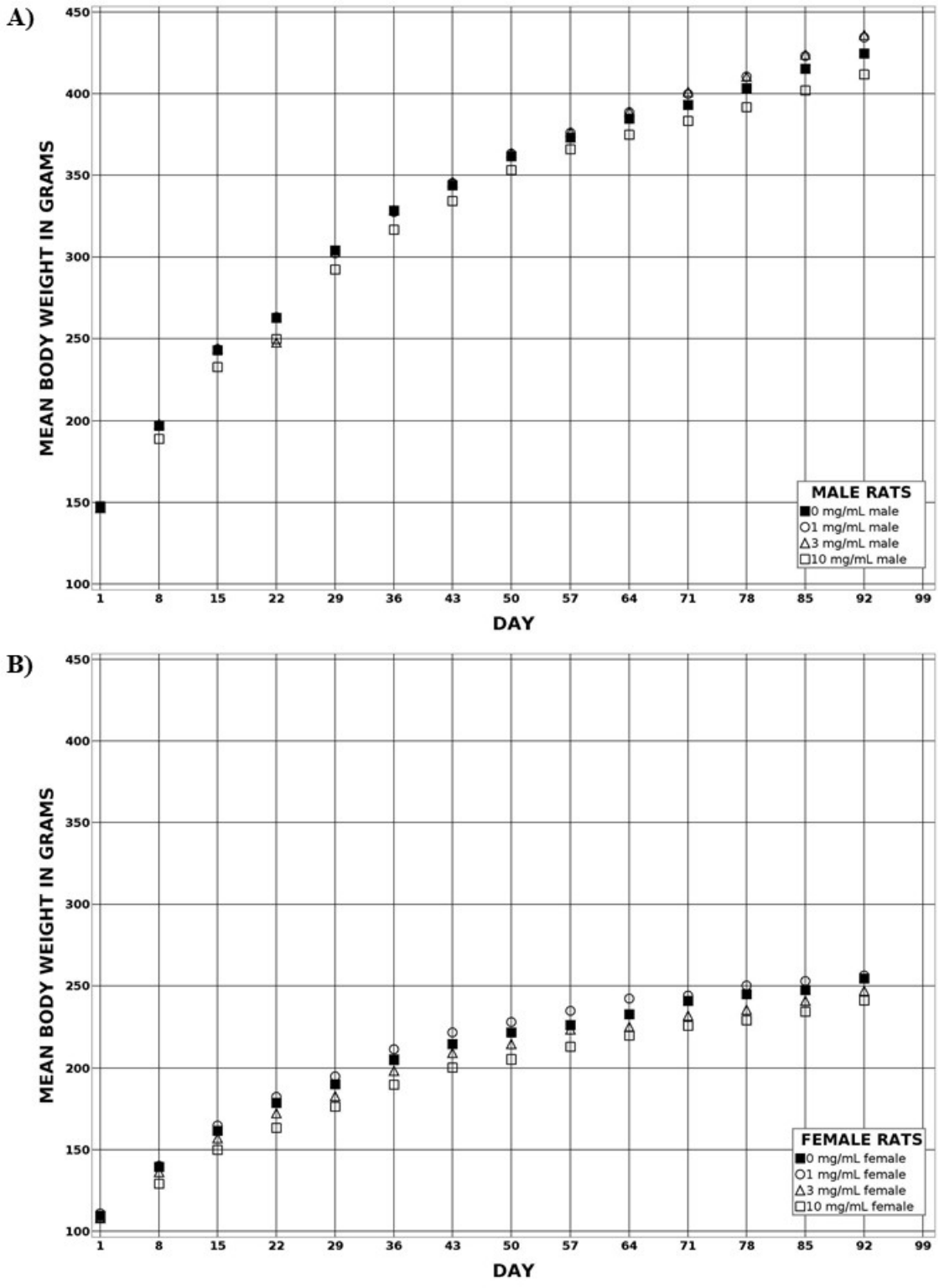


Figure 1. Growth Curves for Male and Female Rats in the Three-month Drinking Water Study of 1-Ethyl-3-Methylimidazolium Chloride

Growth curves are shown for (A) males and (B) females.

Water consumption by male and female rats exposed to 3 or 10 mg/mL Emim-Cl was lower than that of control animals (Table 8). At 1 mg/mL, the mean water consumption was within 10% of the control groups for both males and females. At 3 mg/mL, mean water consumption ranged from 85% to 94% of the control group in males and from 78% to 89% of the control group in females (Appendix E). At the highest exposure concentration of 10 mg/mL, mean water consumption ranged from 71% to 83% of the control group in males and from 59% to 69% of the control group in females. Drinking water concentrations of 1, 3, and 10 mg/mL resulted in estimated average doses of 88, 237, and 668 mg Emim-Cl/kg body weight/day (mg/kg/day) for males and 104, 258, and 691 mg/kg/day for females.

Table 8. Summary of Water and 1-Ethyl-3-Methylimidazolium Chloride Consumption of Male and Female Rats in the Three-month Drinking Water Study

Week	Water (g/day)[a]				Dose (mg/kg/day)[b]		
	0 mg/mL	1 mg/mL	3 mg/mL	10 mg/mL	1 mg/mL	3 mg/mL	10 mg/mL
Male							
1	25.5	25.8	24.0	18.7	175.1	491.6	1,266.5
3	27.7	27.8	25.8	21.2	114.1	317.3	911.6
6	26.1	26.4	23.1	19.4	80.6	210.7	612.3
9	23.9	25.2	21.7	18.1	67.0	172.9	494.5
13	22.4	24.2	20.8	18.5	57.2	147.2	460.0
Female							
1	19.4	20.0	16.8	12.9	180.0	466.3	1,188.3
3	19.2	19.8	15.6	12.7	120.1	298.1	846.8
6	20.0	20.8	16.3	12.4	98.5	246.5	652.8
9	18.7	19.7	15.7	12.0	84.0	210.9	564.0
13	17.7	19.5	15.8	12.3	77.1	197.0	525.0

[a]Mean g of water consumed/animal/day.
[b]Mg of 1-ethyl-3-methylimidazolium chloride consumed/kg body weight/day.

While terminal mean body weights of Emim-Cl-exposed rats were similar to those of control animals, a few organ weight differences were observed in both males and females (Appendix E). The only significant change was in females, wherein the relative right kidney weights were slightly increased in the 3 and 10 mg/mL groups compared to those of control animals (Table 9; Appendix E).

Table 9. Summary of Kidney Weights and Kidney-Weight-to-Body-Weight Ratios for Female Rats in the Three-month Drinking Water Study of 1-Ethyl-3-Methylimidazolium Chloride[a,b]

	0 mg/mL	1 mg/mL	3 mg/mL	10 mg/mL
n	10	10	10	10
Female				
Terminal Body Wt. (g)	254.5 ± 7.3	256.3 ± 7.1	246.9 ± 7.0	241.1 ± 5.6
Right Kidney				
Absolute (g)	0.84 ± 0.03	0.86 ± 0.03	0.87 ± 0.02	0.88 ± 0.02
Relative (mg/g)	3.29 ± 0.06**	3.36 ± 0.07	3.52 ± 0.07*	3.64 ± 0.06**

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Data are presented as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.
[c]Relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.

No changes in rat clinical chemistry parameters were attributable to Emim-Cl exposure (Appendix E). For hematology parameters measured at the end of the 3-month exposure period, significant decreases in the female white blood cell count and differential (i.e., leukocyte counts) were observed relative to control rats (Appendix E). The female control group leukocyte counts for the Emim-Cl study were higher than the control group leukocyte counts for the other three ILs, however, this can be explained by the inherently and relatively high inter- and intra-animal variability of baseline leukocyte counts. In addition, the Emim-Cl leukocyte changes were not observed in mice or male rats, and no histopathological changes were observed in the bone marrow or spleen. Although the decreases in leukocyte counts compared to the control rats appear at first to be exposure-related, they are probably due to biological variability among observed animals. No exposure-related changes were observed in the hematological parameters of male rats.

Estrous cycle length, number of cycles, and percentage of time spent in each respective stage of estrous were not affected by Emim-Cl exposure. Assessment of the lengths of diestrus, proestrus, and estrus using the continuous-time Markov model did not reveal any Emim-Cl-related differences in modeled stage lengths (Appendix E).

No exposure-related gross or histopathological changes were observed in male or female rats exposed to Emim-Cl (Appendix E).

1-Butyl-3-Methylimidazolium Chloride (Bmim-Cl)

Male and female rats were exposed to Bmim-Cl at concentrations of 0, 0.1, 0.3, or 1 mg/mL in drinking water. All rats survived to the end of the 3-month exposure period (Table 10), and no exposure-related clinical observations were noted (Appendix E). Mean body weights of male and female exposed groups remained within 10% of the mean body weights of the control groups throughout the study and were not significantly different at the end of the 3-month exposure period (Table 10; Figure 2; Appendix E).

Table 10. Summary of Survival and Mean Body Weights of Male and Female Rats in the Three-month Drinking Water Study of 1-Butyl-3-Methylimidazolium Chloride

Study Day[a]	0 mg/mL		0.1 mg/mL			0.3 mg/mL			1 mg/mL		
	Av. Wt. (g)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N
Male											
1	141.4	10	140.5	99.3	10	140.1	99.1	10	141.2	99.9	10
8	192.5	10	188.8	98.1	10	186.8	97.1	10	184.4	95.8	10
15	238.8	10	232.7	97.4	10	231.3	96.8	10	228.1	95.5	10
22	275.4	10	267.2	97.0	10	269.2	97.7	10	265.7	96.4	10
29	306.8	10	295.7	96.4	10	296.4	96.6	10	291.0	94.8	10
36	327.8	10	314.5	95.9	10	314.4	95.9	10	311.4	95.0	10
43	347.4	10	332.3	95.6	10	333.4	96.0	10	329.1	94.7	10
50	361.1	10	344.9	95.5	10	348.1	96.4	10	343.4	95.1	10
57	369.3	10	354.1	95.9	10	359.7	97.4	10	353.2	95.6	10
64	380.0	10	365.2	96.1	10	368.3	96.9	10	363.0	95.5	10
71	391.3	10	373.7	95.5	10	380.5	97.2	10	374.0	95.6	10
78	404.4	10	387.5	95.8	10	392.3	97.0	10	382.4	94.5	10
85	413.5	10	394.2	95.3	10	398.4	96.3	10	391.5	94.7	10
EOS	424.7	10	405.0	95.4	10	408.3	96.1	10	398.9	93.9	10
Female											
1	106.3	10	107.7	101.4	10	106.4	100.1	10	106.4	100.2	10
8	134.1	10	133.7	99.7	10	135.3	100.9	10	130.3	97.2	10
15	154.4	10	152.6	98.8	10	156.7	101.5	10	151.0	97.8	10
22	169.5	10	169.2	99.8	10	172.9	102.0	10	166.0	97.9	10
29	188.9	10	183.7	97.2	10	188.6	99.9	10	177.8	94.1	10
36	195.6	10	196.2	100.3	10	199.2	101.8	10	186.0	95.1	10
43	207.0	10	203.0	98.1	10	205.4	99.2	10	193.8	93.7	10
50	214.5	10	210.2	98.0	10	215.6	100.5	10	203.0	94.6	10
57	220.4	10	216.1	98.0	10	221.8	100.6	10	208.6	94.6	10
64	224.4	10	222.1	98.9	10	227.9	101.5	10	212.6	94.7	10
71	229.8	10	225.6	98.2	10	232.8	101.3	10	218.1	94.9	10
78	237.8	10	226.2	95.1	10	237.8	100.0	10	224.3	94.3	10
85	240.7	10	233.8	97.2	10	244.7	101.7	10	228.9	95.1	10
EOS	244.3	10	241.5	98.8	10	250.8	102.7	10	233.3	95.5	10

EOS = end of study.
[a]Study day 1 is the day animals were placed on study.

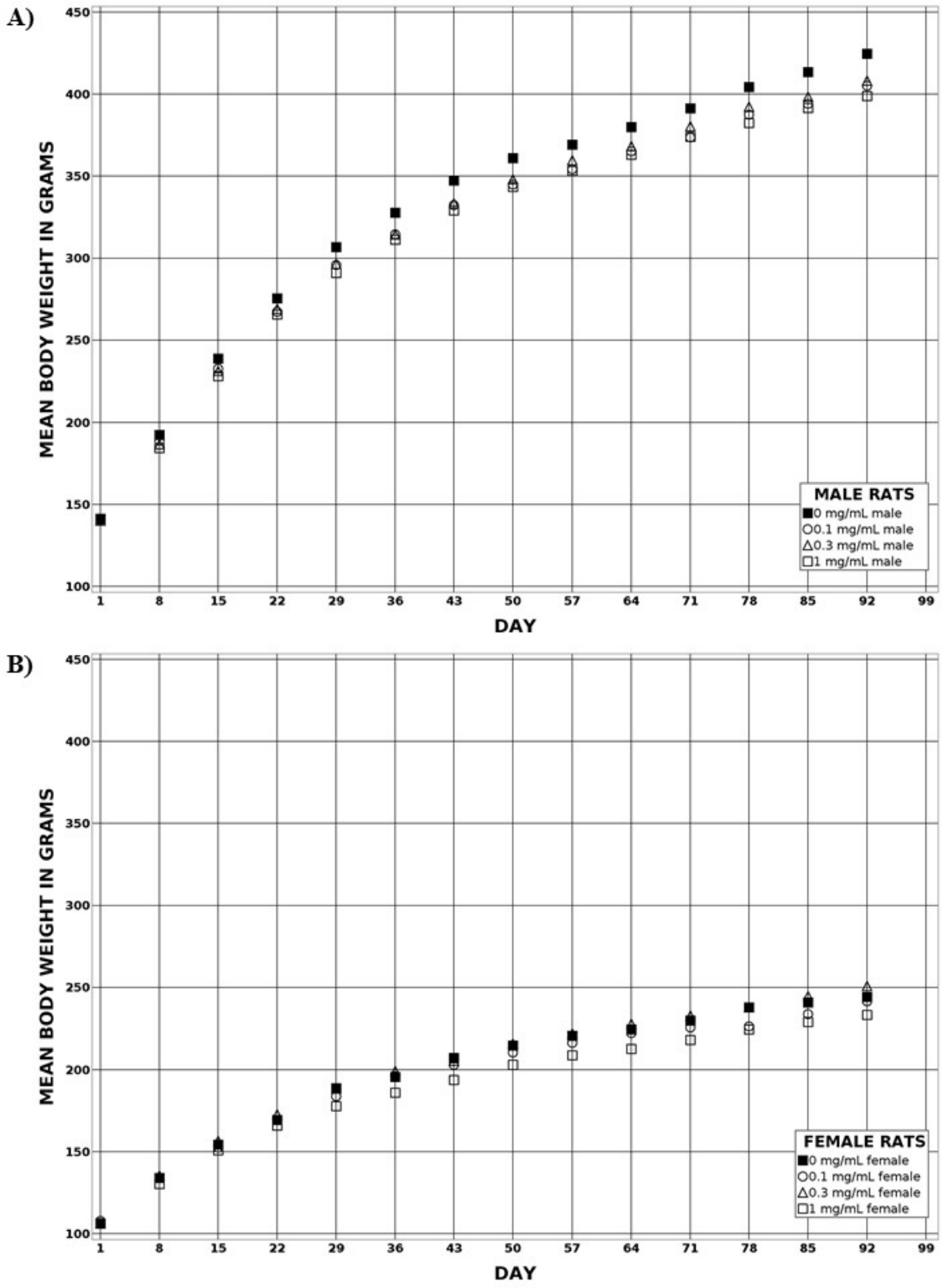


Figure 2. Growth Curves for Male and Female Rats in the Three-month Drinking Water Study of 1-Butyl-3-Methylimidazolium Chloride

Growth curves are shown for (A) males and (B) females.

At the highest Bmim-Cl exposure concentration (1 mg/mL), water consumption was 74%–86% (males) or 75%–87% (females) of the amount consumed by control groups over the course of the study (Table 11; Appendix E). Water consumption was not reduced to this extent in the low (0.1 mg/mL) or moderate (0.3 mg/mL) exposed groups. Drinking water concentrations of 0.1, 0.3, and 1 mg/mL resulted in estimated average doses of 9, 24, and 70 mg/kg/day for males and 11, 31, and 79 mg/kg/day for females, respectively.

Table 11. Summary of Water and 1-Butyl-3-Methylimidazolium Chloride Consumption of Male and Female Rats in the Three-month Drinking Water Study

Week	Water (g/day)[a]				Dose (mg/kg/day)[b]		
	0 mg/mL	0.1 mg/mL	0.3 mg/mL	1 mg/mL	0.1 mg/mL	0.3 mg/mL	1 mg/mL
Male							
1	24.5	28.3	22.4	20.1	20.1	48.0	142.3
3	24.9	28.1	24.5	21.3	12.1	31.8	93.4
6	25.2	24.1	22.0	18.7	7.7	21.0	60.1
9	23.6	23.5	23.6	18.1	6.6	19.7	51.2
13	22.7	22.1	20.6	17.7	5.6	15.5	45.2
Female							
1	18.4	19.7	20.5	15.0	18.3	57.8	140.9
3	16.7	19.9	19.5	14.0	13.0	37.3	92.7
6	16.1	19.1	18.2	13.3	9.7	27.4	71.5
9	16.3	19.0	18.5	12.8	8.8	25.0	61.4
13	16.4	17.7	18.6	14.3	7.6	22.8	62.5

[a]Mean g of water consumed/animal/day.
[b]Mg of 1-butyl-3-methylimidazolium chloride consumed/kg body weight/day.

Although terminal mean body weights of Bmim-Cl-exposed rats were similar to those of control animals, two significant organ weight changes were observed. Absolute heart weights of males exposed to 1 mg/mL were significantly decreased (by 8%) compared to those of control rats (Table 12). Absolute lung weights of females exposed to 1 mg/mL were significantly decreased (by 15%) compared to those of control rats.

Table 12. Summary of Select Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male and Female Rats in the Three-month Drinking Water Study of 1-Butyl-3-Methylimidazolium Chloride[a,b]

	0 mg/mL	0.1 mg/mL	0.3 mg/mL	1 mg/mL
n	10	10	10	10
Male				
Terminal Body Wt. (g)	424.7 ± 8.7	405.0 ± 6.2	408.3 ± 13.5	398.9 ± 8.8
Heart				
Absolute (g)	1.56 ± 0.04*	1.50 ± 0.02	1.45 ± 0.04	1.43 ± 0.03*
Relative (mg/g)[c]	3.66 ± 0.07	3.71 ± 0.04	3.55 ± 0.06	3.60 ± 0.07
Female				
Terminal Body Wt. (g)	244.3 ± 5.6	241.5 ± 4.5	250.8 ± 3.8	233.3 ± 3.5
Lung				
Absolute (g)	1.89 ± 0.10*	1.72 ± 0.06	1.79 ± 0.09	1.60 ± 0.04*
Relative (mg/g)	7.73 ± 0.36	7.13 ± 0.20	7.11 ± 0.31	6.86 ± 0.17

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$.
[a]Data are presented as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.
[c]Relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.

No changes in rat clinical chemistry parameters were attributable to Bmim-Cl exposure (Appendix E). At the end of the 3-month exposure period, there were mild (≤5%) but significant decreases, relative to control rats, in hematocrit, erythrocyte count, and hemoglobin concentrations in female rats exposed to 1 mg/mL (Table 13). Although there were no mean body weight changes observed, these changes in hematological parameters may have indicated a mild suppression in erythropoiesis due to chronic stress of exposure[103]; however, a mild direct effect due to exposure cannot be definitively ruled out. There were no exposure-related changes in the hematological parameters of the male rats (Appendix E).

Table 13. Summary of Select Hematology Data for Female Rats in the Three-month Drinking Water Study of 1-Butyl-3-Methylimidazolium Chloride[a,b]

	0 mg/mL	0.1 mg/mL	0.3 mg/mL	1 mg/mL
n	10	10	9	10
Hematocrit (%)	46.5 ± 0.5**	46.2 ± 0.3	46.7 ± 0.6	44.3 ± 0.4**
Erythrocytes (10^6/μL)	8.35 ± 0.10*	8.29 ± 0.08	8.41 ± 0.08	8.00 ± 0.05*
Hemoglobin (g/dL)	14.8 ± 0.1*	14.8 ± 0.1	14.9 ± 0.2	14.3 ± 0.1*

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Data are presented as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.

Estrous cycle length, number of cycles, and percentage of time spent in each respective stage of estrous were not affected by Bmim-Cl exposure. Assessment of the lengths of diestrus, proestrus, and estrus using the continuous-time Markov model did not reveal any Bmim-Cl-related differences in modeled stage lengths (Appendix E).

No exposure-related gross or histopathological changes were observed in male or female rats exposed to Bmim-Cl (Appendix E).

1-Butyl-1-Methylpyrrolidinium Chloride (Bmpy-Cl)

Male and female rats were exposed to different concentrations of Bmpy-Cl in drinking water due to sex differences observed in the 2-week studies. Male rats were exposed to 0, 0.3, 1, or 3 mg/mL Bmpy-Cl, and female rats were exposed to 0, 1, 3, or 6 mg/mL Bmpy-Cl. All rats survived to the end of the 3-month exposure period (Table 14) and no exposure-related clinical observations were noted (Appendix E). Mean body weights of male rats were within 10% of the control group mean body weights throughout the study, although a 7% decrease in the terminal mean body weight of the 3 mg/mL group was significantly different from the control group (Table 14; Figure 3; Appendix E). Mean body weights of female rats in the 6 mg/mL group were consistently lower than those of control rats throughout the study, and the terminal mean body weight was significantly decreased (12%) relative to the control group. In females exposed to 1 or 3 mg/mL, mean body weights were similar to control animal values throughout the study.

Table 14. Summary of Survival and Mean Body Weights of Male and Female Rats in the Three-month Drinking Water Study of 1-Butyl-1-Methylpyrrolidinium Chloride

Study Day[a]	0 mg/mL		0.3 mg/mL			1 mg/mL			3 mg/mL		
	Av. Wt. (g)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N
Male											
1	157.7	10	159.5	101.1	10	157.0	99.5	10	158.0	100.2	10
8	206.3	10	204.7	99.2	10	201.7	97.8	10	195.5	94.7	10
15	254.0	10	248.3	97.8	10	243.6	95.9	10	236.9	93.3	10
22	288.0	10	282.4	98.1	10	276.8	96.1	10	267.3	92.8	10
29	313.2	10	305.4	97.5	10	302.0	96.4	10	290.7	92.8	10
36	332.5	10	323.1	97.2	10	321.3	96.6	10	308.5	92.8	10
43	350.4	10	341.1	97.4	10	338.1	96.5	10	326.6	93.2	10
50	366.0	10	355.9	97.2	10	351.7	96.1	10	341.1	93.2	10
57	375.1	10	365.0	97.3	10	362.1	96.5	10	354.0	94.4	10
64	387.2	10	378.2	97.7	10	372.9	96.3	10	364.7	94.2	10
71	398.1	10	387.8	97.4	10	381.8	95.9	10	372.2	93.5	10
78	409.4	10	397.0	97.0	10	391.0	95.5	10	382.6	93.5	10
85	418.9	10	408.3	97.5	10	399.7	95.4	10	391.9	93.6	10
EOS	429.6	10	417.0	97.1	10	412.6	96.1	10	399.0	92.9	10
Study Day	**0 mg/mL**		**1 mg/mL**			**3 mg/mL**			**6 mg/mL**		
	Av. Wt. (g)	**N**	**Av. Wt. (g)**	**Wt. (% of Controls)**	**N**	**Av. Wt. (g)**	**Wt. (% of Controls)**	**N**	**Av. Wt. (g)**	**Wt. (% of Controls)**	**N**
Female											
1	104.2	10	104.8	100.6	10	106.9	102.6	10	105.6	101.4	10
8	135.0	10	134.6	99.7	10	132.2	97.9	10	99.2	73.5	10
15	156.5	10	152.9	97.7	10	153.1	97.9	10	124.3	79.5	10
22	176.0	10	171.2	97.3	10	170.5	96.9	10	141.4	80.3	10
29	189.1	10	188.2	99.6	10	184.1	97.4	10	156.1	82.5	10
36	197.0	10	198.8	100.9	10	195.5	99.2	10	167.6	85.1	10
43	207.1	10	206.2	99.5	10	203.8	98.4	10	176.2	85.1	10
50	217.2	10	215.9	99.4	10	215.5	99.2	10	186.5	85.8	10
57	223.4	10	219.7	98.3	10	219.2	98.1	10	192.8	86.3	10
64	230.4	10	225.0	97.7	10	226.4	98.3	10	199.5	86.6	10
71	232.9	10	230.9	99.1	10	230.2	98.8	10	208.1	89.3	10
78	237.3	10	234.5	98.8	10	235.5	99.3	10	210.7	88.8	10
85	243.6	10	243.4	99.9	10	244.8	100.5	10	219.0	89.9	10
EOS	250.1	10	249.4	99.7	10	251.6	100.6	10	220.4	88.1	10

EOS = end of study.
[a]Study day 1 is the day animals were placed on study.

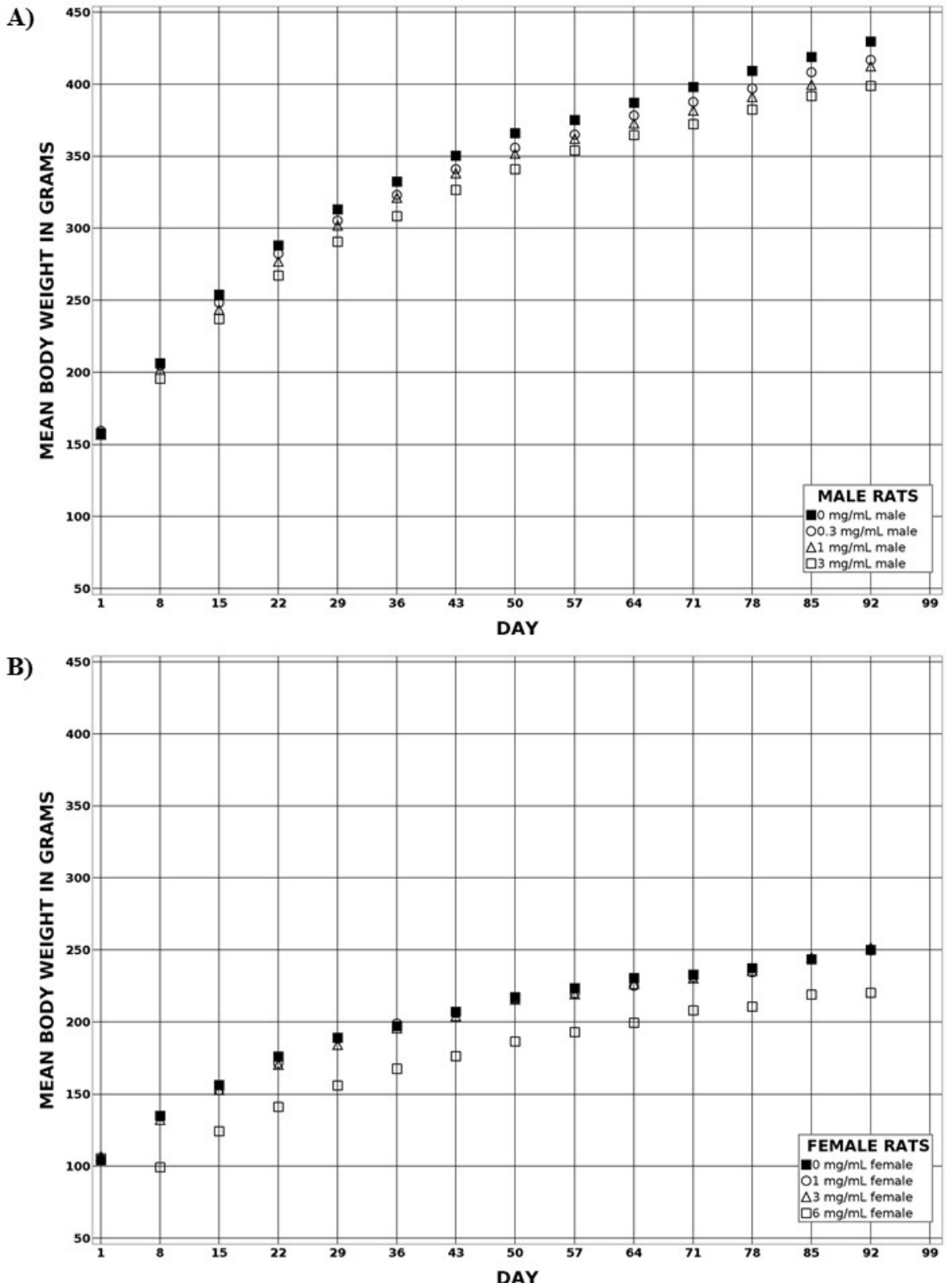


Figure 3. Growth Curves for Male and Female Rats in the Three-month Drinking Water Study of 1-Butyl-1-Methylpyrrolidinium Chloride

Growth curves are shown for (A) males and (B) females.

In general, water consumption by both male and female rats exposed to Bmpy-Cl decreased in an exposure concentration-dependent manner. At any given concentration, however, female rats consumed less water than male rats. Water consumption by male rats exposed to 3 mg/mL Bmpy-Cl, was 75%–85% of control animals (Table 15), whereas water consumption by female rats exposed to 3 mg/mL was 68%–76% of control animals. In female rats exposed to 6 mg/mL, water consumption was reduced even further to 26%–63% of consumption by control animals. The lowest water consumption value of 26% by females was observed during week 1 of the study and was potentially due to the poor palatability of the dose formulation. Drinking water concentrations of 0.3, 1, and 3 mg/mL resulted in estimated average doses of 26, 74, and 201 mg/kg/day, respectively, for males, and drinking water concentrations of 1, 3, and 6 mg/mL resulted in estimated average doses of 89, 222, and 376 mg/kg/day, respectively, for females.

Table 15. Summary of Water and 1-Butyl-1-Methylpyrrolidinium Chloride Consumption of Male and Female Rats in the Three-month Drinking Water Study

Week	Water (g/day)[a]				Dose (mg/kg/day)[b]		
	0 mg/mL	0.3 mg/mL	1 mg/mL	3 mg/mL	0.3 mg/mL	1 mg/mL	3 mg/mL
Male							
1	23.7	24.7	21.7	18.3	46.5	138.2	347.6
3	25.9	26.2	22.3	19.5	31.7	91.5	246.9
6	25.0	25.3	21.7	19.6	23.5	67.5	190.6
9	22.9	24.7	21.1	18.6	20.3	58.3	157.6
13	23.6	25.2	20.9	19.0	18.5	52.3	145.4
	0 mg/mL	**1 mg/mL**	**3 mg/mL**	**6 mg/mL**	**1 mg/mL**	**3 mg/mL**	**6 mg/mL**
Female							
1	17.6	15.2	13.3	4.5	145.1	373.2	255.7
3	17.9	15.9	13.0	9.4	104.0	254.7	453.7
6	18.7	17.8	13.7	11.3	89.5	210.3	404.6
9	19.5	16.0	13.6	11.3	72.8	186.1	351.7
13	19.3	18.0	14.5	11.5	74.0	177.7	315.1

[a]Mean g of water consumed/animal/day.
[b]Mg of 1-butyl-1-methylpyrrolidinium chloride consumed/kg body weight/day.

Significant organ weight changes were observed only in female rats exposed to Bmpy-Cl (Table 16; Appendix E). In female rats exposed to 6 mg/mL, which had significant terminal mean body weight decreases, absolute mean liver weights were significantly decreased by 20% compared to control rats. Relative mean liver weights were also significantly decreased in this group. Furthermore, relative right kidney and heart weights were significantly increased in the 6 mg/mL females compared to control rats; however, these changes were considered secondary effects of decreased body weight and/or water consumption (Appendix E). Despite not experiencing significant decreases in body weights, female rats in the 1 and 3 mg/mL groups had significant decreases in relative liver weights (Table 16).

Table 16. Summary of Liver Weights and Liver-Weight-to-Body-Weight Ratios for Female Rats in the Three-month Drinking Water Study of 1-Butyl-1-Methylpyrrolidinium Chloride[a,b]

	0 mg/mL	1 mg/mL	3 mg/mL	6 mg/mL
n	10	10	10	10
Terminal Body Wt. (g)	250.1 ± 3.8**	249.4 ± 5.1	251.6 ± 6.5	220.4 ± 3.9**
Liver				
Absolute (g)	9.11 ± 0.26**	8.46 ± 0.36	8.49 ± 0.33	7.28 ± 0.21**
Relative (mg/g)[c]	36.41 ± 0.91**	33.78 ± 0.88*	33.69 ± 0.75*	33.00 ± 0.57**

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Data are presented as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.
[c]Relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.

No changes in rat hematology parameters were attributable to Bmpy-Cl exposure. Female rats in the 6 mg/mL group had a significant decrease in alanine aminotransferase activity compared to control rats (Appendix E). Given that the decrease was mild, only present in the highest exposed group, and not observed in male rats, these changes were considered related to biological variability rather than Bmpy-Cl exposure.

Estrous cycle length, number of cycles, and percentage of time spent in each respective stage of estrous were not affected by Bmpy-Cl exposure. Assessment of the lengths of diestrus, proestrus, and estrus using the continuous-time Markov model did not reveal any Bmpy-Cl-related differences in modeled stage lengths. Four females exposed to 6 mg/mL were not cycling, which likely resulted from nonspecific stress, as indicated by the consistently lower mean body weights of these rats compared to control rats (Table 14; Figure 3; Appendix E).

No exposure-related gross or histopathological changes were observed in male or female rats exposed to Bmpy-Cl (Appendix E).

N-Butylpyridinium Chloride (NBuPy-Cl)

Male and female rats were exposed to NBuPy-Cl at concentrations of 0, 0.3, 1, or 3 mg/mL in drinking water. Apart from one death (considered unrelated to exposure) of a male rat exposed to 3 mg/mL on study day 6, all rats survived to the end of the 3-month exposure period (Table 17). No exposure-related clinical observations were noted (Appendix E). Although most body weight changes during the study were within 10% of control animals, significantly decreased terminal mean body weights (approximately 89%–95% of control animals) were noted in the 1 mg/mL NBuPy-Cl-exposed male rats and the 3 mg/mL NBuPy-Cl-exposed male and female rats (Table 17; Figure 4; Appendix E). These were interpreted as lower body weight gain, as opposed to body weight loss, during the exposure period.

Table 17. Summary of Survival and Mean Body Weights of Male and Female Rats in the Three-month Drinking Water Study of N-Butylpyridinium Chloride

Study Day[a]	0 mg/mL		0.3 mg/mL			1 mg/mL			3 mg/mL		
	Av. Wt. (g)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N
Male											
1	157.2	10	156.1	99.3	10	155.4	98.9	10	156.9	99.8	10
8	206.3	10	203.9	98.9	10	199.4	96.7	10	185.4	89.9	9
15	250.2	10	249.0	99.5	10	239.7	95.8	10	224.7	89.8	9
22	285.0	10	284.3	99.7	10	272.3	95.5	10	257.2	90.3	9
29	309.0	10	307.7	99.6	10	293.0	94.8	10	278.5	90.1	9
36	336.9	10	332.7	98.7	10	314.1	93.2	10	299.9	89.0	9
43	352.2	10	348.6	99.0	10	329.7	93.6	10	315.6	89.6	9
50	365.6	10	361.4	98.8	10	343.4	93.9	10	330.2	90.3	9
57	380.7	10	373.5	98.1	10	353.7	92.9	10	339.1	89.1	9
64	390.0	10	380.4	97.5	10	364.0	93.3	10	346.3	88.8	9
71	401.7	10	396.3	98.6	10	381.7	95.0	10	357.9	89.1	9
78	409.9	10	405.2	98.8	10	387.9	94.6	10	363.9	88.8	9
85	417.2	10	413.8	99.2	10	396.5	95.0	10	371.6	89.1	9
EOS	426.8	10	420.7	98.6	10	403.4	94.5	10	379.1	88.8	9
Female											
1	108.2	10	105.7	97.7	10	105.5	97.5	10	106.8	98.7	10
8	137.1	10	132.7	96.8	10	134.4	98.0	10	121.0	88.2	10
15	158.2	10	151.3	95.6	10	154.6	97.7	10	139.7	88.3	10
22	178.1	10	168.6	94.6	10	170.2	95.6	10	155.3	87.2	10
29	189.7	10	181.5	95.6	10	181.8	95.8	10	165.1	87.0	10
36	201.4	10	193.6	96.1	10	192.8	95.7	10	175.8	87.3	10
43	210.7	10	201.9	95.8	10	201.5	95.6	10	184.0	87.3	10
50	218.9	10	212.9	97.3	10	208.2	95.1	10	192.1	87.7	10
57	225.9	10	219.4	97.1	10	216.8	96.0	10	199.3	88.2	10
64	229.7	10	223.3	97.2	10	221.7	96.5	10	204.3	89.0	10
71	234.6	10	228.0	97.2	10	227.4	96.9	10	208.4	88.8	10
78	238.2	10	233.2	97.9	10	228.9	96.1	10	211.7	88.9	10
85	244.5	10	237.9	97.3	10	238.5	97.6	10	217.0	88.7	10
EOS	247.6	10	240.3	97.1	10	242.0	97.7	10	221.8	89.6	10

EOS = end of study.
[a]Study day 1 is the day animals were placed on study.

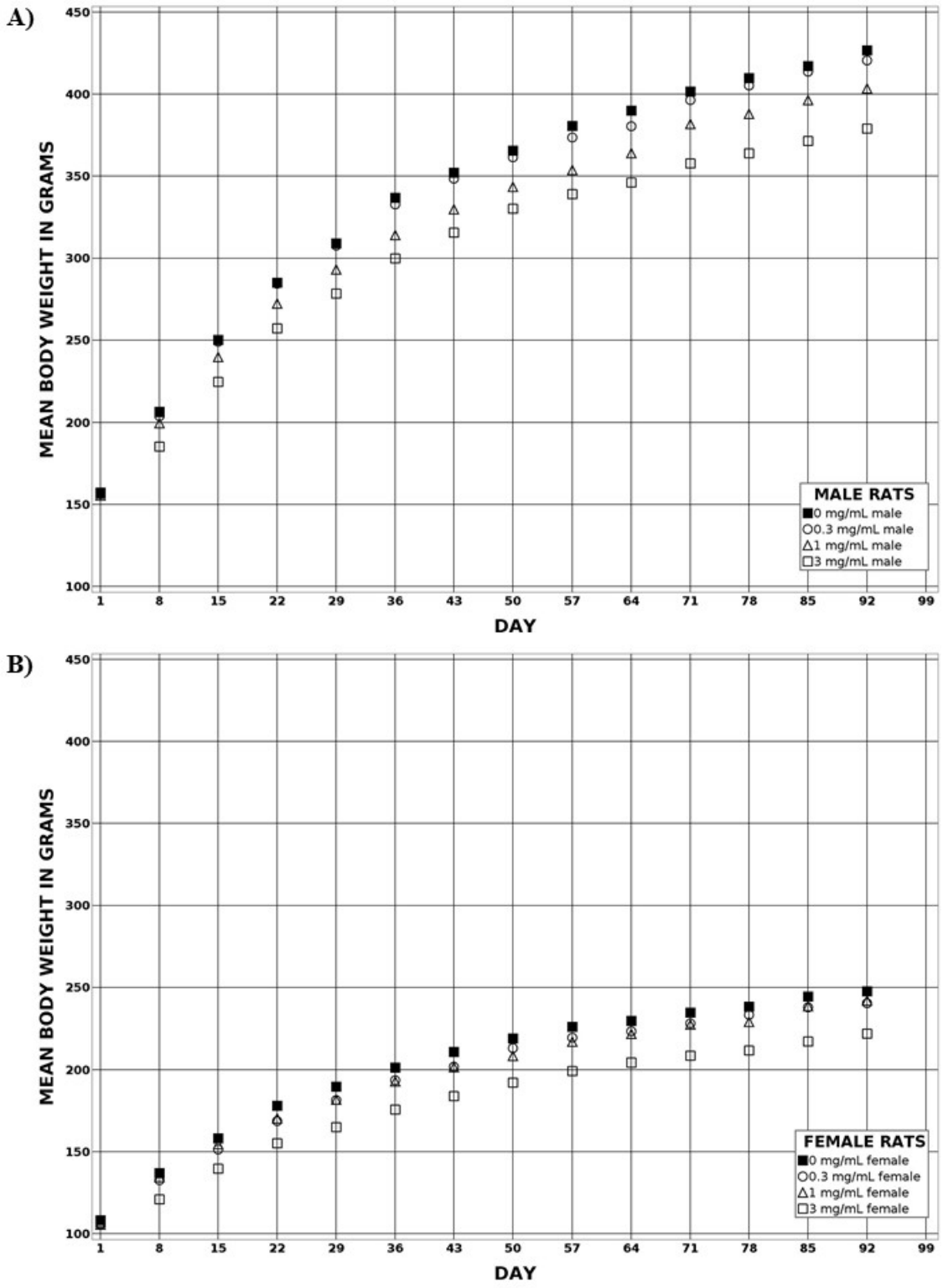


Figure 4. Growth Curves for Male and Female Rats in the Three-month Drinking Water Study of N-Butylpyridinium Chloride

Growth curves are shown for (A) males and (B) females.

Water consumption was lower relative to control groups for males exposed to ≥0.3 mg/mL NBuPy-Cl or females exposed to ≥1 mg/mL and decreased with increasing exposure concentration (Table 18). At the highest exposure concentration of 3 mg/mL, water consumption by male rats was 47%–69% of the control group (Appendix E). Female rats exposed to the highest exposure concentration of 3 mg/mL had mean water consumption ranging from 47% to 63% of the control group (Table 18). The lowest water consumption value of 47% of the control group for both males and females exposed to 3 mg/mL was observed on week 1 of the study and was potentially due to the formulation's poor palatability. Drinking water concentrations of 0.3, 1, and 3 mg/mL resulted in estimated average doses of 23, 73, and 171 mg/kg/day, respectively, for males and 30, 83, and 184 mg/kg/day, respectively, for females.

Table 18. Summary of Water and N-Butylpyridinium Chloride Consumption of Male and Female Rats in the Three-month Drinking Water Study

Week	Water (g/day)[a]				Dose (mg/kg/day)[b]		
	0 mg/mL	0.3 mg/mL	1 mg/mL	3 mg/mL	0.3 mg/mL	1 mg/mL	3 mg/mL
Male							
1	26.8	24.3	21.8	12.5	46.7	140.3	238.9
3	26.7	24.3	21.9	16.9	29.3	91.4	225.6
6	25.8	24.2	20.0	16.6	21.8	63.7	166.0
9	24.3	22.0	20.5	15.8	17.7	58.0	139.8
13	24.0	21.8	20.6	15.8	15.8	52.0	127.6
Female							
1	17.5	18.2	15.2	8.2	51.7	144.1	230.3
3	17.2	17.2	14.1	10.2	34.1	91.2	219.1
6	17.2	18.4	15.0	10.8	28.5	77.8	184.3
9	18.7	18.4	15.4	10.5	25.2	71.0	158.0
13	19.7	20.6	15.8	11.1	26.0	66.2	153.5

[a]Mean g of water consumed/animal/day.
[b]Mg of n-butylpyridinium chloride consumed/kg body weight/day.

Significant organ weight changes were observed in male and female rats exposed to NBuPy-Cl, but all changes were considered secondary effects of decreased body weight and/or water consumption (Appendix E). Alongside a significant decrease in terminal mean body weight, male rats in the 3 mg/mL group had significantly increased relative heart, right kidney, and liver weights. Females in the 3 mg/mL group similarly had a significant decrease in terminal mean body weight accompanied by significantly increased relative heart and right kidney weights. These females also had significantly decreased absolute liver weights. Males in the 1 mg/mL group had a smaller (approximately 5%) significant decrease in terminal mean body weight accompanied by significantly increased relative right kidney and liver weights. Males in the 0.3 mg/mL group, which did not have a significantly different terminal mean body weight, had significantly increased relative right kidney weights. This change was very small, however, and was not considered toxicologically relevant.

At the end of the 3-month exposure period, the 1 and 3 mg/mL male rats had a significant increase in urea nitrogen concentration (Appendix E). This increase was mild and not observed in females; therefore, the change was considered unrelated to NBuPy-Cl exposure and might have indicated mild dehydration due to lower water consumption. There were no changes in rat hematology parameters attributable to NBuPy-Cl administration in drinking water (Appendix E).

Estrous cycle length, number of cycles, and percentage of time spent in each respective stage of estrous were not affected by NBuPy-Cl exposure. Assessment of the lengths of diestrus, proestrus, and estrus using the continuous-time Markov model did not reveal any NBuPy-Cl-related differences in modeled stage lengths (Appendix E).

No exposure-related gross or histopathological changes were observed in male or female rats exposed to NBuPy-Cl (Appendix E).

Mice

1-Ethyl-3-Methylimidazolium Chloride (Emim-Cl)

Male and female mice were exposed to Emim-Cl at concentrations of 0, 3, 10, and 30 mg/mL in drinking water. All mice survived to the end of the 3-month exposure period (Table 19), and no exposure-related clinical observations were noted (Appendix E). Male mice exposed to 10 mg/mL and both male and female mice exposed to 30 mg/mL had significantly decreased terminal mean body weights, relative to control mice, which was interpreted as lower weight gain over the course of the study (Table 19; Figure 5; Appendix E). At the 30 mg/mL exposure concentration, male and female terminal mean body weights were 71% and 79%, respectively, of the control groups. At 10 mg/mL exposure concentration, male terminal body weights were 93% of the control group. Male and female mice exposed to 3 mg/mL and female mice exposed to 10 mg/mL maintained mean body weights within 10% of the control groups, and these differences were not significant (Appendix E).

Table 19. Summary of Survival and Mean Body Weights of Male and Female Mice in the Three-month Drinking Water Study of 1-Ethyl-3-Methylimidazolium Chloride

Study Day[a]	0 mg/mL		3 mg/mL			10 mg/mL			30 mg/mL		
	Av. Wt. (g)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N
Male											
1	23.2	10	23.1	99.9	10	23.1	99.8	10	23.1	99.7	10
8	24.5	10	24.5	100.0	10	24.1	98.3	10	21.8	88.8	10
15	25.6	10	25.9	101.3	10	25.5	99.8	10	22.7	88.8	10
22	26.9	10	27.2	101.3	10	26.6	99.1	10	23.6	88.0	10
29	28.0	10	28.6	102.1	10	27.9	99.9	10	24.3	86.8	10
36	28.6	10	29.6	103.6	10	28.4	99.4	10	24.5	85.7	10
43	30.4	10	31.4	103.3	10	29.7	98.0	10	22.3	73.5	10
50	31.6	10	32.5	102.8	10	30.8	97.6	10	24.0	75.8	10
57	33.2	10	34.0	102.3	10	32.2	96.8	10	25.2	75.8	10
64	34.3	10	35.1	102.2	10	32.9	95.8	10	26.3	76.7	10
71	36.0	10	36.7	101.9	10	34.2	95.0	10	26.9	74.9	10
78	37.2	10	37.9	101.9	10	34.6	93.2	10	27.1	73.0	10
85	38.6	10	39.2	101.4	10	36.1	93.4	10	27.7	71.7	10
EOS	38.9	10	39.1	100.6	10	36.2	93.0	10	27.8	71.3	10
Female											
1	17.4	10	17.3	99.8	10	16.9	97.5	10	17.0	97.8	10
8	18.5	10	18.4	99.4	10	18.6	100.4	10	16.4	88.7	10
15	19.5	10	19.3	99.2	10	19.5	100.0	10	17.3	88.5	10
22	20.4	10	20.3	99.1	10	20.7	101.3	10	18.5	90.3	10
29	21.7	10	21.3	98.1	10	21.6	99.7	10	19.3	89.0	10
36	22.3	10	21.8	97.8	10	22.0	98.9	10	20.0	89.8	10
43	23.7	10	23.0	97.2	10	23.2	98.0	10	20.8	87.6	10
50	24.5	10	23.0	93.9	10	23.4	95.5	10	21.2	86.6	10
57	25.4	10	24.3	95.7	10	25.0	98.7	10	21.8	86.1	10
64	26.6	10	24.3	91.4	10	25.4	95.4	10	22.2	83.4	10
71	28.9	10	26.4	91.3	10	26.4	91.3	10	22.9	79.3	10
78	28.5	10	25.6	89.9	10	26.2	92.2	10	22.5	79.1	10
85	28.8	10	26.3	91.2	10	27.0	93.6	10	23.0	79.7	10
EOS	29.2	10	26.6	91.0	10	27.8	95.2	10	23.0	78.8	10

EOS = end of study.
[a]Study day 1 is the day animals were placed on study.

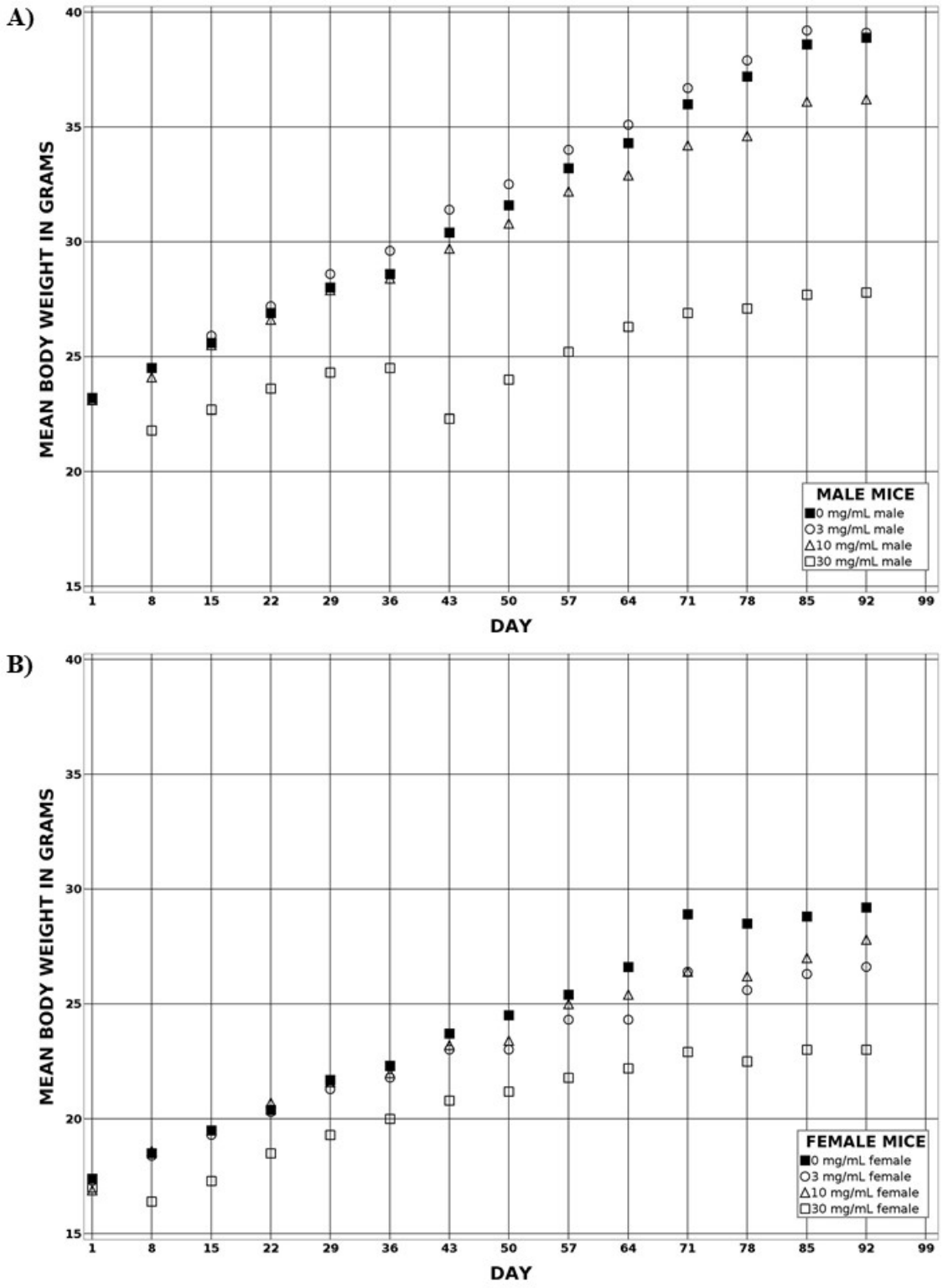


Figure 5. Growth Curves for Male and Female Mice in the Three-month Drinking Water Study of 1-Ethyl-3-Methylimidazolium Chloride

Growth curves are shown for (A) males and (B) females.

In general, water consumption decreased in an exposure concentration-dependent manner for both male and female mice exposed to Emim-Cl (Table 20; Appendix E). In the highest exposed group (30 mg/mL), mean water consumption ranged from 44% to 66% of control animals for males and from 43% to 87% of control animals for females. Drinking water concentrations of 3, 10, and 30 mg/mL resulted in estimated average doses of 341, 1,039, and 2,283 mg/kg/day, respectively, for males and 383, 1,198, and 2,457 mg/kg/day, respectively, for females.

Table 20. Summary of Water and 1-Ethyl-3-Methylimidazolium Chloride Consumption of Male and Female Mice in the Three-month Drinking Water Study

Week	Water (g/day)[a]				Dose (mg/kg/day)[b]		
	0 mg/mL	3 mg/mL	10 mg/mL	30 mg/mL	3 mg/mL	10 mg/mL	30 mg/mL
Male							
2[c]	3.4	3.5	3.2	1.8	428.0	1,327.3	2,479.3
3	3.5	3.6	3.2	1.8	416.7	1,253.4	2,377.8
6	3.5	3.6	3.1	2.1	364.4	1,090.8	2,570.4
9	3.3	3.5	2.9	2.0	308.9	901.2	2,381.0
13	3.3	3.4	3.3	1.9	260.5	914.9	2,058.5
Female							
1	2.6	2.7	2.8	1.2	467.4	1,654.8	2,120.1
3	2.8	2.7	2.4	1.4	418.8	1,230.8	2,433.4
6	3.7	2.9	2.6	1.6	399.6	1,181.8	2,402.4
9	3.0	3.0	2.7	1.8	371.0	1,078.3	2,473.7
13	3.0	2.8	2.8	2.0	319.8	1,037.8	2,613.2

[a]Mean g of water consumed/animal/day.
[b]Mg of 1-ethyl-3-methylimidazolium chloride consumed/kg body weight/day.
[c]Week 1 data unavailable for male mice.

Terminal mean body weights of mice exposed to Emim-Cl were significantly decreased at the highest exposure concentration of 30 mg/mL (Appendix E). Accordingly, significant organ weight changes were observed for both male and female mice exposed to 30 mg/mL; however, these were considered secondary effects of decreased body weight and/or water consumption (Appendix E). Males exposed to 30 mg/mL had significantly decreased absolute right kidney and liver weights (by 19% and 23%, respectively) compared to the control group. These males also had significantly increased relative heart, right kidney, liver, lung, and right testis weights. Females exposed to 30 mg/mL had significantly decreased absolute heart and liver weights (by 13% and 20%, respectively), as well as significantly increased relative right kidney weights, compared to control animals. Males in the 10 mg/mL group had a smaller (7%) significant decrease in terminal mean body weights compared to control animals, accompanied by a significant increase in relative right testis weight. Again, this organ weight change was considered a secondary effect of decreased body weight and/or water consumption.

At the end of the 3-month exposure period, there was a mild increase in the erythron of the 30 mg/mL male mice (Table 21). These changes included significant increases in the manual hematocrit, erythrocyte count, and reticulocyte count compared to control animals and were consistent with mild hemoconcentration due to lower water consumption. Similarly, a

significant increase in erythrocyte counts and reticulocyte counts were observed in the 30 mg/mL female mice.

Table 21. Summary of Select Hematology Data for Male and Female Mice in the Three-month Drinking Water Study of 1-Ethyl-3-Methylimidazolium Chloride[a,b]

	0 mg/mL	3 mg/mL	10 mg/mL	30 mg/mL
n	10	10	10	10
Male				
Manual Hematocrit (%)	48.7 ± 0.3*	49.3 ± 0.4	48.8 ± 0.4	50.2 ± 0.4*
Erythrocytes (10^6/μL)	11.05 ± 0.09**	11.13 ± 0.14	11.07 ± 0.08	11.62 ± 0.11**
Reticulocytes (10^3/μL)	241.9 ± 8.0**	233.2 ± 7.4	253.7 ± 7.4	273.8 ± 3.7**
Female				
Erythrocytes (10^6/μL)	10.84 ± 0.20**	11.06 ± 0.17	11.45 ± 0.29	11.99 ± 0.28**
Reticulocytes (10^3/μL)	231.5 ± 17.4*	270.9 ± 14.7	291.3 ± 18.8*	280.7 ± 11.5

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Data are presented as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.

Emim-Cl exposure in female mice did not affect estrous cycle length, number of cycles, and percentage of time spent in each respective stage of estrous. Assessment of the lengths of diestrus, proestrus, and estrus using the continuous-time Markov model did not reveal any Emim-Cl-related differences in modeled stage lengths (Appendix E).

Limited histopathological changes were observed in male and female mice exposed to Emim-Cl (Table 22). There were significant increases in the incidences of chronic progressive nephropathy and renal tubule cytoplasmic alteration in males exposed to 30 mg/mL relative to the control group. There was also a positive trend in the incidences of renal infarct in male mice. Similar histological findings were not observed in the kidneys of female mice. However, there was a significant increase in the incidence of adrenal cortex persistent X-zone in females exposed to 30 mg/mL compared to the control group. The morphological features of the lesions discussed in this section are presented in the Histopathological Descriptions of Mice section.

Table 22. Incidences of Nonneoplastic Lesions of the Kidney and Adrenal Gland in Male and Female Mice in the Three-month Drinking Water Study of 1-Ethyl-3-Methylimidazolium Chloride

	0 mg/mL	3 mg/mL	10 mg/mL	30 mg/mL
n[a]	10	10	10	10
Male				
Kidney				
Infarct[b]	0*	0	0	2 (1.0)[c]
Nephropathy, chronic progressive	1** (1.0)	1 (1.0)	2 (1.0)	8** (1.0)
Renal tubule, cytoplasmic alteration	0**	0	0	9** (1.7)
Female				
Adrenal Gland				
Adrenal cortex, X-zone, persistent	0**	1	0	8**

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control indicates a significant trend test.
*Statistically significant at $p \leq 0.05$ by the Cochran-Armitage (trend) or Fisher's exact (pairwise) test; **$p \leq 0.01$.
[a]Number of animals examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of observed lesion in affected animals; 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

1-Butyl-3-Methylimidazolium Chloride (Bmim-Cl)

Male and female mice were exposed to Bmim-Cl at concentrations of 0, 0.3, 1, and 3 mg/mL in drinking water. All mice survived to the end of the 3-month exposure period (Table 23) and no exposure-related clinical observations were noted (Appendix E). Male mice exposed to 3 mg/mL had a significantly decreased terminal mean body weight (77% of the control group), which was interpreted as lower weight gain over the course of the study (Table 23; Figure 6; Appendix E). Female terminal mean body weight at this exposure concentration was significantly decreased at 91% of the control group. Mice exposed to 0.3 or 1 mg/mL maintained body weights within 10% of the control groups.

Table 23. Summary of Survival and Mean Body Weights of Male and Female Mice in the Three-month Drinking Water Study of 1-Butyl-3-Methylimidazolium Chloride

Study Day[a]	0 mg/mL		0.3 mg/mL			1 mg/mL			3 mg/mL		
	Av. Wt. (g)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N
Male											
1	21.9	10	21.7	99.2	10	21.8	99.4	10	21.7	99.0	10
8	23.9	10	23.7	99.2	10	23.3	97.7	10	22.6	94.7	10
15	25.5	10	25.6	100.6	10	25.1	98.6	10	23.8	93.4	10
22	26.9	10	27.1	100.6	10	26.4	98.1	10	24.3	90.3	10
29	28.0	10	28.0	100.0	10	27.1	96.9	10	25.3	90.4	10
36	29.6	10	29.2	98.9	10	28.2	95.4	10	25.4	85.9	10
43	30.6	10	30.5	99.5	10	29.4	95.9	10	25.8	84.3	10
50	32.0	10	31.5	98.7	10	30.4	95.2	10	26.7	83.5	10
57	33.0	10	32.8	99.4	10	31.2	94.7	10	27.2	82.5	10
64	34.7	10	34.5	99.4	10	32.8	94.5	10	28.0	80.8	10
71	36.3	10	35.6	98.0	10	34.0	93.8	10	28.5	78.7	10
78	37.2	10	36.6	98.2	10	34.6	92.9	10	29.2	78.4	10
85	38.0	10	37.7	99.2	10	35.9	94.3	10	29.7	78.1	10
EOS	38.8	10	38.6	99.6	10	36.5	94.0	10	29.7	76.6	10
Female											
1	17.2	10	17.0	99.2	10	16.7	97.3	10	16.9	98.2	10
8	18.3	10	17.8	97.6	10	17.9	98.2	10	17.7	97.0	10
15	19.6	10	18.9	96.4	10	19.3	98.7	10	18.3	93.5	10
22	20.3	10	19.7	96.7	10	20.3	99.6	10	19.1	94.1	10
29	21.5	10	20.7	96.4	10	21.1	98.4	10	19.7	91.7	10
36	22.2	10	22.0	99.1	10	22.2	99.7	10	20.7	93.1	10
43	23.2	10	22.5	97.2	10	22.8	98.5	10	21.6	93.4	10
50	23.6	10	23.1	97.7	10	23.3	98.7	10	22.0	93.0	10
57	24.4	10	23.8	97.4	10	23.9	98.0	10	22.7	93.0	10
64	24.7	10	24.6	99.5	10	24.6	99.6	10	23.3	94.3	10
71	26.5	10	25.6	96.6	10	25.5	96.0	10	23.4	88.3	10
78	26.0	10	26.4	101.3	10	25.9	99.4	10	23.4	90.0	10
85	26.5	10	25.7	97.3	10	25.9	97.8	10	23.6	89.2	10
EOS	26.8	10	26.3	97.9	10	26.3	98.1	10	24.4	91.0	10

EOS = end of study.
[a]Study day 1 is the day animals were placed on study.

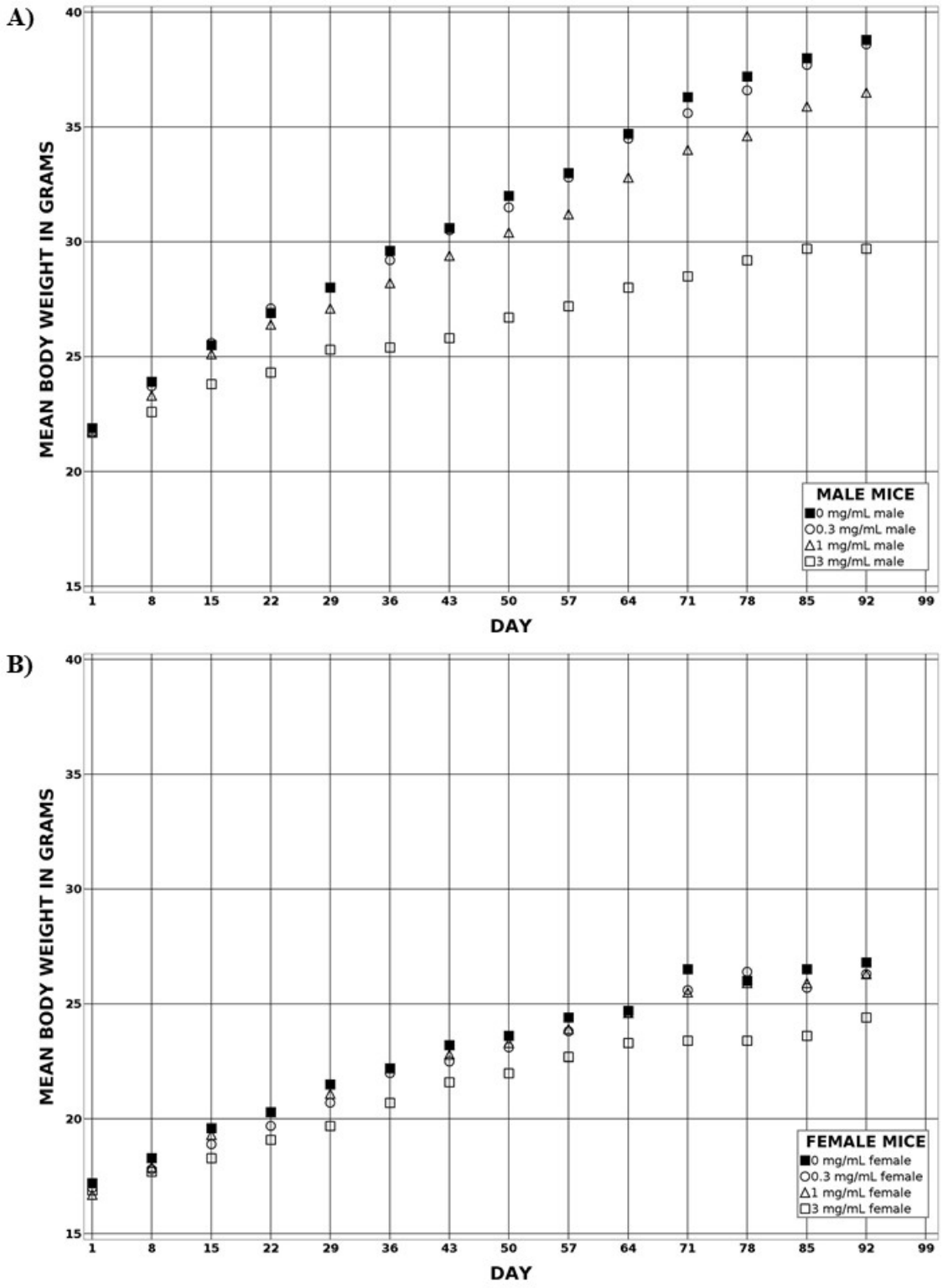


Figure 6. Growth Curves for Male and Female Mice in the Three-month Drinking Water Study of 1-Butyl-3-Methylimidazolium Chloride

Growth curves are shown for (A) males and (B) females.

In general, water consumption decreased in an exposure concentration-dependent manner for both male and female mice exposed to Bmim-Cl (Table 24; Appendix E). At the highest exposure concentration of 3 mg/mL, water consumption by male mice was 56%–77% of the control group. In female mice exposed to 3 mg/mL, water consumption was 47%–69% of the control group. Drinking water concentrations of 0.3, 1, and 3 mg/mL resulted in estimated average doses of 37, 116, and 262 mg/kg/day, respectively, for males and 39, 125, and 270 mg/kg/day, respectively, for females.

Table 24. Summary of Water and 1-Butyl-3-Methylimidazolium Chloride Consumption of Male and Female Mice in the Three-month Drinking Water Study

Week	Water (g/day)[a]				Dose (mg/kg/day)[b]		
	0 mg/mL	0.3 mg/mL	1 mg/mL	3 mg/mL	0.3 mg/mL	1 mg/mL	3 mg/mL
Male							
2[c]	3.7	3.8	3.5	2.7	48.1	150.1	358.1
3	3.7	4.0	3.7	2.6	46.8	147.4	327.9
6	3.6	3.6	3.2	2.0	36.9	113.5	236.2
9	3.5	3.6	3.4	2.1	33.0	108.9	231.4
13	3.5	3.8	3.5	2.7	30.2	97.6	272.6
Female							
1	2.9	2.4	2.6	1.8	42.3	155.6	320.3
3	3.8	2.6	2.7	1.8	41.3	139.7	294.9
6	3.0	3.0	2.9	1.9	40.9	130.9	275.8
9	2.9	2.9	2.9	2.0	36.6	121.1	264.3
13	3.1	2.8	2.8	1.9	32.6	108.2	241.6

[a]Mean g of water consumed/animal/day.
[b]Mg of 1-butyl-3-methylimidazolium chloride consumed/kg body weight/day.
[c]Week 1 data unavailable for male mice.

Terminal mean body weight was significantly decreased in mice exposed to 3 mg/mL Bmim-Cl (Appendix E). Significant organ weight changes, considered secondary effects of decreased body weight and/or water consumption, were observed for both male and female mice exposed to this concentration. In males, absolute right kidney and liver weights were significantly decreased compared to the control group. Relative heart, right kidney, liver, lung, and right testis weights were significantly increased compared to the control group. Absolute lung weights were also significantly decreased in 3 mg/mL females compared to the control group. Additionally, relative heart and right kidney weights were significantly increased in 3 mg/mL females.

No changes in mice hematology parameters were attributable to Bmim-Cl exposure (Appendix E).

Estrous cycle length, number of cycles, and percentage of time spent in each respective stage of estrous were not affected by Bmim-Cl exposure. Assessment of the lengths of diestrus, proestrus, and estrus using the continuous-time Markov model did not reveal any Bmim-Cl-related differences in modeled stage lengths (Appendix E).

Limited histopathological changes were observed in male and female mice exposed to Bmim-Cl (Table 25). In the kidney, there was a positive trend in the incidences of chronic progressive nephropathy in both male and female mice. Additionally, a significant increase in the incidence of adrenal gland persistent X-zone was observed in females exposed to 3 mg/mL relative to the control group. The morphological features of the lesions discussed in this section are presented in the Histopathological Descriptions of Mice section.

Table 25. Incidences of Nonneoplastic Lesions of the Kidney and Adrenal Gland in Male and Female Mice in the Three-month Drinking Water Study of 1-Butyl-3-Methylimidazolium Chloride

	0 mg/mL	0.3 mg/mL	1 mg/mL	3 mg/mL
n[a]	10	10	10	10
Male				
Kidney				
Nephropathy, chronic progressive[b]	1* (1.0)[c]	3 (1.0)	0	5 (1.0)
Female				
Kidney				
Nephropathy, chronic progressive	0*	0	1 (1.0)	3 (1.0)
Adrenal Gland				
Adrenal cortex, X-zone, persistent	0**	1	0	5*

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control indicates a significant trend test.
*Statistically significant at $p \leq 0.05$ by the Cochran-Armitage (trend) or Fisher's exact (pairwise) test; **$p \leq 0.01$.
[a]Number of animals examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of observed lesion in affected animals; 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

1-Butyl-1-Methylpyrrolidinium Chloride (Bmpy-Cl)

Male and female mice were exposed to different concentrations of Bmpy-Cl. Male mice were exposed to 0, 1, 3, or 10 mg/mL, and females were exposed to 0, 1, 3, or 6 mg/mL in drinking water. All mice survived to the end of the 3-month exposure period (Table 26), and no exposure-related clinical observations were noted (Appendix E). Only male mice exposed to 10 mg/mL had a significantly decreased terminal mean body weight compared to the control group; this finding was interpreted as lower weight gain throughout the study (Table 26; Figure 7; Appendix E). At the end of the study, the mean body weight of male mice exposed to 10 mg/mL was 84% of the control group. The terminal mean body weight of female mice exposed to 6 mg/mL was 94% of the control group.

Table 26. Summary of Survival and Mean Body Weights of Male and Female Mice in the Three-month Drinking Water Study of 1-Butyl-1-Methylpyrrolidinium Chloride

Study Day[a]	0 mg/mL		1 mg/mL			3 mg/mL			10 mg/mL		
	Av. Wt. (g)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N
Male											
1	22.6	10	22.7	100.4	10	22.5	99.8	10	22.3	98.6	10
8	24.1	10	24.2	100.4	10	24.1	100.1	10	21.8	90.6	10
15	25.5	10	25.3	99.1	10	25.3	99.3	10	23.0	90.0	10
22	26.4	10	26.4	100.0	10	26.4	99.8	10	23.6	89.3	10
29	27.4	10	26.6	96.8	10	27.6	100.5	10	24.7	90.1	10
36	28.8	10	28.2	97.8	10	28.5	99.1	10	25.5	88.6	10
43	29.5	10	29.1	98.8	10	29.3	99.4	10	26.0	88.0	10
50	30.8	10	30.0	97.6	10	30.3	98.6	10	26.9	87.4	10
57	31.9	10	31.4	98.4	10	31.7	99.3	10	27.4	86.0	10
64	33.0	10	33.0	99.9	10	33.2	100.6	10	28.0	84.9	10
71	33.6	10	33.5	99.7	10	33.4	99.4	10	28.8	85.6	10
78	34.7	10	34.7	99.9	10	34.6	99.6	10	29.2	84.2	10
85	35.7	10	36.1	101.2	10	35.8	100.3	10	29.9	83.7	10
EOS	36.6	10	37.2	101.6	10	36.8	100.6	10	30.6	83.7	10
Study Day	**0 mg/mL**		**1 mg/mL**			**3 mg/mL**			**6 mg/mL**		
	Av. Wt. (g)	**N**	**Av. Wt. (g)**	**Wt. (% of Controls)**	**N**	**Av. Wt. (g)**	**Wt. (% of Controls)**	**N**	**Av. Wt. (g)**	**Wt. (% of Controls)**	**N**
Female											
1	17.2	10	17.3	100.9	10	17.1	99.5	10	17.3	100.9	10
8	18.0	10	18.5	102.8	10	17.8	98.9	10	18.0	100.1	10
15	19.5	10	19.5	100.1	10	18.7	95.8	10	18.7	96.2	10
22	20.6	10	20.5	99.6	10	19.8	96.1	10	19.7	95.7	10
29	21.8	10	21.6	99.2	10	20.9	96.1	10	20.2	92.6	10
36	22.8	10	22.9	100.6	10	21.9	95.9	10	22.2	97.5	10
43	23.2	10	23.6	101.9	10	22.5	97.2	10	22.5	97.2	10
50	24.2	10	24.0	99.3	10	21.6	89.1	10	23.4	96.8	10
57	25.5	10	24.8	97.6	10	23.2	91.2	10	24.1	94.7	10
64	26.0	10	25.8	99.3	10	25.4	97.7	10	24.7	95.0	10
71	26.8	10	26.6	99.4	10	25.8	96.2	10	25.5	95.3	10
78	27.6	10	26.7	96.7	10	27.0	97.8	10	26.1	94.7	10
85	27.7	10	26.8	97.0	10	26.7	96.7	10	25.8	93.2	10
EOS	27.9	10	27.0	97.0	10	26.5	95.2	10	26.1	93.7	10

EOS = end of study.
[a]Study day 1 is the day animals were placed on study.

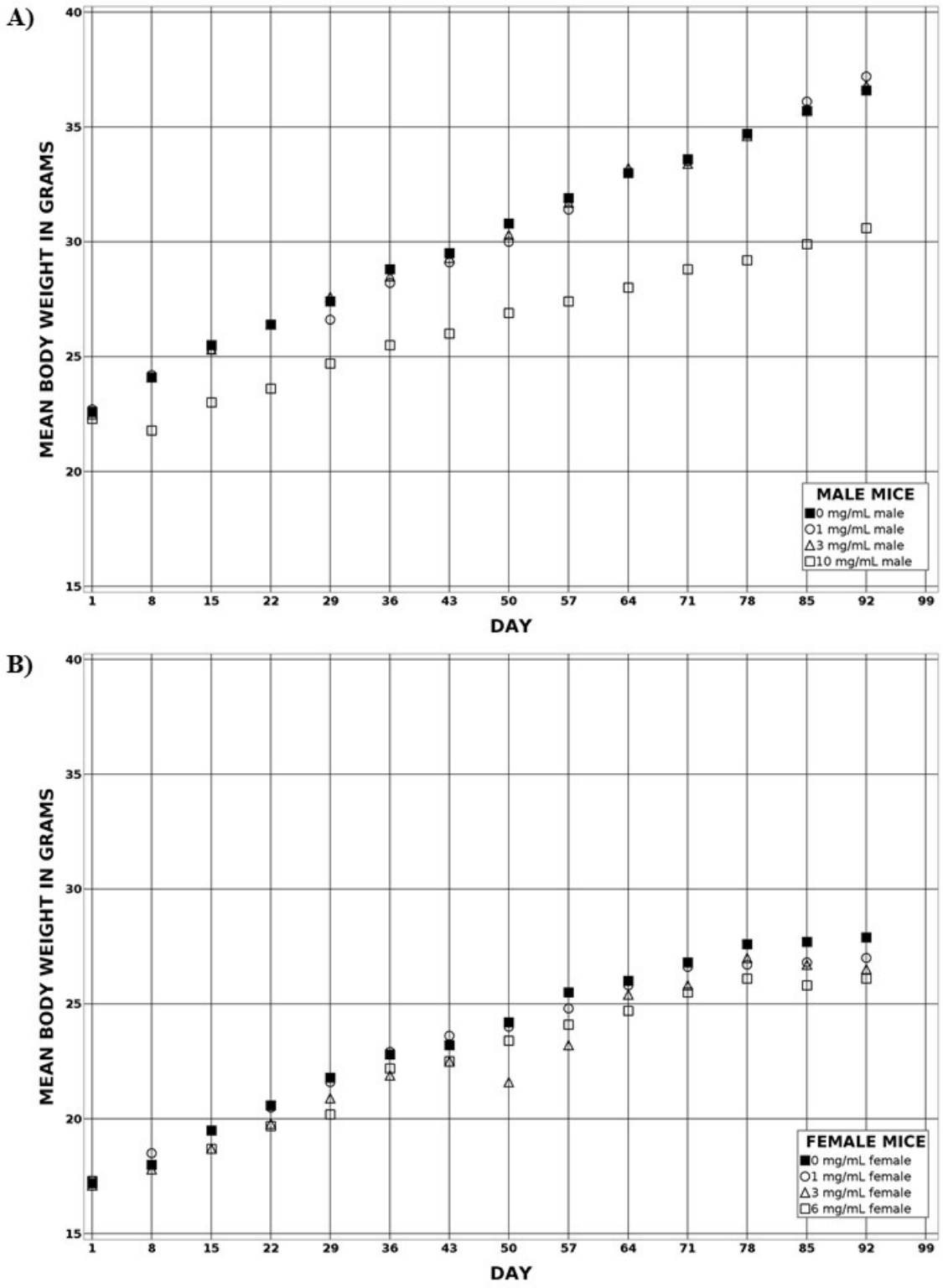


Figure 7. Growth Curves for Male and Female Mice in the Three-month Drinking Water Study of 1-Butyl-1-Methylpyrrolidinium Chloride

Growth curves are shown for (A) males and (B) females.

Water consumption by male and female mice exposed to Bmpy-Cl at the highest exposure concentrations of 10 mg/mL and 6 mg/mL, respectively, was lower than that of the control groups (Table 27). Mean water consumption ranged from 61% to 81% of the control group for males and from 81% to 97% of the control group for females (Appendix E). Drinking water concentrations of 1, 3, and 10 mg/mL resulted in estimated average doses of 123, 364, and 942 mg/kg/day for males, respectively. For females, drinking water concentrations of 1, 3, and 6 mg/mL resulted in estimated average doses of 130, 403, and 696 mg/kg/day, respectively.

Table 27. Summary of Water and 1-Butyl-1-Methylpyrrolidinium Chloride Consumption of Male and Female Mice in the Three-month Drinking Water Study

Week	Water (g/day)[a]				Dose (mg/kg/day)[b]		
	0 mg/mL	1 mg/mL	3 mg/mL	10 mg/mL	1 mg/mL	3 mg/mL	10 mg/mL
Male							
2[c]	3.3	3.6	3.5	2.0	148.8	435.1	916.2
3	3.3	3.6	3.4	2.2	142.3	402.5	957.8
6	3.4	3.9	3.6	2.4	138.5	378.5	941.2
9	3.2	3.5	3.5	2.6	111.5	331.4	947.9
13	3.6	3.8	3.7	2.6	105.2	310.3	871.0
	0 mg/mL	**1 mg/mL**	**3 mg/mL**	**6 mg/mL**	**1 mg/mL**	**3 mg/mL**	**6 mg/mL**
Female							
1	2.9	2.7	3.9	2.4	156.0	685.4	831.9
3	2.5	2.6	2.2	2.1	133.3	353.3	672.4
6	2.9	2.8	2.5	2.4	122.1	342.9	647.8
9	3.1	3.1	2.9	2.8	124.8	374.5	696.8
13	3.2	3.4	3.2	3.0	126.8	359.1	698.5

[a]Mean g of water consumed/animal/day.
[b]Mg of 1-butyl-1-methylpyrrolidinium chloride consumed/kg body weight/day.
[c]Week 1 data unavailable for male mice.

Absolute organ weights were not significantly altered in males or females exposed to Bmpy-Cl (Table 28; Appendix E); however, there were several significantly increased relative organ weights, primarily in animals exposed at the highest concentrations. Males exposed to 10 mg/mL had significantly increased relative right kidney, lung, right testis, and thymus weights compared to control animals. Because male mice exposed to 10 mg/mL also had significantly decreased terminal mean body weight, these changes were considered secondary effects of decreased body weight and/or water consumption. Males exposed to 10 mg/mL Bmpy-Cl also had significantly increased relative liver weights. This significant increase in relative liver weights was additionally observed in male mice exposed to 1 or 3 mg/mL Bmpy-Cl, despite their terminal mean body weights remaining similar to those of the control group. In females, the only significant difference observed was in relative right kidney weights, which increased in the 6 mg/mL group compared to the control group (Table 28).

Table 28. Summary of Select Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male and Female Mice in the Three-month Drinking Water Study of 1-Butyl-1-Methylpyrrolidinium Chloride[a,b]

	0 mg/mL	1 mg/mL	3 mg/mL	10 mg/mL
n	10	10	10	10
Male				
Terminal Body Wt. (g)	36.6 ± 0.8**	37.2 ± 1.4	36.8 ± 1.1	30.6 ± 0.7**
Liver				
Absolute (g)	1.59 ± 0.04	1.71 ± 0.07	1.69 ± 0.06	1.46 ± 0.03
Relative (mg/g)[c]	43.51 ± 0.51**	46.01 ± 0.59**	45.90 ± 0.70**	47.62 ± 0.49**
	0 mg/mL	**1 mg/mL**	**3 mg/mL**	**6 mg/mL**
Female				
Terminal Body Wt. (g)	27.9 ± 0.9	27.0 ± 0.9	26.5 ± 0.7	26.1 ± 0.9
Kidney				
Absolute (g)	0.18 ± 0.00	0.17 ± 0.01	0.17 ± 0.01	0.18 ± 0.01
Relative (mg/g)	6.31 ± 0.13*	6.41 ± 0.15	6.55 ± 0.09	6.80 ± 0.15*

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Data are presented as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.
[c]Relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.

At the end of the 3-month exposure period, there were positive trends and significant but minimal (<3%) increases in the male mice hematocrit and erythrocyte count in the 3 mg/mL and/or 10 mg/mL groups compared to the control group (Appendix E). While these changes may represent mild hemoconcentration due to lower water consumption, they were small in magnitude and might have resulted from biological variability.

Estrous cycle length and number of cycles were not affected by Bmpy-Cl exposure (Appendix E). The 1 mg/mL group displayed a greater percentage of time spent in estrus, and the continuous-time Markov model comparison of estrus stage length attained significance and was increased by 0.6 days. Inspection of the daily cyclicity observations revealed five control mice that displayed apparently longer cycles, resulting in fewer observations of estrus and metestrus. Although this response is common in control mice, five is a higher frequency than typically observed in a group size of 10; a frequency of two or three is more common, as observed in the 3 and 6 mg/mL groups. In contrast, all mice in the 1 mg/mL group displayed prototypical cycling. Given this finding, the apparent response in the 1 mg/mL group is considered spurious and not the result of Bmpy-Cl exposure.

Limited histopathological changes were observed in male and female mice exposed to Bmpy-Cl (Table 29). In the kidney, a significant increase in the incidence of renal tubule cytoplasmic alteration was found in males exposed to 10 mg/mL compared to the control group. In the adrenal gland of female mice, significant increases in the incidences of adrenal cortex persistent X-zone were found in the 3 and 6 mg/mL groups compared to the control group. The morphological features of the lesions discussed in this section are presented in the Histopathological Descriptions of Mice section.

Table 29. Incidences of Nonneoplastic Lesions of the Kidney and Adrenal Gland in Male and Female Mice in the Three-month Drinking Water Study of 1-Butyl-1-Methylpyrrolidinium Chloride

	0 mg/mL	1 mg/mL	3 mg/mL	10 mg/mL
n[a]	10	10	10	10
Male				
Kidney				
Renal tubule, cytoplasmic alteration[b]	0**	0	0	5* (1.8)[c]
	0 mg/mL	**1 mg/mL**	**3 mg/mL**	**6 mg/mL**
Female				
Adrenal Gland				
Adrenal cortex, X-zone, persistent	0*	1	4*	4*

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control indicates a significant trend test.
*Statistically significant at $p \leq 0.05$ by the Cochran-Armitage (trend) or Fisher's exact (pairwise) test; **$p \leq 0.01$.
[a]Number of animals examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of observed lesion in affected animals; 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

N-Butylpyridinium Chloride (NBuPy-Cl)

Male and female mice were exposed to NBuPy-Cl at concentrations of 0, 1, 3, or 6 mg/mL in drinking water. All mice survived to the end of the 3-month exposure period (Table 30), and no exposure-related clinical observations were noted (Appendix E). Decreases in terminal mean body weights, interpreted as lower weight gain over the course of the study, were noted for male and female mice (Table 30; Figure 8; Appendix E). Terminal mean body weights were 98%, 94%, and 72% of control males for the 1, 3, and 6 mg/mL groups, respectively. Females in the same exposed groups had terminal mean body weights that were 95%, 91%, and 83%, respectively, of the control group. These changes were significant in the 3 mg/mL NBuPy-Cl-exposed females and the 6 mg/mL NBuPy-Cl-exposed males and females.

Table 30. Summary of Survival and Mean Body Weights of Male and Female Mice in the Three-month Drinking Water Study of N-Butylpyridinium Chloride

Study Day[a]	0 mg/mL		1 mg/mL			3 mg/mL			6 mg/mL		
	Av. Wt. (g)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N
Male											
1	23.3	10	23.7	101.7	10	23.6	101.1	10	23.5	100.9	10
8	25.1	10	25.0	99.9	10	24.9	99.3	10	21.7	86.4	10
15	25.9	10	26.1	100.9	10	26.2	101.0	10	22.9	88.3	10
22	27.0	10	27.1	100.4	10	26.5	98.3	10	23.4	86.6	10
29	28.3	10	28.5	100.6	10	27.8	98.2	10	24.3	85.7	10
36	29.2	10	29.5	101.3	10	28.6	98.2	10	23.2	79.6	10
43	30.7	10	30.8	100.4	10	29.7	96.9	10	23.3	76.0	10
50	31.6	10	31.8	100.9	10	31.0	98.2	10	25.2	79.7	10
57	33.0	10	32.9	99.5	10	32.0	97.0	10	25.4	77.0	10
64	33.8	10	33.8	99.9	10	32.6	96.5	10	25.7	76.1	10
71	35.2	10	34.9	99.1	10	33.8	95.8	10	26.6	75.5	10
78	35.6	10	35.3	99.2	10	34.0	95.6	10	26.9	75.4	10
85	36.7	10	36.1	98.4	10	34.7	94.6	10	26.6	72.6	10
EOS	38.0	10	37.1	97.6	10	35.6	93.7	10	27.3	71.7	10
Female											
1	17.7	10	17.5	98.8	10	17.5	98.6	10	17.2	97.0	10
8	18.7	10	18.4	98.2	10	18.3	97.6	10	16.5	88.0	10
15	20.1	10	19.7	98.0	10	19.4	96.6	10	17.6	87.4	10
22	20.7	10	20.4	98.6	10	20.2	97.5	10	18.8	90.8	10
29	22.0	10	21.8	99.4	10	21.2	96.5	10	19.5	88.8	10
36	23.0	10	22.4	97.3	10	22.1	96.3	10	20.2	88.1	10
43	24.4	10	23.4	96.2	10	22.9	94.1	10	20.9	85.8	10
50	24.5	10	24.0	98.0	10	23.2	94.4	10	21.6	88.1	10
57	25.4	10	24.7	97.0	10	24.2	95.0	10	22.2	87.2	10
64	26.1	10	25.0	95.9	10	24.1	92.5	10	22.3	85.5	10
71	27.3	10	26.2	96.0	10	25.1	91.9	10	22.9	83.8	10
78	27.8	10	25.9	93.1	10	24.7	88.6	10	22.4	80.4	10
85	27.7	10	26.4	95.3	10	24.9	89.7	10	22.5	81.2	10
EOS	27.5	10	26.2	95.4	10	25.1	91.4	10	22.8	82.9	10

EOS = end of study.
[a]Study day 1 is the day animals were placed on study.

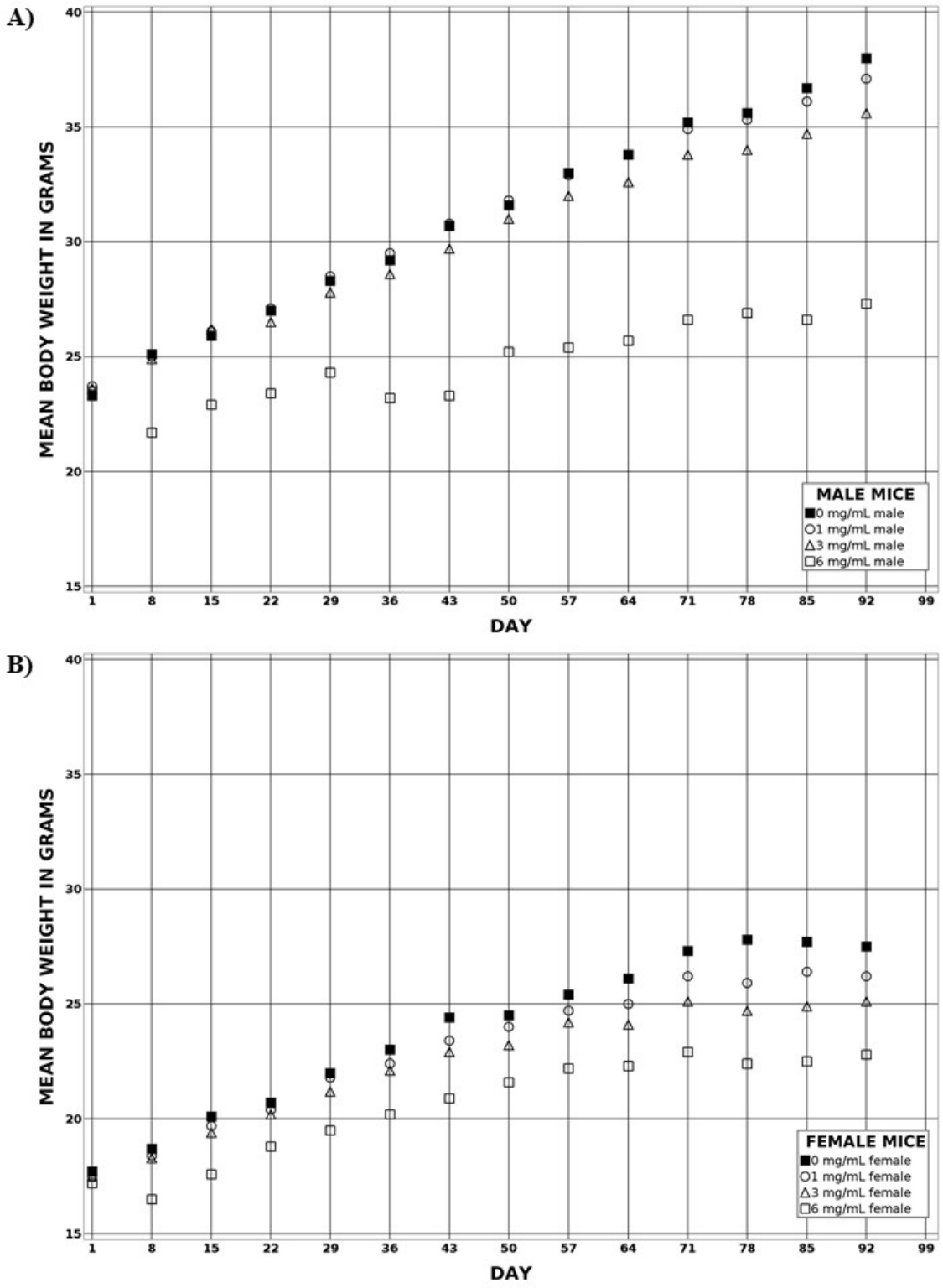


Figure 8. Growth Curves for Male and Female Mice in the Three-month Drinking Water Study of N-Butylpyridinium Chloride

Growth curves are shown for (A) males and (B) females.

In general, water consumption decreased in an exposure concentration-dependent manner for both male and female mice exposed to NBuPy-Cl (Table 31; Appendix E). In the highest exposed groups (6 mg/mL), water consumption by male mice ranged from 49% to 65% of control males, and water consumption by female mice ranged from 46% to 63% of control females. Drinking water concentrations of 1, 3, and 6 mg/mL resulted in estimated average doses of 118, 310, and 483 mg/kg/day, respectively, for males and 125, 331, and 518 mg/kg/day, respectively, for females.

Table 31. Summary of Water and N-Butylpyridinium Chloride Consumption of Male and Female Mice in the Three-month Drinking Water Study

Week	Water (g/day)[a]				Dose (mg/kg/day)[b]		
	0 mg/mL	1 mg/mL	3 mg/mL	6 mg/mL	1 mg/mL	3 mg/mL	6 mg/mL
Male							
2[c]	3.7	3.7	3.4	1.8	147.8	410.0	498.8
3	3.4	3.5	2.9	2.0	133.9	332.6	524.7
6	3.2	3.4	3.0	2.0	115.1	314.2	516.8
9	3.6	3.7	2.9	1.9	112.6	271.5	448.5
13	3.5	3.4	3.0	1.9	94.1	259.2	427.9
Female							
1	2.8	2.7	2.4	1.3	154.6	412.6	454.5
3	2.6	2.6	2.0	1.5	132.0	309.1	512.5
6	3.1	2.7	2.4	1.8	120.8	325.2	533.6
9	3.3	3.0	2.7	1.9	121.6	335.3	514.0
13	3.4	3.3	2.7	2.0	124.9	325.8	533.1

[a]Mean g of water consumed/animal/day.
[b]Mg of n-butylpyridinium chloride consumed/kg body weight/day.
[c]Week 1 data unavailable for male mice.

Various significant organ weight changes were observed for both male and female mice exposed to NBuPy-Cl (Table 32; Appendix E). Male mice exposed to 6 mg/mL had a significantly decreased terminal mean body weight compared to the control group, accompanied by significantly decreased absolute right kidney, liver, lung, and thymus weights and significantly increased relative right kidney, liver, and right testis weights. Female mice exposed to 6 mg/mL had a significantly decreased terminal mean body weight compared to the control group, accompanied by significantly decreased absolute lung weights and significantly increased relative heart, right kidney, and liver weights. Female mice exposed to 3 mg/mL also had a significantly decreased terminal mean body weight and significantly increased relative heart and right kidney weights. All these organ weight changes were considered secondary effects of decreased body weight and/or water consumption (Appendix E). In contrast, male mice exposed to 3 mg/mL did not have a significantly decreased terminal mean body weight but did have significantly increased relative right kidney and liver weights compared to the control group (Table 32).

Table 32. Summary of Select Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male Mice in the Three-month Drinking Water Study of N-Butylpyridinium Chloride[a,b]

	0 mg/mL	1 mg/mL	3 mg/mL	6 mg/mL
n	10	10	10	10
Terminal Body Wt. (g)	38.0 ± 1.3**	37.1 ± 1.1	35.6 ± 1.5	27.3 ± 0.6**
Right Kidney				
Absolute (g)	0.31 ± 0.01*	0.32 ± 0.01	0.32 ± 0.01	0.27 ± 0.01*
Relative (mg/g)[c]	8.16 ± 0.21**	8.57 ± 0.16	8.91 ± 0.20**	9.89 ± 0.15**
Liver				
Absolute (g)	1.73 ± 0.06**	1.66 ± 0.05	1.70 ± 0.08	1.40 ± 0.03**
Relative (mg/g)	45.65 ± 0.67**	44.88 ± 0.44	47.74 ± 0.64*	51.22 ± 0.73**

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Data are presented as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.
[c]Relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.

At the end of the 3-month exposure period, neutrophil counts in male mice exposed to 3 or 6 mg/mL were significantly decreased relative to the control group (Appendix E). Given that these changes were small in magnitude and not observed in the rats or female mice, the toxicological significance of the decreased neutrophil counts in the male mice is unknown. No hematological effects were observed in female mice.

Estrous cycle length, number of cycles, and percentage of time spent in each respective stage of estrous were not affected by NBuPy-Cl exposure. Assessment of the lengths of diestrus, proestrus, and estrus using the continuous-time Markov model did not reveal any NBuPy-Cl-related differences in modeled stage lengths (Appendix E).

Limited histopathological changes were observed in male and female mice exposed to NBuPy-Cl (Table 33). Significant increases in the incidences of chronic progressive nephropathy and renal tubule cytoplasmic alteration were observed in the kidneys of male mice exposed to 6 mg/mL compared to the control group. There was also a positive trend in the incidence of chronic progressive nephropathy in female mice. In the adrenal gland of female mice, there was a significant increase in the incidence of adrenal cortex persistent X-zone at 6 mg/mL compared to the control group. The morphological features of the lesions discussed in this section are presented in the Histopathological Descriptions of Mice section.

Table 33. Incidences of Nonneoplastic Lesions of the Kidney and Adrenal Gland in Male and Female Mice in the Three-month Drinking Water Study of N-Butylpyridinium Chloride

	0 mg/mL	1 mg/mL	3 mg/mL	6 mg/mL
n[a]	10	10	10	10
Male				
Kidney				
Nephropathy, chronic progressive[b]	2** (1.0)[c]	0	4 (1.0)	8* (1.0)
Renal tubule, cytoplasmic alteration	0**	0	0	10** (2.0)
Female				
Kidney				
Nephropathy, chronic progressive	2* (1.0)	0	2 (1.0)	5 (1.0)
Adrenal Gland				
Adrenal cortex, X-zone, persistent	0**	2	3	9**

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control indicates a significant trend test.
*Statistically significant at $p \leq 0.05$ by the Cochran-Armitage (trend) or Fisher's exact (pairwise) test; **$p \leq 0.01$.
[a]Number of animals examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of observed lesion in affected animals; 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

Histopathological Descriptions of Mice

In general, the histopathological changes, when present in the kidney and adrenal gland, were morphologically similar across studies.

Kidney: Chronic progressive nephropathy (CPN) was generally characterized by minimal focal to multifocal cytoplasmic basophilia and nuclear crowding of the proximal and distal tubular epithelium (Figure 9). The tubular basophilia is evidence of cellular regeneration. CPN has been more extensively studied in rats than in mice; its incidence and/or severity is influenced by a variety of factors, such as hormones, caloric intake, protein content of diet, and some xenobiotics.[104]

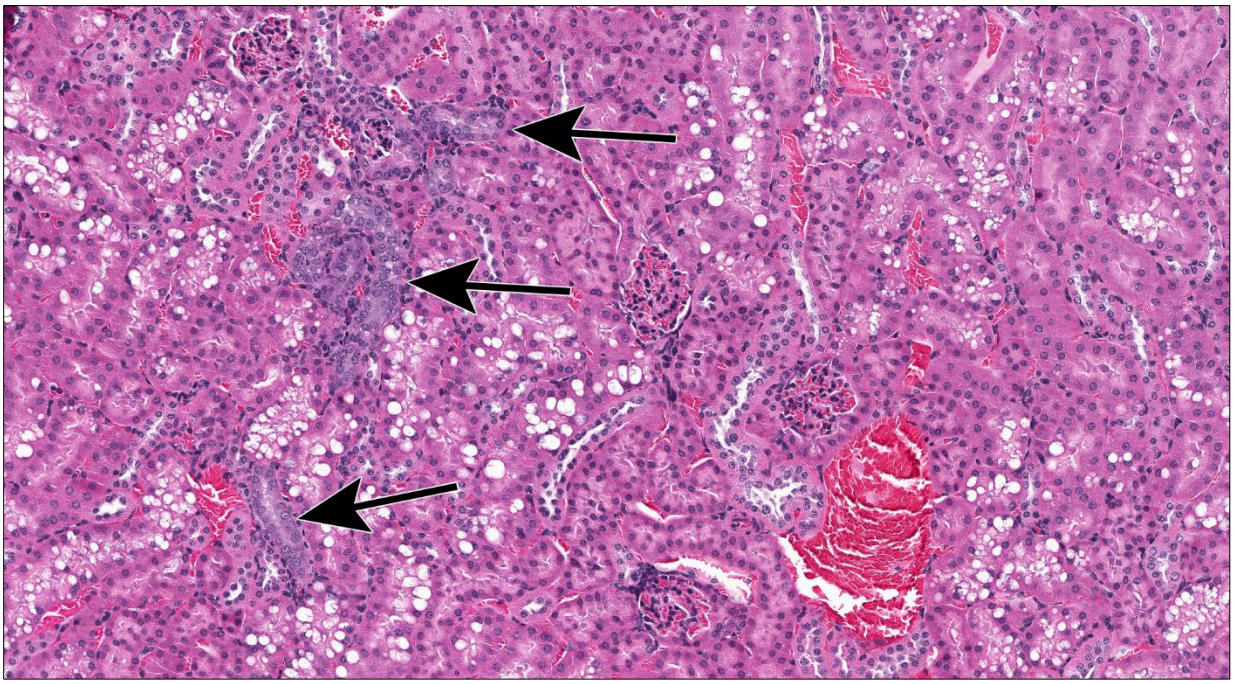
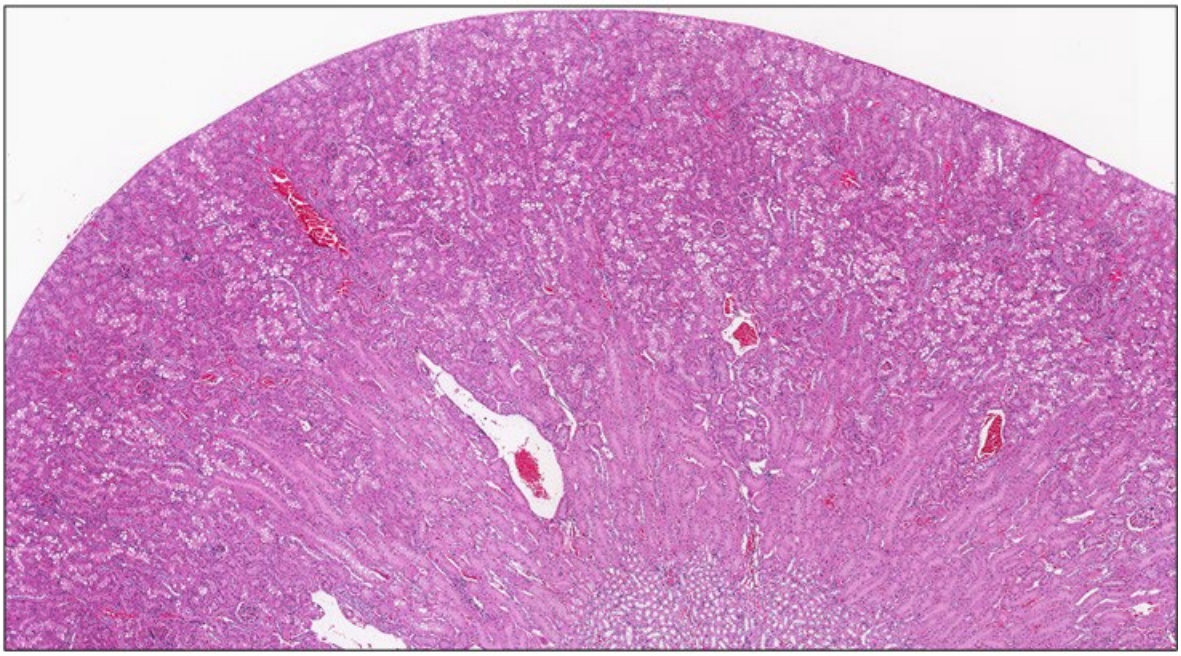


Figure 9. Representative Image of Chronic Progressive Nephropathy in the Kidney of a Male Mouse in the Three-month Drinking Water Study of 1-Butyl-3-Methylimidazolium Chloride (H&E)

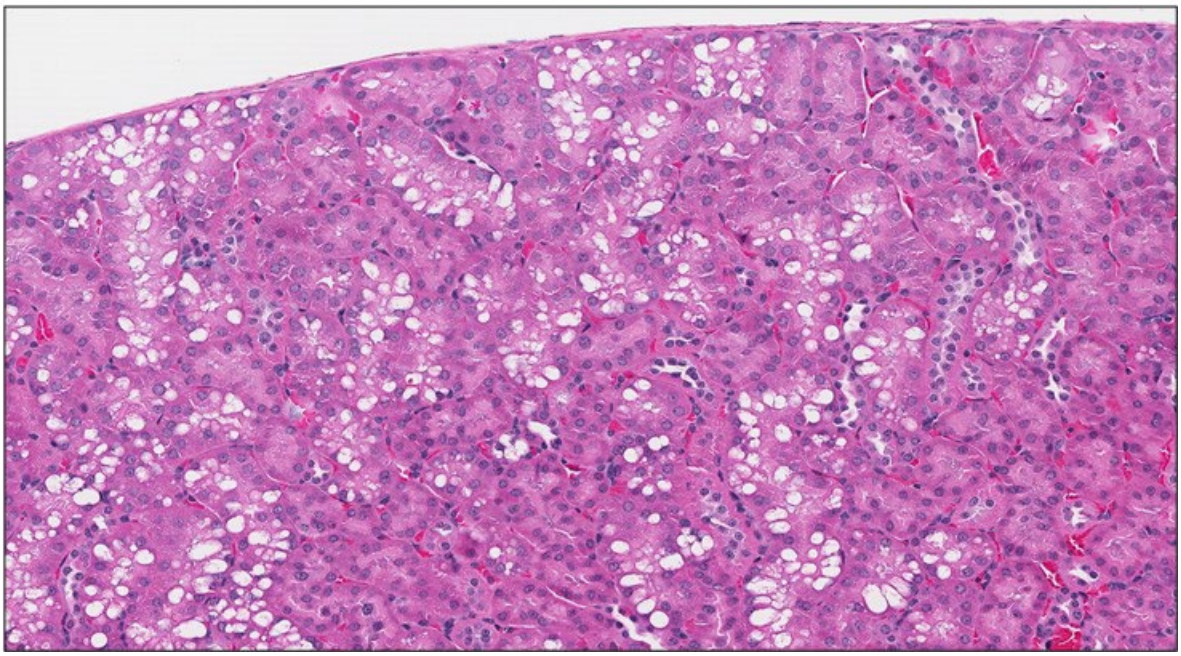
Chronic progressive nephropathy was characterized by focal to multifocal cytoplasmic basophilia and nuclear crowding of the proximal and distal tubular epithelium (arrows) as illustrated in this image from the kidney of a male mouse exposed to 3 mg/mL 1-butyl-3-methylimidazolium chloride (14.8x). H&E = hematoxylin and eosin stain.

Renal tubule cytoplasmic alteration was generally characterized by a reduction in the renal tubule cytoplasmic vacuolization normally seen in the outer cortical tubules of male mice (Figure 10). The presence of cytoplasmic vacuoles is a sexually dimorphic lesion, occurring only in males, and is thought to represent autophagic vacuoles associated with the normal degeneration of renal tubular epithelial cells.[105]

A)



B)



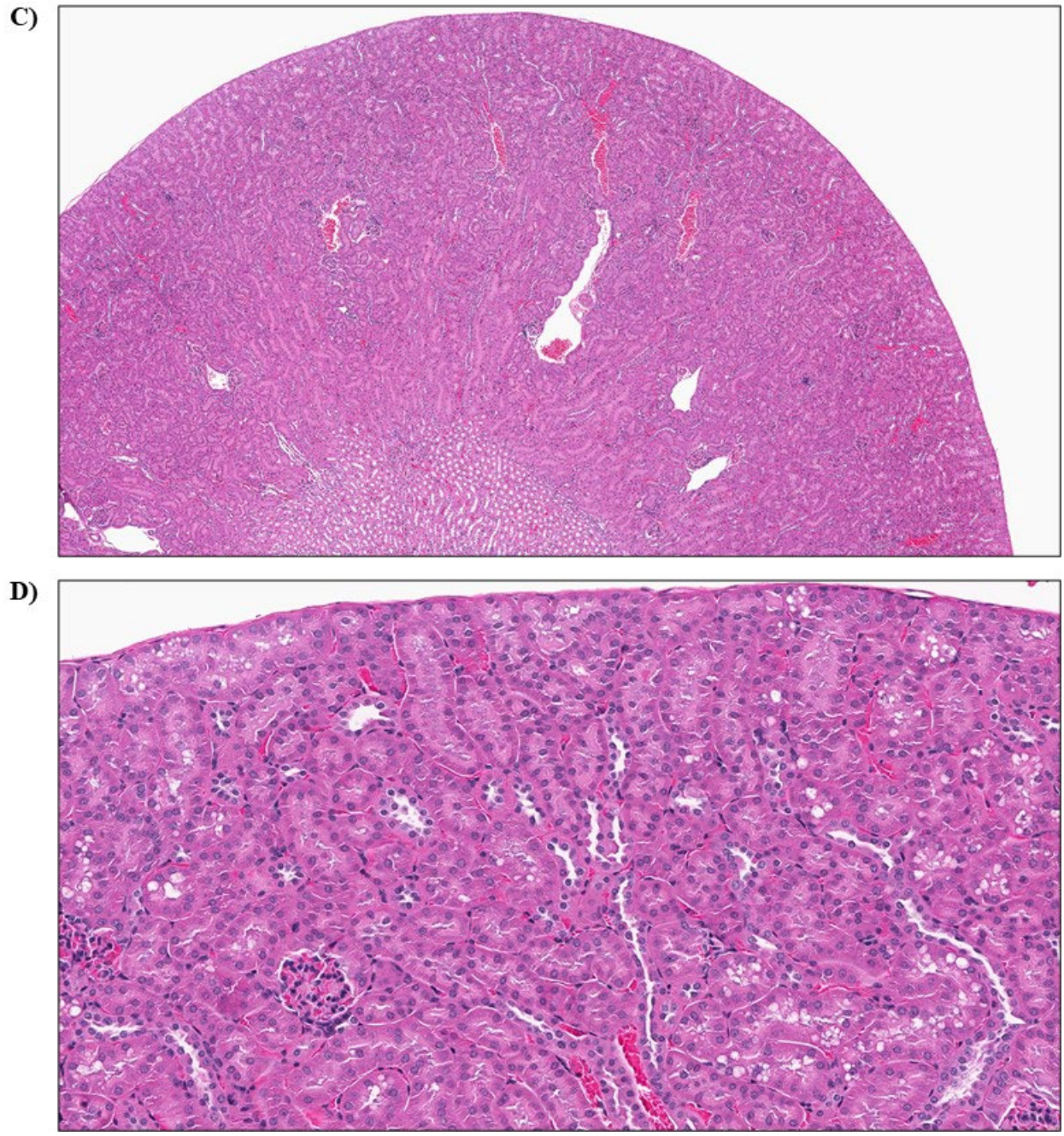
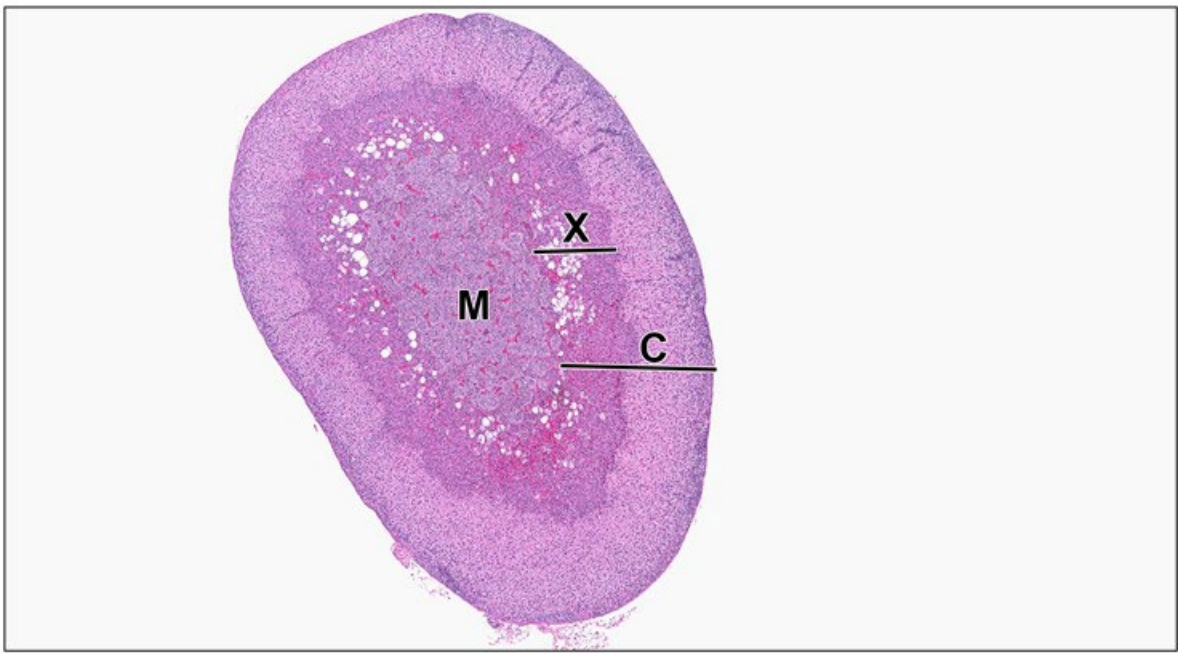


Figure 10. Representative Images of Renal Tubule Cytoplasmic Alteration in the Kidney of Male Mice in the Three-month Drinking Water Study of 1-Butyl-1-Methylpyrrolidinium Chloride (H&E)

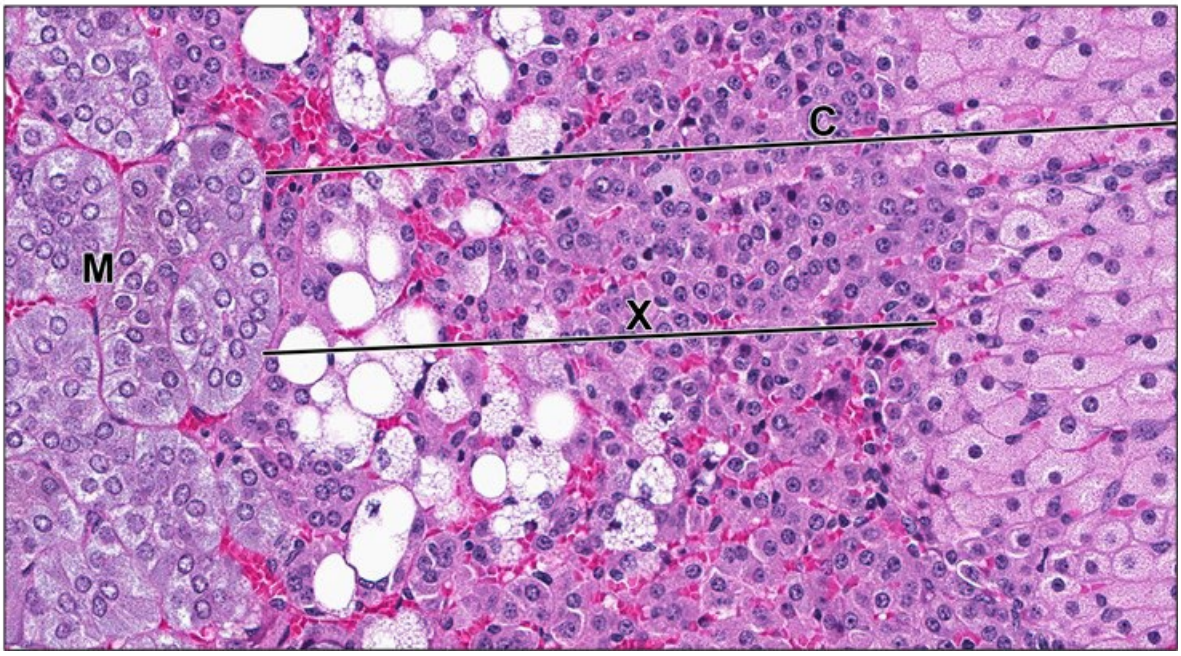
Cytoplasmic alteration of the renal tubules is a lesion specific to the kidneys of male mice, characterized by a reduction in the renal tubule cytoplasmic vacuolization that is normally seen in the outer cortical tubules. Compare normal vacuoles in cortical renal tubules in a control male mouse (panel A at 4x and panel B at 20x) to the markedly fewer vacuoles from a male mouse exposed to 10 mg/mL 1-butyl-1-methylpyrrolidinium chloride (panel C at 4x and panel D at 20x). H&E = hematoxylin and eosin stain.

Adrenal gland: Adrenal cortex persistent X-zone was characterized by reduced numbers or the absence of X-zone cells undergoing vacuolar degeneration (Figure 11). The X-zone is a transient region in young mice at the cortical-medullary boundary of the adrenal gland, appearing a few days after birth in both sexes. The cells in the X-zone are darker and smaller, and the cytoplasm is more basophilic than those in the zona fasciculata. The function of the X-zone is not known, but during development, these fetal cortical cells are gradually replaced by newly formed adult cortical cells that develop into outer definitive zones. In the male mouse, this zone disappears rapidly with the approach of puberty (approximately 5 weeks of age) due to androgen signaling via the androgen receptor.[106] In female virgins, the X-zone continues to grow in size until about 9 weeks of age and then regresses gradually.

A)



B)



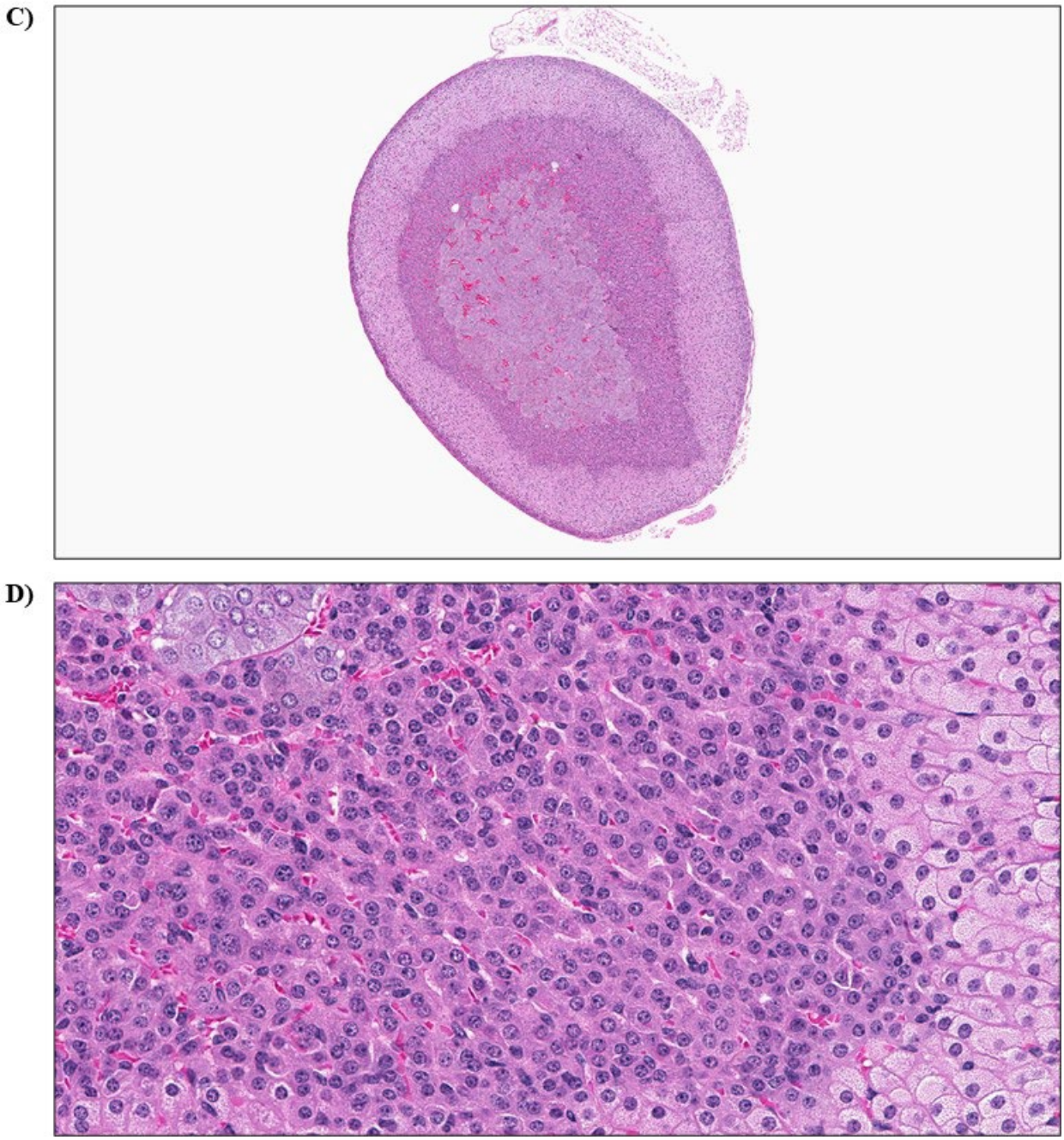


Figure 11. Representative Images of Persistent X-zone in the Adrenal Gland of Female Mice in the Three-month Drinking Water Study of N-Butylpyridinium Chloride (H&E)

Persistent X-zone was characterized by reduced numbers or the absence of cells at the cortico-medullary boundary of the adrenal gland undergoing vacuolar degeneration. Compare the normal degenerating vacuolated cells at the cortico-medullary junction in a control female mouse (panel A at 4x and panel B at 36.4x) to the markedly fewer vacuolated cells from a female mouse exposed to 6 mg/mL n-butylpyridinium chloride (panel C at 4x and panel D at 36.4x). H&E = hematoxylin and eosin stain, M = medulla, X = X-zone, C = cortex.

Genetic Toxicology

All four ILs were tested for induction of gene mutations in three strains of bacteria (*Salmonella typhimurium* strains TA98 and TA100, and *Escherichia coli* strain WP2 *uvr*A [pKM101]), as well as induction of structural or numerical chromosomal damage in the rodent peripheral blood erythrocyte micronucleus (MN) assay using samples collected from the 3-month study in rats and mice. Data from all NTP genetic toxicity tests with Emim-Cl, Bmim-Cl, Bmpy-Cl, and NBuPy-Cl are presented in Appendix D.

None of the four ILs induced mutations in the three strains of bacteria in studies conducted with and without induced rat liver S9 mix at doses up to 10,000 μg/plate.

Results of the MN assay were somewhat variable across the four ILs. Emim-Cl did not increase the frequency of micronucleated erythrocytes in male or female rats or in male mice. In female mice, the MN assay response was also judged to be negative, although a small, significant increase was observed in micronucleated mature erythrocytes; this response was not considered of sufficient magnitude to be biologically relevant and was well within the laboratory historical control 95% confidence interval for female B6C3F1 mice.

No increases in micronucleated erythrocytes were seen in blood samples from Bmim-Cl-exposed rats and mice.

For Bmpy-Cl-exposed animals, a clearly positive response was seen in the immature erythrocyte population in male rat peripheral blood samples (the appropriate cell population used to assess MN in rats). Results were negative for female rats as well as male and female mice.

The response in NBuPy-Cl-exposed male rats was judged to be equivocal. Although there was a significant increase in micronucleated mature erythrocytes, the results for immature erythrocytes were negative. Because this response pattern is in direct contrast to what would be expected in rats for a truly positive compound, the increases observed were within the laboratory 95% confidence interval for male rats, and there is no biological explanation for the observation (such as altered spleen function) in these rats, the response was judged to be equivocal. No differences in MN frequencies were observed in female rats or male and female mice for either cell population.

Discussion

Since their nomination to the National Toxicology Program (NTP), the use of ionic liquids (ILs) in commercial processes and consumer products has expanded. ILs are finding their way into many households through electronics, batteries, perfumes, haircare products, pharmaceuticals, and antimicrobial cleaning agents as the movement for more "green" and "natural" consumer products gains momentum.[3; 39; 40; 107; 108] Occupational exposure is also increasing as ILs are used to replace volatile organic compounds in many new and existing industrial applications.[2; 5] Despite the potential for increased human exposure, limited mammalian toxicity data are available for this class of chemicals. To address this data gap, NTP evaluated the in vivo toxicity of four ILs—1-ethyl-3-methylimidazolium chloride (Emim-Cl), 1-butyl-3-methylimidazolium chloride (Bmim-Cl), 1-butyl-1-methylpyrrolidinium chloride (Bmpy-Cl), and n-butylpyridinium chloride (NBuPy-Cl) —in 2-week and 3-month drinking water studies in rats and mice. These studies facilitate comparison of toxicity among the three most common cations used in ILs (imidazolium, pyrrolidinium, and pyridinium) and among differing alkyl side chain lengths.

In the 2-week studies, rats and mice were exposed to various concentrations of Emim-Cl, Bmim-Cl, Bmpy-Cl, or NBuPy-Cl in drinking water. This oral exposure route was used to simulate exposure to ILs through contamination of public water supplies.[109; 110] All animals survived until the end of the 2-week exposure period. Notably, for each of the four ILs, lower mean body weights of exposed animals were directly related to lower water consumption, which in turn was related to the concentration of the IL tested. Furthermore, clinical findings, including thinness and ruffled fur, were considered related to lower body weight rather than direct toxicity. At an equivalent exposure concentration (3 mg/mL), Bmim-Cl-exposed male and female rats exhibited the lowest water consumption and terminal mean body weights compared to the control groups, followed by rats exposed to NBuPy-Cl, Bmpy-Cl, and then Emim-Cl. Water consumption by, and terminal mean body weights of, male and female mice at 3 mg/mL followed a similar pattern. Thus, lower terminal mean body weights of IL-exposed rats and mice were possibly related to poor palatability of the ILs, resulting in lower water consumption.

While estimated chemical intake increased as IL concentrations in drinking water increased, chemical intake did not increase in direct proportion to exposure concentration because of the lower water consumption at higher IL concentrations. Although feed consumption was not measured, previous research suggests that poor palatability of a compound in drinking water can negatively affect both water and feed consumption, thereby reducing body weight gain.[111] Under these circumstances, it is difficult to determine whether lower terminal mean body weights resulted primarily from IL-induced toxicity or were secondary to dehydration or poor appetite. Other endpoints observed across all ILs at the highest exposure concentrations, such as organ weight changes, similarly appear to reflect lower mean body weights and potentially decreased nutritional and caloric intake. Body weight and water consumption results from the 2-week range-finding study enabled selection of exposure concentrations for the 3-month drinking water studies in rats and mice.

As a guideline toxicity study, the 3-month exposure studies assessed specific endpoints of toxicity, namely: mortality, body weights, water consumption, organ weights, clinical chemistry, hematology, vaginal cytology, histopathology, and genotoxicity. There were no significant toxicological effects of IL exposure on mortality, clinical chemistry, hematology, vaginal

cytology, or genotoxicity; details on these data are presented in the results, Appendix D, and Appendix E. Endpoints significantly affected by IL exposure (body weights, water consumption, organ weights, and histopathology) are further discussed below.

Exposure of rats and mice to Emim-Cl, Bmim-Cl, Bmpy-Cl, or NBuPy-Cl for 3 months resulted in an overarching pattern of lower mean body weights and water consumption similar to the 2-week studies. In general, water consumption by both rats and mice exposed to ILs for 3 months was lower than water consumption by control animals. Often, this observation was exposure concentration-dependent, with rats consuming on average 54%–80% of the control groups' water consumption at the highest IL exposure concentrations (10 mg/mL Emim-Cl, 1 mg/mL Bmim-Cl, 3 mg/mL Bmpy-Cl in males, 6 mg/mL Bmpy-Cl in females, 3 mg/mL NBuPy-Cl) and mice consuming 56%–87% of the control groups' water consumption at the highest IL exposure concentrations (30 mg/mL Emim-Cl, 3 mg/mL Bmim-Cl, 10 mg/mL Bmpy-Cl in males, 6 mg/mL Bmpy-Cl in females, 6 mg/mL NBuPy-Cl). Despite both species consuming less water with increased IL exposure concentrations, body weight changes were more variable and did not always decrease as IL exposure concentration increased. These body weight changes were potentially related to the ingested dose. Estimated compound consumption was increased at higher exposure concentrations but not always in direct proportion to the exposure concentration because of decreased water consumption. Estimated compound consumption values show that mice received a higher dose of ILs than rats; for example, rats exposed to 3 mg/mL Emim-Cl, Bmpy-Cl, or NBuPy-Cl consumed an estimated average of 171–258 mg/kg/day, whereas mice exposed to 3 mg/mL of these ILs consumed an estimated average of 310–403 mg/kg/day. For each of these exposed groups, males consumed an estimated 6%–11% less compound than females.

Changes in organ weights were observed at the highest exposure concentration of most ILs for rats and mice. These changes followed the pattern of lower body weights with higher IL exposure, which coincided with lower water consumption. In a few instances, in which absolute organ weight decreases were observed, these findings were not accompanied by histological changes in rats or mice; thus, their toxicological significance is not clear.

Histopathological results showed species- and sex-specific responses to the highest exposure concentrations of ILs. Neither male nor female rats exposed to Emim-Cl, Bmim-Cl, Bmpy-Cl, or NBuPy-Cl displayed any significant histopathological findings. Mice, however, had lesions in the kidney and adrenal gland (discussed in detail below). It is unclear whether this species-specific difference resulted from higher internal doses in mice compared to rats at the same exposure concentration, or higher sensitivity of mice tissues to ILs. Interestingly, the lesions all suggest steroid, potentially androgen, perturbation following IL exposure.

Exposure to high concentrations of ILs resulted in kidney lesions in both male and female mice. At the highest tested exposure concentrations of Emim-Cl (30 mg/mL), Bmpy-Cl (10 mg/mL), and NBuPy-Cl (6 mg/mL), male mice displayed cytoplasmic alteration in the kidney. This diagnosis is defined by a reduction in the renal tubular cytoplasmic vacuoles that are normally present in certain strains of young male mice but are uncommon in female mice or in male or female rats. The vacuoles are thought to be autophagic and involved in the sequestration and degradation of organelles associated with the normal degeneration of renal tubular epithelial cells.[105; 112] In male mice, these vacuoles decrease and eventually disappear as the animal ages. The accumulation of lysosomes in the outer renal tubules of male mice, seen microscopically as

vacuolation, has been shown to depend on endogenous testosterone; female mice administered testosterone accumulated lysosomes, resulting in kidneys phenotypically similar to male mice.[112] In the present study, there were markedly fewer vacuoles (cytoplasmic alteration) in male mice in the highest exposure concentration groups for 3 of the 4 ILs (Emim-Cl, Bmpy-Cl, and NBuPy-Cl), such that the kidneys of the male mice were phenotypically similar to the kidneys of female mice. This phenotypic observation was also true for one male mouse at the highest exposure of Bmim-Cl. This lack of testosterone-responsive vacuolation in the renal cortical tubules in male mice after exposure to all four ILs suggests a potential perturbation of androgens.

Another kidney lesion observed in IL-exposed mice was chronic progressive nephropathy (CPN). The incidences of CPN were significantly increased at the highest exposure concentration in Emim-Cl- and NBupy-Cl-exposed male mice; a positive trend was also observed in Bmim-Cl-exposed male mice as well as Bmim-Cl- and NBuPy-Cl-exposed female mice. CPN is primarily tubulointerstitial nephritis, common as a background lesion in rats, but also occurring in mice.[104] The incidence and severity of CPN are generally greater in rats than in mice, and greater in males than in females.[113] No evidence of exposure-related increases in the incidence or severity of CPN in rats was noted in this study; however, a higher incidence was found in male mice than in female mice. The etiology of CPN is unknown, but factors such as xenobiotic administration, genetic background, increased age, protein content of diet, and hormones can modulate its occurrence and severity.[113] The presence of androgens has also been associated with the risk of developing CPN.[114] In male rats, CPN severity is enhanced by the administration of testosterone and abrogated by castration.[114; 115] As with the cytoplasmic alteration in the kidney, the lack of sexually dimorphic enhancement of CPN severity in male rats compared to female rats in this study also suggests a perturbation of androgens.

Numerous female mice exposed to each of the four ILs displayed a persistent X-zone in their adrenal gland, whereas no adrenal gland lesions were observed in male mice. Mammalian adrenal glands have three morphologically distinct zones with separate functions: the outer zona glomerulosa that secretes mineralocorticoids, such as aldosterone; an intermediate zone fasciculata that secretes glucocorticoids, such as cortisol; and an inner zona reticularis that secretes adrenal androgens, mainly dehydroepiandrosterone (DHEA).[116] Mice lack a functional, distinct zona reticularis and lack 17α-hydroxylase (CYP17), the key enzyme in the production of androgenic steroids, and therefore mice do not synthesize adrenal androgens.[117] Instead, mice have a unique transient region, called the "X-zone," at the junction of the cortex and medulla. The analogous structure in humans is the "fetal zone."[116; 118] In mice, the X-zone appears a few days after birth and is fully developed by weaning. The cells in this region are denser and more basophilic than the cells in the adjacent zona fasciculata.[119] In male mice, the X-zone disappears at approximately 5 weeks of age with the onset of puberty and does not undergo vacuolization. In female mice, the X-zone reaches its maximum size at about 9 weeks of age and then gradually regresses in virgins or rapidly at first pregnancy. During the process of regression in female mice, these cells develop lipid droplets with peculiar mitochondrial complexes and a smooth endoplasmic reticulum characteristic of steroid-secreting cells.[120] There is a delay in the involution of the X-zone in prepubertal male and female mice following gonadectomy and, in female mice, hypophysectomy will result in X-zone involution.[119] Androgen administration, such as androstenedione, will result in the rapid disappearance of the X-zone in female mice, and removing androgens with ovariectomy will prolong the persistence of the X-zone in female mice.[121-124] While the function of the X-zone is unknown, its normal development and regression

are mediated by a variety of factors, including sex steroid hormones, luteinizing hormones, and thyroid hormones.[118] This is the first known study in which exposure to ILs has been linked to this type of lesion in the adrenal gland.

The toxicity of ILs is dependent on the anion, cation, concentration, substituents, and the investigated biosystem.[108] Most studies examining the toxicity of ILs use in silico or in vitro approaches, and early in vivo toxicity studies more commonly used nonmammalian animal models.[108] Although somewhat limited by the number of chemicals evaluated here, this study can be used to make general comparisons to better understand in vivo mammalian toxicity associated with different cations or alkyl side chain length. Specifically, body weight and histological changes in response to IL exposure can be compared among the four ILs included in this study. Rats only had significant changes in terminal mean body weights after exposure to two of the four ILs. These changes were within 12% of the control groups and occurred at the higher exposure concentrations (1–6 mg/mL), for which average daily water consumption was 48%–84% of the control groups. Furthermore, no evidence of histopathological changes was found in rats following the 3-month exposure period. The rat studies, therefore, did not allow comparisons that might support conclusions relating cation type or alkyl chain length to toxicity. In contrast, mice exposed to all four ILs did exhibit body weight and histopathological changes, providing an opportunity to compare the toxicity of each IL (by cation type or alkyl chain length).

Zhao et al. first proposed that imidazolium-based ILs with longer alkyl chain substituents are more toxic because longer alkyl side chains increase chemical hydrophobicity.[125] This hypothesis was investigated by Bailey and colleagues, who evaluated the potential for Emim-Cl, Bmim-Cl, and the longer chain 1-decyl-3-methylimidazolium chloride (Dmim-Cl) to induce developmental toxicity in pregnant CD-1 mice.[68] Bailey et al.[68] reported that Emim-Cl was not toxic, but ILs with longer alkyl chain lengths had increased toxicity. Using short-term in vitro inhibition respiration assays, Gomez-Herrero et al.[57] further showed the toxicity of imidazolium-based ILs increased as alkyl chain length increased. The imidazolium-based IL with the shortest alkyl chain was 24-fold less toxic than the IL with the longest alkyl chain; Emim-Cl (2 carbons), Bmim-Cl (4 carbons), 1-hexyl-3-methylimidazolium chloride (Hmim-Cl; 6 carbons), and 1-octyl-3-methylimidazolium chloride (Omim-Cl; 8 carbons) had median effective concentration (EC_{50}) values of 4.19, 1.79, 0.91, and 0.17 mM, respectively. This pattern of toxicity is corroborated by several other in vitro assays and a myriad of other biological systems, such as microorganisms, plants, and human cell lines.[4; 57; 58; 126-129] In the present studies, mice exposed to Bmim-Cl experienced significantly decreased terminal mean body weights and/or histopathological changes at exposure concentrations 10 times lower than mice exposed to Emim-Cl, supporting the hypothesis that ILs with longer alkyl chains are more toxic. Specifically, male mice exposed to Emim-Cl had a lowest-observed-effect level (LOEL) of 10 mg/mL when focusing on significant terminal mean body weight changes and 30 mg/mL when focusing on histopathological changes (Figure 12). Male mice exposed to Bmim-Cl had a LOEL of 3 mg/mL for significant terminal mean body weight changes; male mice exposed to Bmim-Cl had no significant histopathological changes and therefore no corresponding LOEL could be determined (Figure 12). Female mice exposed to Emim-Cl had a LOEL of 30 mg/mL when focusing on significant terminal mean body weight changes and histopathological changes, whereas female mice exposed to Bmim-Cl had a LOEL of 3 mg/mL for the same endpoints. Although no deaths were reported in Bmim-Cl-exposed rats or mice in this study, Landry et al.[46] estimated an oral median lethal dose (LD_{50}) for Bmim-Cl of 550 mg/kg in female Fischer 344 (F344) rats. Rats

and mice in the present study were exposed to Emim-Cl at higher concentrations and much higher estimated average internal doses (up to 691 mg/kg/day in rats and 2,457 mg/kg/day in mice) than the reported Bmim-Cl LD_{50}, but no deaths were reported for this chemical, suggesting Emim-Cl, with a 2-carbon alkyl side chain, may be better tolerated and less toxic than Bmim-Cl, with a 4-carbon alkyl side chain.

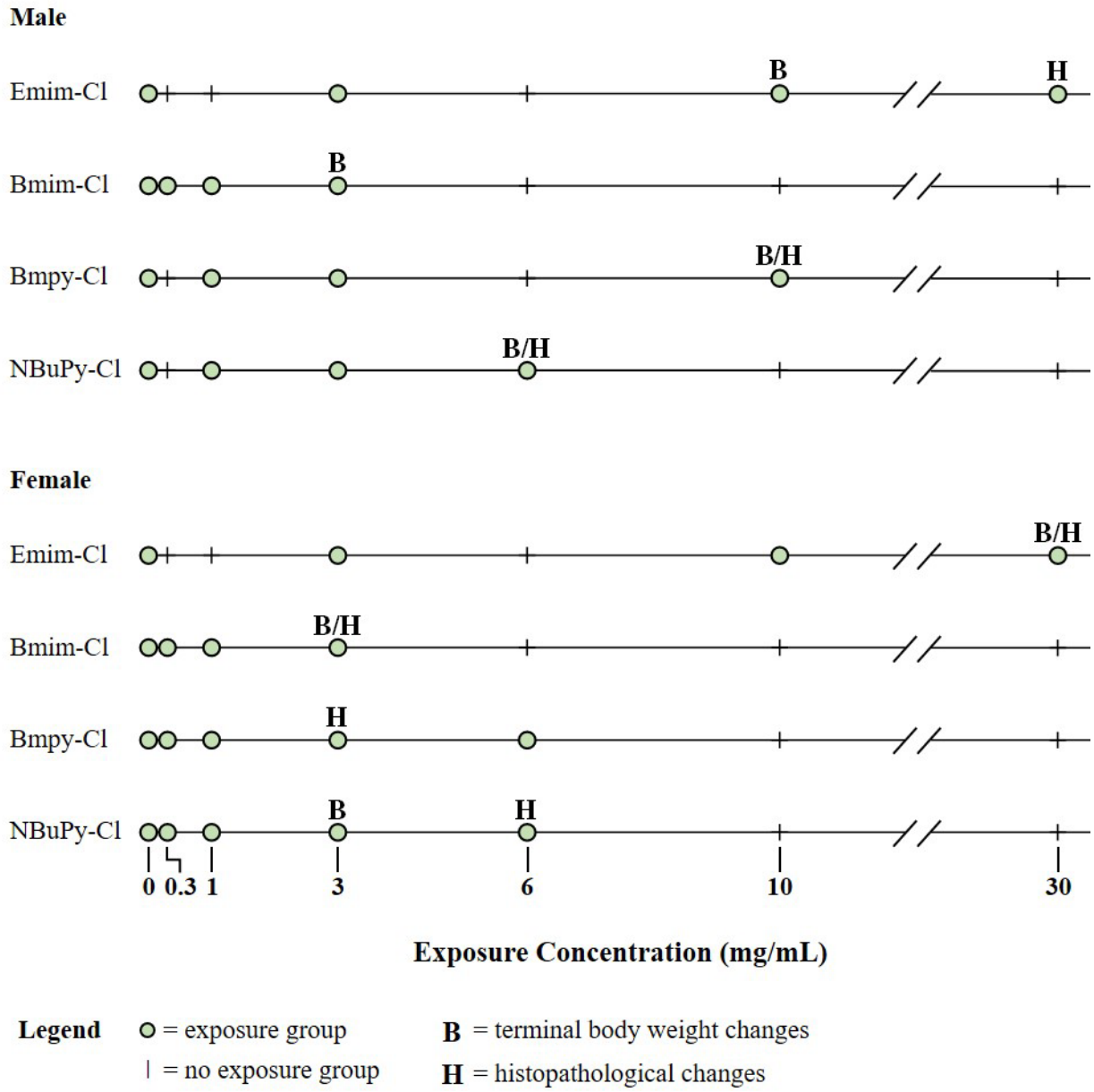


Figure 12. Lowest-observed-effect Levels Related to Significant Decreases in Terminal Mean Body Weight and/or Histopathological Changes in Male and Female Mice

Emim-Cl = 1-ethyl-3-methylimidazolium chloride; Bmim-Cl = 1-butyl-3-methylimidazolium chloride; Bmpy-Cl = 1-butyl-1-methylpyrrolidinium chloride; NBuPy-Cl = n-butylpyridinium chloride.

Additional research by NTP investigating this question in a dermal sensitization study demonstrated similar results; in vivo and in vitro assays for skin sensitization determined that Emim-Cl was not toxic, whereas Bmim-Cl was classified as a weak sensitizer.[69] The relationship between alkyl chain length and toxicity is further supported by high-throughput in vitro screening assays conducted by NTP on 197 ILs. In general, as IL alkyl chain length increases (up to 18 carbons), toxicity and activity in Tox21 assays increase.[130; 131] It should be noted that the four ILs tested in this report had very weak activities in these in vitro assays,[132-135] potentially supporting the conclusion of their relatively low toxicity in vivo. One caveat to the present studies is that while differing alkyl chain lengths were tested for methylimidazoliums, ILs with <5 carbons (e.g., both Emim-Cl [2 carbons] and Bmim-Cl [4 carbons]) are typically thought to have limited toxicity, whereas ILs with >6 carbons are thought to have linearly increased toxicity up to 10 carbons, wherein toxicity plateaus.[62; 63] It may be worthwhile, therefore, to assess in vivo mammalian toxicity in ILs with longer alkyl chain lengths in future studies. Gomez-Herrero et al.[57] suggested longer alkyl chains may interact with membrane proteins as well as with the lipid bilayer, altering integrity and native state of proteins in the membrane. Interestingly, this ability of some long-chain ILs to perturb cell membranes can be mitigated by rational design, and this quality is being utilized in the pharmaceutical industry to enhance transcellular and paracellular drug transport.[108]

Another goal of the present studies was to compare the toxicity of different cations (i.e., imidazolium, pyrrolidinium, and pyridinium). To ensure the cation could be properly evaluated, Bmim-Cl, Bmpy-Cl, and NBuPy-Cl all contained a 4-carbon alkyl side chain and the same anion. Various studies have suggested that aromatic-based ILs, such as imidazolium and pyridinium, are more toxic in vitro than nonaromatic ILs, such as pyrrolidinium, due to increased hydrophobicity of head groups.[4; 57; 58] Quantitative structure-property relationship (QSPR) modeling predicts that imidazolium cations are more toxic than pyridinium cations due to the difference in the number of nitrogen atoms.[4; 127] In NTP studies investigating these ILs for dermal sensitization, both Bmim-Cl and Bmpy-Cl were classified as weak sensitizers, whereas NBuPy-Cl was classified as an irritant,[69] but results from the Frawley et al. study did not distinguish toxicity by cation type.

In the 3-month drinking water study presented here, toxicological findings varied across male and female mice for all three cation types. When comparing body weight changes, male mice exposed to Bmim-Cl (imidazolium) displayed significant changes at lower exposure concentrations than NBuPy-Cl (pyridinium) or Bmpy-Cl (pyrrolidinium) (Figure 12); significantly decreased terminal mean body weights were observed at 3, 6, and 10 mg/mL in Bmim-Cl-, NBuPy-Cl-, and Bmpy-Cl-exposed male mice, respectively. In contrast, while no significant histopathological changes were observed in Bmim-Cl-exposed male mice, NBuPy-Cl- and Bmpy-Cl-exposed male mice had significant increases in the incidence of kidney lesions at 6 mg/mL and 10 mg/mL, respectively (note these are the same LOELs as for terminal mean body weight changes). In female mice, a significant decrease in terminal mean body weight was observed in the groups exposed to 3 mg/mL Bmim-Cl or NBuPy-Cl, but not Bmpy-Cl. Significant increases in adrenal lesions were also observed in all three cation groups at 3 mg/mL in Bmim-Cl- and Bmpy-Cl-exposed female mice and at 6 mg/mL in NBuPy-Cl-exposed female mice (the same as in NBuPy-Cl-exposed males). On the basis of these endpoints and on the exposure concentrations tested, it is unclear whether imidazolium-, pyrrolidinium- or pyridinium-based IL exposure is most toxic.

In the aforementioned Tox21 high-throughput in vitro assays conducted by NTP, cation type was not a good predictor of cytotoxicity or activity. Other researchers have speculated that the toxicity of ILs with a bioactive cation may depend more on the cationic head group than on the alkyl side chain, whereas toxicity in less bioactive cations (e.g., imidazolium, pyridinium) may be modulated more strongly by the side chain.[108; 136] For example, in the promyelocytic leukemia rat cell line IPC-81, toxicity of imidazolium, pyridinium, piperidinium, pyrrolidinium, and morpholinium are similar.[108; 137] Therefore, it is reasonable that the cations tested in the current study exhibit minimal differences in toxicity.

Overall, the results from this comparative study suggest that Emim-Cl, Bmim-Cl, Bmpy-Cl, and NBuPy-Cl in drinking water have minimal effects on rats and mice at low exposure concentrations (<3 mg/mL). Exposure to higher IL concentrations (≥3 mg/mL) results in lower body weights and nonneoplastic lesions in the kidneys of male and female mice and in the adrenal glands of female mice. The lowest-observed-effect levels (LOELs) were determined using these data. The LOELs were assigned as 10 mg/mL for Emim-Cl-exposed male mice, 3 mg/mL for Bmim-Cl-exposed male mice, 10 mg/mL for Bmpy-Cl-exposed male mice, and 6 mg/mL for NBuPy-Cl-exposed male mice. The LOELs for female mice differed slightly. The LOELs were 30 mg/mL for Emim-Cl-exposed female mice, 3 mg/mL for Bmim-Cl-exposed female mice (the same as for Bmim-Cl-exposed male mice), 3 mg/mL for Bmpy-Cl-exposed female mice, and 3 mg/mL for NBuPy-Cl-exposed female mice. These studies indicate that IL-induced toxicity may be attributable to alkyl chain length and cation type. ILs with longer alkyl chains typically exhibit increased toxicity compared to ILs with shorter alkyl chains. In this report, mice exposed to Bmim-Cl (an IL with a 4-carbon alkyl chain) had a lower LOEL than did mice exposed to Emim-Cl (an IL with a 2-carbon alkyl chain). The difference in toxicity among cations (imidiazolium vs. pyrrolidinium vs. pyridinium), however, is less clear and is both sex- and endpoint-dependent.

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Appendix A. Chemical Characterization and Dose Formulation Studies

Table of Contents

Tables

Figures

A.1. Procurement and Characterization of Ionic Liquids

A.1.1. 1-Ethyl-3-Methylimidazolium Chloride (Emim-Cl)

1-Ethyl-3-methylimidazolium chloride (Emim-Cl) was obtained from Sigma-Aldrich (St. Louis, MO) in three lots (S37784, S46787, and STBB3624). Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at MRIGlobal (Kansas City, MO). Reports on analyses performed in support of the ionic liquid (IL) studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

Lot S37784 of Emim-Cl was a faint yellow solid, lot S46787 was a brown solidified mass, and lot STBB3624 was a yellow-brown solid. The chemical identity was confirmed using infrared (IR) and ^{1}H nuclear magnetic resonance (NMR) spectroscopies. The IR and ^{1}H NMR spectra (Figure A-1, Figure A-2, Figure A-3) were consistent with reference spectra (SDBSWeb: https://sdbs.db.aist.go.jp, National Institute of Advanced Industrial Science and Technology, accessed November 1, 2010) and the anticipated structure of Emim-Cl. In addition, mass spectrometry (MS) and elemental analysis were performed to aid in chemical identity confirmation. Mass spectra supported the presence of the cation $[Emim]^{+}$ at m/z 111.0 for lot S37784, m/z 111.1 for lot S46787, and m/z 111.0 for lot STBB3624. Elemental analysis was performed by Prevalere Life Sciences, Inc. (Whitesboro, NY). The relative amounts of carbon, hydrogen, nitrogen, and chlorine for lot S37784 (49.01%, 7.46%, 18.60%, and 24.17%), lot S46787 (49.17%, 7.82%, 18.81%, and 24.37%), and lot STBB3624 (47.27%, 7.86%, 17.66%, and 22.19%) were within 2% of the theoretical values.

The moisture content of lots S37784, S46787, and STBB3624 was determined by Karl Fischer titration. The purity profiles of the three lots were determined using high-performance liquid chromatography (HPLC) with evaporative light scattering detection (ELSD) and HPLC with ultraviolet (UV) detection. Chloride content of each lot was quantified using ion chromatography (IC). Weight loss on drying was attempted to determine volatile content, but constant weight of the test article could not be achieved due to its hygroscopic nature. The volatile content was determined by headspace gas chromatography (GC/headspace) with flame ionization detection (FID) for lot STBB3624.

For lot S37784, Karl Fischer titration yielded a water content of approximately 0.2%. This value is an estimate due to the hygroscopic nature of the compound. No impurities were detected in lot S37784 with HPLC/ELSD (Table A-1, System D). The IC analysis determined the chloride concentration for lot S37784 to be 23.80% ± 0.14(sd)% (sd = standard deviation) (Table A-1, System F). The overall purity of the lot was determined to be >99%. Lot S37784 was reanalyzed at 20 months after the first analysis using HPLC/UV (Table A-1, System B), and the purity was determined to be 99.0% ± 0.9(sd)%.

For lot S46787, Karl Fischer titration yielded a water content of approximately 0.6%. No impurities were detected in lot S46787 with HPLC/ELSD (Table A-1, System D). The IC analysis determined the chloride concentration for lot S46787 to be 23.50% ± 0.08(sd)% (Table A-1, System F). The overall purity of the test article was >99%.

For lot STBB3624, Karl Fischer titration yielded a water content of approximately 1.1%. No impurities were detected in lot STBB3624 with HPLC/ELSD (Table A-1, System C). The IC analysis determined the chloride concentration for lot STBB3624 to be 22.26% ± 0.33(sd)%

(Table A-1, System G). The GC/headspace analysis for residual solvent content determined that acetonitrile, isopropanol, and ethyl acetate were not present at concentrations >0.001% (Table A-2, System H). The overall purity of the lot was determined to be >99%. The purity of lot STBB3624 was reevaluated prior to use in the 3-month study using HPLC/UV (Table A-1, System B) and determined to be 101.0% ± 0.5(sd)% relative to a frozen reference sample of the same lot.

Accelerated stability studies of Emim-Cl were conducted on samples of lot STBB3624 stored under an inert headspace in amber glass vials sealed with Teflon®-lined screw-top lids at frozen (−20°C), refrigerated (5°C), room (25°C), and elevated (60°C) temperatures. After 2 weeks, samples were analyzed by HPLC/UV (Table A-1, System B). Stability of the bulk chemical was confirmed for at least 2 weeks when stored under an inert headspace and protected from light at temperatures from −20°C to 60°C.

The two bottles of lot S37784 were completely melted and thoroughly blended. They were then transferred into amber glass bottles and sealed with an inert gas headspace and Teflon®-lined lids. A single drum of lot STBB3624 was completely melted, thoroughly blended, and transferred into wide-mouth amber glass bottles and sealed with an inert gas headspace and Teflon®-lined lids. All containers were then placed in two plastic bags with the outermost bag containing Drierite desiccant and stored under inert gas at room temperature.

A.1.2. 1-Butyl-3-Methylimidazolium Chloride (Bmim-Cl)

1-Butyl-3-methylimidazolium chloride (Bmim-Cl) was obtained from Solvent Innovations (Koln, Germany) in a single lot (99/787) and from Sigma-Aldrich (St. Louis, MO) in a single lot (STBB3444). Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at MRIGlobal (Kansas City, MO). Reports on analyses performed in support of the IL studies are on file at NIEHS.

Lot 99/787 of Bmim-Cl was a pale amber solid in 1- to 4-inch pieces, and lot STBB3444 was a solid white mass with small orange streaks. The lot identities were confirmed using IR and ^{1}H NMR spectroscopies. The IR and ^{1}H NMR spectra (Figure A-4, Figure A-5, Figure A-6) were consistent with the proposed structure. No reference spectra were available for comparison. In addition, MS and elemental analysis were performed to aid in chemical identity confirmation. Mass spectra supported the presence of the cation $[Bmim]^{+}$ at m/z 139.0 for lot 99/787 and m/z 139.1 for lot STBB3444. Elemental analysis was performed by Prevalere Life Sciences, Inc. (Whitesboro, NY). The relative amounts of carbon, hydrogen, nitrogen, and chlorine for lot 99/787 (54.64%, 8.91%, 15.83%, and 19.91%) and lot STBB3444 (53.72%, 8.71%, 14.98%, and 18.92%) were within 2% of the theoretical values.

The moisture content of lots 99/787 and STBB3444 was determined by Karl Fischer titration. The purity profiles of lots 99/787 and STBB3444 were determined using HPLC/ELSD and HPLC/UV, and the chloride content was quantified using IC. The volatile content was determined by GC/headspace with FID for lot STBB3444.

For lot 99/787, Karl Fischer titration yielded a water content of approximately 0.5%. This value is an estimate due to the hygroscopic nature of the compound. No impurities were detected in lot 99/787 with HPLC/ELSD (Table A-1, System C). The IC analysis determined the percent chloride concentration for lot 99/787 to be 20.01% ± 0.02(sd)% and detected no bromide

(Table A-1, System G). Weight loss on drying was attempted to determine volatile content for lot 99/787, but constant weight of the test article could not be achieved due to its hygroscopic nature. The overall purity of lot 99/787 was determined to be >99%. Lot 99/787 was reanalyzed 26 months later using HPLC/UV (Table A-1, System B) and determined to be 98.6% ± 0.5($\bar{d}$)% ($\bar{d}$) is the average deviation between two samples) relative to a frozen reference sample of the same lot.

For lot STBB3444, Karl Fischer titration yielded a water content of approximately 0.7%. No significant response was obtained from the test article with HPLC/UV detection at 240 nm (Table A-1, System A). No impurities ≥0.05% were detected in lot STBB3444 with HPLC/ELSD (Table A-1, System C). The IC analysis determined the percent chloride concentration for lot STBB3444 to be 19.28% ± 0.43(sd)% (Table A-1, System G). The GC/headspace analysis for residual solvent content determined that acetonitrile, isopropanol, and ethyl acetate were not present at concentrations >0.001% (Table A-2, System H). The overall purity of lot STBB3444 was determined to be >99%. The purity of lot STBB3444 was reevaluated prior to use in the 3-month study using HPLC/UV (Table A-1, System B) and determined to be 101.4% ± 2.0(sd)% relative to a frozen reference sample of the same lot.

Accelerated stability studies of Bmim-Cl were conducted on samples of lot STBB3444 stored under an inert headspace in amber glass vials sealed with Teflon®-lined screw-top lids at frozen (−20°C), refrigerated (5°C), room (25°C), and elevated (60°C) temperatures. After 2 weeks, samples were analyzed by HPLC/UV (Table A-1, System B). Stability of the bulk chemical was confirmed for at least 2 weeks when stored under an inert headspace and protected from light at temperatures from −20°C to 60°C.

Lot 99/787 was completely melted, thoroughly blended, and then stored in secondary containment under an inert atmosphere at room temperature. Lot STBB3444 also was completely melted and then thoroughly blended. After homogenization, it was transferred into wide-mouth amber glass bottles and sealed with an inert gas headspace and Teflon®-lined lids. All containers were then placed in two plastic bags with the outermost bag containing Drierite desiccant stored under inert gas at room temperature.

A.1.3. 1-Butyl-1-Methylpyrrolidinium Chloride (Bmpy-Cl)

1-Butyl-1-methylpyrrolidinium chloride (Bmpy-Cl) was obtained from Solvent Innovations (Koln, Germany) in a single lot (99/831) and from Promy Chemical, Inc. (El Sobrante, CA) in a single lot (20100610). Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at MRIGlobal (Kansas City, MO). Reports on analyses performed in support of the IL studies are on file at NIEHS.

Lot 99/831 of Bmpy-Cl was a white chunky powder, and lot 20100610 was a pale-yellow crystalline powder. The chemical identity was confirmed using IR and ^{1}H NMR spectroscopies. The IR and ^{1}H NMR spectra (Figure A-7, Figure A-8, Figure A-9) were consistent with the proposed structure. No reference spectra were available for comparison. In addition, MS and elemental analysis were performed to confirm chemical identity. Mass spectra supported the presence of the cation $[Bmpy]^{+}$ at m/z 141.9 for lot 99/831 and at m/z 142.1 for lot 20100610. Elemental analysis was performed by Prevalere Life Sciences, Inc. (Whitesboro, NY). The relative amounts of carbon, hydrogen, nitrogen, and chlorine for lot 99/831 (60.29%, 11.66%,

7.78%, and 19.97%) and lot 20100610 (60.43%, 11.26%, 7.50%, and 20.67%) were within 1% of the theoretical values.

The moisture content of lots 99/831 and 20100610 was determined by Karl Fischer titration, and the purity profiles of both lots were determined using HPLC/ELSD and HPLC with charged aerosol detection (CAD). Chloride content was quantified using IC. The volatile content was determined by weight loss on drying for lot 99/831 and GC/headspace with FID for lot 20100610.

For lot 99/831, Karl Fischer titration yielded a water content of approximately 0.5%. This value is an estimate due to the hygroscopic nature of the compound. No impurities were detected in lot 99/831 with HPLC/ELSD (Table A-1, System C). The IC analysis determined the percent chloride concentration for lot 99/831 to be 19.71% ± 0.27(sd)% and detected no bromide (Table A-1, System G). Weight loss on drying determined a volatile content for lot 99/831 of 0.75% ± 0.12($\bar{d}$)% after 2 hours of drying. The overall purity of lot 99/831 was determined to be >99%. The purity of lot 99/831 was reevaluated prior to use in the 3-month study using HPLC/ELSD (Table A-1, System D) and determined to be 101.3% ± 1.2(sd)% relative to a frozen reference sample of the same lot.

For lot 20100610, Karl Fischer titration yielded a water content of approximately 1.2%. No impurities ≥0.05% were detected in lot 20100610 with HPLC/ELSD (Table A-1, System C). The IC analysis determined the chloride concentration for lot 20100610 to be 19.16% ± 0.65(sd)% (Table A-1, System G). The GC/headspace analysis for residual solvent content determined that acetonitrile, isopropanol, and ethyl acetate were not present at concentrations >0.001% (Table A-2, System H). The overall purity of lot 20100610 was determined to be >98%. The purity of lot 20100610 was reevaluated prior to use in the 3-month study using HPLC/CAD (Table A-1, System E) and determined to be 99.9% ± 0.3(sd)% relative to a frozen reference sample of the same lot.

Accelerated stability studies of Bmpy-Cl were conducted on samples of lot 20100610 stored under an inert headspace in amber glass vials sealed with Teflon®-lined screw-top lids at frozen (−20°C), refrigerated (5°C), room (25°C), and elevated (60°C) temperatures. After 2 weeks, samples were analyzed by HPLC/ELSD (Table A-1, System D). Stability of the bulk chemical was confirmed for at least 2 weeks when stored under an inert headspace and protected from light at temperatures from −20°C to 60°C.

Details of the homogenization of lot 99/831 were not reported. Lot 99/831 was stored at room temperature under inert gas. Lot 20100610 was manually blended by hand kneading the unopened Mylar bag containing the bulk material for 3 to 4 minutes. After homogenization, the material was transferred into wide-mouth amber glass bottles and sealed with an inert gas headspace and Teflon®-lined lids. All containers were then placed in two plastic bags with the outermost bag containing Drierite desiccant and stored at room temperature.

A.1.4. N-Butylpyridinium Chloride (NBuPy-Cl)

N-Butylpyridinium chloride (NBuPy-Cl) was obtained from Solvent Innovations (Koln, Germany) in a single lot (99/830) and from Promy Chemical, Inc. (El Sobrante, CA) in a single lot (20100610). Identity, purity, and stability analyses were conducted by the analytical

chemistry laboratory at MRIGlobal (Kansas City, MO). Reports on analyses performed in support of the IL studies are on file at NIEHS.

Lot 99/830 of NBuPy-Cl was an off-white solid with a peach hue, and lot 20100610 was a pale-yellow crystalline powder. The chemical identity was confirmed using IR and ^{1}H NMR spectroscopies. The IR and ^{1}H NMR spectra (Figure A-10, Figure A-11, Figure A-12) were consistent with the proposed structure. No reference IR spectra were available for comparison; the ^{1}H spectrum was consistent with a reference spectrum (SDBSWeb: https://sdbs.db.aist.go.jp, National Institute of Advanced Industrial Science and Technology, accessed November 1, 2010). In addition, MS and elemental analysis were performed to aid in chemical identity confirmation. Mass spectra supported the presence of the cation $[NBuPy]^{+}$ at m/z 135.9 for lot 99/830 and at m/z 136.1 for lot 20100610. Elemental analysis was performed by Prevalere Life Sciences, Inc. (Whitesboro, NY). The relative amounts of carbon, hydrogen, nitrogen, and chlorine for lot 99/830 (62.75%, 8.31%, 7.99%, and 20.57%) and lot 20100610 (62.66%, 8.21%, 7.72%, and 20.49%) were within 1% of the theoretical values.

The moisture content of lots 99/830 and 20100610 was determined by Karl Fischer titration. The purity profiles of lots 99/830 and 20100610 were determined using HPLC/UV and HPLC/ELSD, and the chloride content was quantified using IC. The volatile content was determined by GC/headspace with FID for lot 20100610.

For lot 99/830, Karl Fischer titration yielded a water content of approximately 0.3%. This value is an estimate due to the hygroscopic nature of the compound. Purity of the test article with HPLC/UV was 99.23%, with one reportable impurity of 0.61% (Table A-1, System A). No impurities were detected in lot 99/830 with HPLC/ELSD (Table A-1, System C). The IC analysis determined the chloride concentration for lot 99/830 to be 20.47% ± 0.13(sd)% and detected no bromide (Table A-1, System G). Weight loss on drying was attempted to determine volatile content for lot 99/830, but constant weight of the test article could not be achieved due to its hygroscopic nature. The purity of lot 99/830 was reevaluated prior to use in the 3-month study using HPLC/UV (Table A-1, System B) and determined to be 101.1% ± 2.3(sd)% relative to a frozen reference sample of the same lot. The overall purity was >99%.

For lot 20100610, Karl Fischer titration yielded a water content of approximately 0.5%. Purity of the test article with HPLC/UV detection was 99.27%, with one impurity of 0.73% (Table A-1, System A). No impurities were detected in lot 20100610 with HPLC/ELSD (Table A-1, System C). The IC analysis determined the chloride concentration for lot 20100610 to be 20.25% ± 0.11(sd)% (Table A-1, System G). The GC/headspace analysis for residual solvent content detected six impurities using two procedures (Table A-2, System I, System J). Two of these were identified as methanol (<0.01%) and pyridine (0.3%). The test material was further analyzed for impurities with GC/MS (Table A-2, System K). Two more impurities were tentatively identified as ethyl ether and 1-chlorobutane on the basis of the observed mass spectra. The overall purity of the lot was determined to be >98%. The purity of lot 20100610 was reevaluated prior to use in the 3-month study using HPLC/UV (Table A-1, System B) and determined to be 100.6% ± 0.7(sd)% relative to a frozen reference sample of the same lot.

Accelerated stability studies of NBuPy-Cl were conducted on samples of lot 20100610 stored under an inert headspace in amber glass vials sealed with Teflon®-lined screw-top lids at frozen (−20°C), refrigerated (5°C), room (25°C), and elevated (60°C) temperatures. After 2 weeks,

samples were analyzed by HPLC/UV (Table A-1, System B). Stability of the bulk chemical was confirmed for at least 2 weeks when stored under an inert headspace and protected from light at temperatures from −20°C to 60°C.

Lot 99/830 was blended manually by shaking the bottle vigorously prior to opening to ensure homogeneity and was stored under inert gas at room temperature. The original bulk material in a Mylar bag (containing inner Mylar bags) of lot 20100610 was manually blended for 3 to 4 minutes by hand kneading the unopened bag. After homogenization, the bulk material of lot 20100610 was transferred into wide-mouth amber glass bottles and sealed with an inert gas headspace and Teflon®-lined lids. All containers were then placed in two plastic bags with the outermost bag containing Drierite desiccant and stored at room temperature.

A.2. Preparation and Analysis of Dose Formulations

A.2.1. Two-week Studies

The dose formulations for the 2-week study were prepared once at MRIGlobal (Kansas City, MO) by mixing Emim-Cl, Bmim-Cl, Bmpy-Cl, or NBuPy-Cl with tap water to give the required concentrations (Table A-3). Dose formulations of Emim-Cl were prepared at concentrations of 3, 10, and 30 mg/mL with lot S37784 and 100 mg/mL with a combination of lots S37784 and S46787. Dose formulations of Bmim-Cl were prepared at concentrations of 0.3, 1, 3, and 6 mg/mL with lot 99/787. Dose formulations of Bmpy-Cl were prepared at concentrations of 1, 3, 6, and 10 mg/mL with lot 99/831. Dose formulations of NBuPy-Cl were prepared at concentrations of 0, 1, 3, and 6 mg/mL with lot 99/830. All dose formulations were stored protected from light at refrigerated temperatures (2°C–8°C) for up to 7 days until shipment to the study laboratory (BioReliance, Rockville, MD). At the study laboratory, all dose formulations were stored protected from light in a plastic carboy at refrigerated temperatures (2°C–8°C) and used within 42 days.

The homogeneity and stability of the dose formulations for lots S37784 (Emim-Cl), 99/787 (Bmim-Cl), 99/831 (Bmpy-Cl), and 99/830 (NBuPy-Cl) were determined by the analytical laboratory using HPLC/UV or HPLC/ELSD (Table A-1, Systems B, B, D, and B, respectively). Homogeneity was confirmed for the highest and lowest dose formulations of each ionic liquid. Dose formulations were considered stable if the measured concentrations were statistically no different from day 0 concentrations using the test variability limit statistical analysis. The storage stability of the 3 mg/mL Emim-Cl dose formulation was confirmed when protected from light for ≤35 days at room temperature, ≤21 days under refrigeration, and for ≤7 days under simulated animal room temperature. Storage stability of 0.3 mg/mL Bmim-Cl and 1 mg/mL Bmpy-Cl dose formulations were confirmed for ≤42 days at room and refrigerated temperatures and for ≤7 days under simulated animal room conditions. The storage stability of 1 mg/mL NBuPy-Cl dose formulation was confirmed for ≤35 days at room and refrigerated temperatures and for ≤4 days under simulated animal room conditions.

Analyses of preadministration and postadministration dose formulations for the 2-week studies of Emim-Cl, Bmim-Cl, and NBuPy-Cl were conducted once for each study by MRIGlobal analytical chemistry laboratory using HPLC/UV (Table A-1, System B). HPLC/ELSD (Table A-1, System D) was used to conduct analysis of preadministration and postadministration dose formulations of Bmpy-Cl. Postadministration dose formulation analysis results are averages

from animal room drinking water bottles and bulk containers. All dose formulations were within 10% of the target concentrations for all four chemicals (Emim-Cl [Table A-5], Bmim-Cl [Table A-8], Bmpy-Cl [Table A-11], and NBuPy-Cl [Table A-14]). The pH of Emim-Cl and Bmpy-Cl dose formulations were measured postadministration and are reported in Table A-17 and Table A-18, respectively.

A.2.2. Three-month Studies

The dose formulations for the 3-month studies were prepared at the Battelle study laboratory (Columbus, OH) by mixing Emim-Cl, Bmim-Cl, Bmpy-Cl, or NBuPy-Cl with tap water to give the required concentrations for each species (Table A-4). Dose formulations of Emim-Cl were prepared at four concentrations of 0, 1, 3, and 10 mg/mL for the rat study and 0, 3, 10, and 30 mg/mL for the mouse study with lot STBB3624. Dose formulations of Bmim-Cl were prepared at four concentrations of 0, 0.1, 0.3, and 1 mg/mL for the rat study and 0, 0.3, 1, and 3 mg/mL for the mouse study with lot STBB3444. Dose formulations of Bmpy-Cl were prepared at five concentrations of 0, 0.3, 1, 3, and 6 mg/mL for the rat study and 0, 1, 3, 6, and 10 mg/mL for the mouse study with lot 20100610. Dose formulations of NBuPy-Cl were prepared at four concentrations of 0, 0.3, 1, and 3 mg/mL for the rat study and 0, 1, 3, and 6 mg/mL for the mouse study with lot 20100610. The formulations were stored protected from light in a plastic carboy at refrigerated temperatures (2°C–8°C). Emim-Cl and NBuPy-Cl formulations were used within 35 days of preparation, and Bmim-Cl and Bmpy-Cl formulations were used within 42 days of preparation.

The storage stability of 1 mg/mL Emim-Cl, 0.1 mg/mL Bmim-Cl, and 0.3 mg/mL NBuPy-Cl dose formulations at refrigerated and room temperatures were analyzed by the study laboratory using HPLC/UV (Table A-1, System B). HPLC/CAD (Table A-1, System E) was used to conduct similar analyses of 0.3 mg/mL Bmpy-Cl dose formulations. Dose formulations were considered stable if the measured concentrations were within 10% of the day 0 concentrations and no signs of degradation were detected in the chromatograms. All four IL dose formulations were considered stable for ≤42 days when stored refrigerated or at room temperature, protected from light.

Analyses of preadministration and postadministration dose formulations for the 3-month studies of Emim-Cl, Bmim-Cl, and NBuPy-Cl were conducted once after each new formulation preparation at the Battelle study laboratory using HPLC/UV (Table A-1, System B). HPLC/CAD (Table A-1, System E) was used to conduct similar analyses of the dose formulations for the 3-month study of Bmpy-Cl. All preadministration dose formulations for all four chemicals were within 10% of the target concentrations. All animal room samples for Emim-Cl (Table A-6, Table A-7) and Bmim-Cl (Table A-9, Table A-10) were within 10% of the target concentrations. All animal room samples for Bmpy-Cl (Table A-12, Table A-13) were within 10% of the target concentrations, with the exception of three samples for the rat study and five samples for the mouse study. For the rat study, one 0.3 mg/mL formulation was 14.0% and 15.8% above target for the animal room and carboy samples, respectively, and one 1 mg/mL formulation was 10.7% above target for the carboy sample. For the mouse study, two 10 mg/mL formulations were 11.7% and 12.3% above target in the animal room and 13.7% and 14.3% above target in the carboys. Additionally, one 1 mg/mL formulation was 10.7% above target for the carboy sample. All animal room samples for NBuPy-Cl (Table A-15, Table A-16) were within 10% of the target

concentrations, with the exception of one carboy sample for the rat study, which was 13.1% above the target concentration of 0.3 mg/mL.

Table A-1. Liquid and Ion Chromatography Systems Used in the Two-week and Three-month Drinking Water Studies of Ionic Liquids

Chromatography	Detection System	Column	Mobile Phase
High-performance Liquid Chromatography Systems			
System A			
High-performance liquid chromatography	Ultraviolet (240 nm)	Supelco Discovery Cyano (150 mm × 4.6 mm ID, 5 µm particle size)	A: Acetonitrile B: Aqueous ~33 mM ammonium acetate, acidified with acetic acid to pH ~4.0 Gradient program: A:B Isocratic 85:15, 0.8 mL/min flow rate
System B			
High-performance liquid chromatography	Ultraviolet (210 nm)	Supelco Discovery Cyano (150 mm × 4.6 mm ID, 5 µm particle size)	A: Acetonitrile B: Aqueous ~40 mM ammonium acetate with 0.5% acetic acid Gradient program: A:B Isocratic 90:10, 0.8–1.0 mL/min flow rate
System C			
High-performance liquid chromatography	Evaporative light scattering	Supelco Discovery Cyano (150 mm × 4.6 mm ID, 5 µm particle size)	A: Acetonitrile B: Aqueous ~33 mM ammonium acetate, acidified with acetic acid to pH ~4.0 Gradient program: A:B Isocratic 85:15, 0.8 mL/min flow rate
System D			
High-performance liquid chromatography	Evaporative light scattering	Supelco Discovery Cyano (150 mm × 4.6 mm ID, 5 µm particle size)	A: Acetonitrile B: Aqueous ~40 mM ammonium acetate, acidified with 0.5% acetic acid Gradient program: A:B Isocratic 90:10, 0.8–1.0 mL/min flow rate

Chromatography	Detection System	Column	Mobile Phase
System E			
High-performance liquid chromatography	Charged aerosol detector	Supelco Discovery Cyano (150 mm × 4.6 mm ID, 5 µm particle size)	A: Acetonitrile B: ~40 mM Ammonium acetate with 0.5% acetic acid Gradient program: A:B Isocratic 90:10, 0.8 mL/min flow rate
Ion Chromatography Systems			
System F			
Ion chromatography	Conductivity	Transgenomic ICSep AN2 (250 mm × 4.6 mm ID)	Mobile Phase: Aqueous ~3.2 mM sodium carbonate and ~1.0 mM sodium bicarbonate, 1 mL/min flow rate External Water: ~0.5% Aqueous sulfuric acid, 4 mL/min flow rate
System G			
Ion chromatography	Conductivity	Transgenomic ICSep AN2 (250 mm × 4.6 mm ID) with Transgenomic ICSep AN2 Guard (50 mm × 4.6 mm ID)	Mobile Phase: Aqueous ~3.2 mM sodium carbonate and ~1.0 mM sodium bicarbonate, 1 mL/min flow rate External Water: ~0.5% Aqueous sulfuric acid, 4 mL/min flow rate

ID = internal diameter.

Table A-2. Gas Chromatography Systems Used in the Two-week and Three-month Drinking Water Studies of Ionic Liquids

Detection System	Column	Carrier Gas	Oven Temperature Program
System H			
Flame ionization (260°C)	Phenomenex Zebron ZB-624 (30 m × 0.53 mm ID, 3.0-µm film thickness)	Helium, 2.55 mL/min	50°C for 6 min, then 8°C/min to 210°C, held for 4 min
System I			
Flame ionization (250°C)	Rxi624SilMS (30 m × 0.32 mm ID, 1.8-µm film thickness)	Helium, 2.37 mL/min	40°C for 20 min, then 10°C/min to 240°C, held for 20 min

Detection System	Column	Carrier Gas	Oven Temperature Program
System J			
Flame ionization (250°C)	Stabilwax (30 m × 0.32 mm ID, 0.25-µm film thickness)	Helium, 2.46 mL/min	50°C for 20 min, then 6°C/min to 165°C, held for 20 min
System K			
Electron ionization mass spectrometry	Rxi-624Sil mS (30 m × 0.32 mm ID, 1.8-µm film thickness)	Helium, 3.3 mL/min	40°C for 20 min, then 10°C/min to 240°C, held for 20 min

ID = internal diameter.

Table A-3. Preparation and Storage of Dose Formulations in the Two-week Drinking Water Studies of Ionic Liquids

Emim-Cl	Bmim-Cl	Bmpy-Cl	NBuPy-Cl
Preparation			
The appropriate amount of test article was weighed and transferred into a 1-L volumetric flask. The contents were diluted to volume with tap water and mixed by inversion. The formulation was transferred to a 10-L plastic carboy. After using the same volumetric flask to weigh and transfer nine additional 1-L aliquots to the carboy, the 10-L formulation was mixed well. The doses were prepared once.			
Chemical Lot Number			
S37784 and S46787[a]	99/787	99/831	99/830
Maximum Storage Time[b]			
42 days			
Storage Conditions			
Plastic carboys covered with aluminum foil. Stored refrigerated at 2°C–8°C.			
Analytical Laboratory			
MRIGlobal (Kansas City MO)			
Study Laboratory			
BioReliance (Rockville, Maryland)			

Emim-Cl = 1-ethyl-3-methylimidazolium chloride; Bmim-Cl = 1-butyl-3-methylimidazolium chloride; Bmpy-Cl = 1-butyl-1-methylpyrrolidinium chloride; NBuPy-Cl = n-butylpyridinium chloride.
[a]Lot S37784 was used for the 3, 10, and 30 mg/mL formulations. Lots S37784 and S46787 were combined to make the 100 mg/mL formulation.
[b]The expiration date used for all formulations. Emim-Cl and NBuPy-Cl were determined to be stable when refrigerated for up to 21 days and 35 days, respectively, in the stability study conducted before the 2-week study. All formulations were determined to be stable when refrigerated for up to 42 days during the stability study conducted before the 3-month study.

Table A-4. Preparation and Storage of Dose Formulations in the Three-month Drinking Water Studies of Ionic Liquids

Emim-Cl	Bmim-Cl	Bmpy-Cl	NBuPy-Cl
Preparation			
An appropriate amount of vehicle (tap water) was added to the container holding the preweighed test article. The container was sealed, shaken, and mixed by sonication and/or vortexing until the contents were a solution. The solution was then transferred into a plastic carboy containing approximately half the required amount of vehicle. The test article container was rinsed with vehicle at least three times, and the rinses were transferred into the carboy. Approximately 90% of the final total vehicle volume was added to the carboy, and the formulation was stirred with an overhead stirrer. Approximately 200 mL was dispensed through the spigot into a beaker and poured back into the carboy. The beaker was rinsed with vehicle thoroughly, and the rinse was added into the carboy. The carboy was diluted to final volume with vehicle. The formulation was stirred with an overhead stirrer.			
Chemical Lot Number			
STBB3624	STBB3444	20100610	20100610
Maximum Storage Time			
35 days	42 days	42 days	35 days
Storage Conditions			
Plastic carboys stored refrigerated at 2°C–8°C.			
Study Laboratory			
Battelle (Columbus, OH)			

Emim-Cl = 1-ethyl-3-methylimidazolium chloride; Bmim-Cl = 1-butyl-3-methylimidazolium chloride; Bmpy-Cl = 1-butyl-1-methylpyrrolidinium chloride; NBuPy-Cl = n-butylpyridinium chloride.

Table A-5. Results of Analyses of Dose Formulations Administered to Male and Female Rats and Mice in the Two-week Drinking Water Studies of 1-Ethyl-3-Methylimidazolium Chloride

Date Prepared	Date Analyzed[a]	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[b]	Difference from Target (%)
February 26, 2009	March 4, 2009	3	3.0 ± 0.03	−0.4
		10	10.1 ± 0.1	1.2
		30	30.8 ± 0.2	2.8
		100	100.6 ± 0.9	0.6
Animal Room Samples (Rats)[c]				
February 26, 2009	April 2, 2009	3	3.0 ± 0.0	−0.8
		10	10.0 ± 0.0	−0.2
		30	30.5 ± 0.2	1.6
		100	100.1 ± 0.4	0.1
Animal Room Samples (Mice)[c]				
February 26, 2009	April 2, 2009	3	3.0 ± 0.0	−1.5
		10	10.0 ± 0.0	−0.3
		30	30.3 ± 0.1	1.2
		100	100.1 ± 0.6	0.1

[a]Analysis dates assumed from laboratory report dates. Preadministration formulations were analyzed in triplicate within 1 week of preparation.
[b]Average and standard deviation of all sample measurements.
[c]Preadministration samples were taken from carboys. Postadministration samples were taken from carboys and drinking water bottles.

Table A-6. Results of Analyses of Dose Formulations Administered to Male and Female Rats in the Three-month Drinking Water Study of 1-Ethyl-3-Methylimidazolium Chloride

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[a,b]	Difference from Target (%)
April 19, 2013	April 19, 2013	0	BLOQ	NA
		1	0.999 ± 0.002	−0.1
		3	2.97 ± 0.01	−0.9
		10	10.2 ± 0.1	2.3
June 4, 2013	June 6, 2013	0	BLOQ	NA
		1	1.00 ± 0.01	0.2
		3	3.04 ± 0.00	1.3
		10	10.0 ± 0.1	0.3
July 15, 2013	July 16, 2013	0	BLOQ	NA
		1	0.992 ± 0.002	−0.8
		3	2.93 ± 0.01	−2.5
		10	10.1 ± 0.1	0.7
Animal Room Samples				
April 19, 2013 (Drinking Water Bottle)	May 24, 2013	0	BLOQ	NA
		1	1.03 ± 0.00	3.0
		3	3.03 ± 0.01	0.9
		10	10.2 ± 0.1	2.3
April 19, 2013 (Carboy)	May 24, 2013	0	BLOQ	NA
		1	1.03 ± 0.0	3.0
		3	3.03 ± 0.01	1.1
		10	10.3 ± 0.1	2.7
June 4, 2013 (Drinking Water Bottle)	July 9, 2013	0	BLOQ	NA
		1	0.998 ± 0.002	–0.2
		3	3.03 ± 0.01	1.1
		10	10.2 ± 0.1	1.7
June 4, 2013 (Carboy)	July 9, 2013	0	BLOQ	NA
		1	0.986 ± 0.009	–1.4
		3	2.99 ± 0.01	−0.2
		10	10.0 ± 0.1	0.4
July 15, 2013 (Drinking Water Bottle)	August 9, 2013	0	BLOQ	NA
		1	0.996 ± 0.007	−0.4
		3	2.95 ± 0.01	−1.5
		10	10.2 ± 0.0	2.0

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[a,b]	Difference from Target (%)
July 15, 2013 (Carboy)	August 9, 2013	0	BLOQ	NA
		1	1.01 ± 0.01	1.0
		3	2.98 ± 0.02	−0.7
		10	10.2 ± 0.1	1.7

BLOQ = below the limit of quantification; NA = not applicable.
[a]Average and standard deviation of triplicate analysis.
[b]The limit of quantification of the analytical method, estimated as 10 times the standard deviation of the lowest vehicle standard, was 0.001 mg/mL.

Table A-7. Results of Analyses of Dose Formulations Administered to Male and Female Mice in the Three-month Drinking Water Study of 1-Ethyl-3-Methylimidazolium Chloride

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[a,b]	Difference from Target (%)
April 19, 2013	April 19, 2013	0	BLOQ	NA
		3	2.97 ± 0.01	−0.9
		10	10.2 ± 0.1	2.3
		30	30.4 ± 0.1	1.4
June 4, 2013	June 6, 2013	0	BLOQ	NA
		3	3.04 ± 0.00	1.3
		10	10.0 ± 0.1	0.3
		30	30.2 ± 0.2	0.7
July 15, 2013	July 16, 2013	0	BLOQ	NA
		3	2.93 ± 0.01	−2.5
		10	10.1 ± 0.1	0.7
		30	30.0 ± 0.1	0
Animal Room Samples				
April 19, 2013 (Drinking Water Bottle)	May 24, 2013	0	BLOQ	NA
		3	3.04 ± 0.00	1.3
		10	10.2 ± 0.1	1.7
		30	31.3 ± 0.1	4.2
April 19, 2013 (Carboy)	May 24, 2013	0	BLOQ	NA
		3	3.03 ± 0.01	1.1
		10	10.3 ± 0.1	2.7
		30	31.1 ± 0.1	3.7
June 4, 2013 (Drinking Water Bottle)	July 9, 2013	0	BLOQ	NA
		3	2.99 ± 0.01	−0.5
		10	10.1 ± 0.1	0.7
		30	29.6 ± 0.2	−1.4

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[a,b]	Difference from Target (%)
June 4, 2013 (Carboy)	July 9, 2013	0	BLOQ	NA
		3	2.99 ± 0.01	−0.2
		10	10.0 ± 0.1	0.4
		30	29.3 ± 0.1	−2.3
July 15, 2013 (Drinking Water Bottle)	August 9, 2013	0	BLOQ	NA
		3	2.93 ± 0.01	−2.2
		10	10.2 ± 0.0	2
		30	30.2 ± 0.2	0.6
July 15, 2013 (Carboy)	August 9, 2013	0	BLOQ	NA
		3	2.98 ± 0.02	−0.7
		10	10.2 ± 0.1	1.7
		30	30.1 ± 0.0	0.3

BLOQ = below the limit of quantification; NA = not applicable.
[a]Average and standard deviation of triplicate analysis.
[b]The limit of quantification of the analytical method, estimated as 10 times the standard deviation of the lowest vehicle standard, was 0.001 mg/mL.

Table A-8. Results of Analyses of Dose Formulations Administered to Male and Female Rats and Mice in the Two-week Drinking Water Studies of 1-Butyl-3-Methylimidazolium Chloride

Date Prepared	Date Analyzed[a]	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[b]	Difference from Target (%)
February 25, 2009	February 25, 2009	0.3	0.3 ± 0.0	5.1
		1	1.0 ± 0.0	1.8
		3	3.1 ± 0.0	3.0
		6	6.2 ± 0.1	3.1
Animal Room Samples (Rats)[c]				
February 25, 2009	April 6, 2009	0.3	0.3 ± 0.0	2.8
		1	1.0 ± 0.0	0.0
		3	3.0 ± 0.0	1.0
		6	NA	NA
Animal Room Samples (Mice)[c]				
February 25, 2009	April 6, 2009	0.3	NA	NA
		1	1.0 ± 0.0	−0.3
		3	3.0 ± 0.0	0.9
		6	5.9 ± 0.2	−2.1

NA = not applicable.
[a]Analysis dates assumed from laboratory report dates. Preadministration formulations were analyzed in triplicate within 1 week of preparation.
[b]Average and standard deviation of all sample measurements.
[c]Preadministration samples were taken from carboys. Postadministration samples were taken from carboys and drinking water bottles.

Table A-9. Results of Analyses of Dose Formulations Administered to Male and Female Rats in the Three-month Drinking Water Study of 1-Butyl-3-Methylimidazolium Chloride

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[a,b]	Difference from Target (%)
April 15, 2013	April 16, 2013	0	BLOQ	NA
		0.1	0.0989 ± 0.0003	−1.1
		0.3	0.306 ± 0.000	2.0
		1	0.999 ± 0.001	−0.1
May 6, 2013	May 7, 2013	0	BLOQ	NA
		0.1	0.0981 ± 0.0006	−1.9
		0.3	0.302 ± 0.000	0.7
		1	1.02 ± 0.00	2.0
June 17, 2013	June 17, 2013	0	BLOQ	NA
		0.1	0.102 ± 0.001	1.7
		0.3	0.307 ± 0.001	2.2
		1	1.03 ± 0.01	3.3
Animal Room Samples				
April 15, 2013 (Drinking Water Bottle)	May 17, 2013	0	BLOQ	NA
		0.1	0.0991 ± 0.0001	−0.9
		0.3	0.312 ± 0.000	4.0
		1	1.02 ± 0.00	2.0
April 15, 2013 (Carboy)	May 17, 2013	0	BLOQ	NA
		0.1	0.0993 ± 0.0002	−0.7
		0.3	0.311 ± 0.001	3.5
		1	1.02 ± 0.01	1.7
May 6, 2013 (Drinking Water Bottle)	June 7, 2013	0	BLOQ	NA
		0.1	0.0953 ± 0.0005	−4.7
		0.3	0.301 ± 0.001	0.2
		1	0.997 ± 0.001	−0.3
May 6, 2013 (Carboy)	June 7, 2013	0	BLOQ	NA
		0.1	0.0951 ± 0.0003	−4.9
		0.3	0.300 ± 0.000	0.0
		1	0.994 ± 0.002	−0.6
June 17, 2013 (Drinking Water Bottle)	July 26, 2013	0	BLOQ	NA
		0.1	0.108 ± 0.001	7.7
		0.3	0.329 ± 0.003	9.6
		1	1.08 ± 0.01	8.3

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[a,b]	Difference from Target (%)
June 17, 2013 (Carboy)	July 26, 2013	0	BLOQ	NA
		0.1	0.106 ± 0.001	6.3
		0.3	0.325 ± 0.001	8.5
		1	1.08 ± 0.00	8.0

BLOQ = below the limit of quantification; NA = not applicable.
[a]Average and standard deviation of triplicate analysis.
[b]The limit of quantification of the analytical method, estimated as 10 times the standard deviation of the lowest vehicle standard, was 0.003 mg/mL.

Table A-10. Results of Analyses of Dose Formulations Administered to Male and Female Mice in the Three-month Drinking Water Study of 1-Butyl-3-Methylimidazolium Chloride

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[a,b]	Difference from Target (%)
April 15, 2013	April 16, 2013	0	BLOQ	NA
		0.3	0.306 ± 0.000	2.0
		1	0.999 ± 0.001	−0.1
		3	2.92 ± 0.00	−2.7
May 6, 2013	May 7, 2013	0	BLOQ	NA
		0.3	0.302 ± 0.000	0.7
		1	1.02 ± 0.00	2.0
		3	3.06 ± 0.0	2.0
June 17, 2013	June 17, 2013	0	BLOQ	NA
		0.3	0.307 ± 0.001	2.2
		1	1.03 ± 0.01	3.3
		3	3.14 ± 0.00	4.7
Animal Room Samples				
April 15, 2013 (Drinking Water Bottle)	May 17, 2013	0	BLOQ	NA
		0.3	0.309 ± 0.001	2.9
		1	1.02 ± 0.00	2.0
		3	2.97 ± 0.01	−0.9
April 15, 2013 (Carboy)	May 17, 2013	0	BLOQ	NA
		0.3	0.311 ± 0.001	3.5
		1	1.02 ± 0.01	1.7
		3	2.98 ± 0.00	−0.7
May 6, 2013 (Drinking Water Bottle)	June 7, 2013	0	BLOQ	NA
		0.3	0.304 ± 0.000	1.3
		1	1.00 ± 0.00	0.0
		3	2.99 ± 0.01	−0.5

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[a,b]	Difference from Target (%)
May 6, 2013 (Carboy)	June 7, 2013	0	BLOQ	NA
		0.3	0.300 ± 0.000	0.0
		1	0.994 ± 0.002	−0.6
		3	2.98 ± 0.00	−0.7
June 17, 2013 (Drinking Water Bottle)	July 26, 2013	0	BLOQ	NA
		0.3	0.306 ± 0.000	2.0
		1	1.02 ± 0.00	2.0
		3	3.11 ± 0.01	3.5
June 17, 2013 (Carboy)	July 26, 2013	0	BLOQ	NA
		0.3	0.325 ± 0.001	8.5
		1	1.08 ± 0.00	8.0
		3	3.12 ± 0.00	4.0

BLOQ = below the limit of quantification; NA = not applicable.
[a]Average and standard deviation of triplicate analysis.
[b]The limit of quantification of the analytical method, estimated as 10 times the standard deviation of the lowest vehicle standard, was 0.003 mg/mL.

Table A-11. Results of Analyses of Dose Formulations Administered to Male and Female Rats and Mice in the Two-week Drinking Water Studies of 1-Butyl-1-Methylpyrrolidinium Chloride

Date Prepared	Date Analyzed[a]	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[b]	Difference from Target (%)
February 24, 2009	March 2, 2009	1	1.1 ± 0.0	5.6
		3	3.3 ± 0.0	10.0
		6	6.4 ± 0.1	7.1
		10	10.3 ± 0.1	3.2
Animal Room Samples (Rats)[c]				
February 24, 2009	April 6, 2009	1	1.1 ± 0.0	5.6
		3	3.3 ± 0.0	9.4
		6	6.5 ± 0.1	8.1
		10	NA	NA
Animal Room Samples (Mice)[c]				
February 24, 2009	April 6, 2009	1	NA	NA
		3	3.3 ± 0.0	9.1
		6	6.3 ± 0.1	5.6
		10	10.2 ± 0.1	2.2

NA = not applicable.
[a]Analysis dates assumed from laboratory report dates. Preadministration formulations were analyzed in triplicate within 1 week of preparation.
[b]Average and standard deviation of all sample measurements.
[c]Preadministration samples were taken from carboys. Postadministration samples were taken from carboys and drinking water bottles.

Table A-12. Results of Analyses of Dose Formulations Administered to Male and Female Rats in the Three-month Drinking Water Study of 1-Butyl-1-Methylpyrrolidinium Chloride

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[a,b]	Difference from Target (%)
August 5, 2013	August 7, 2013	0	BLOQ	NA
		0.3	0.292 ± 0.003	−2.7
		1	1.05 ± 0.01	5.3
		3	3.08 ± 0.01	2.5
		6	6.09 ± 0.08	1.5
September 25, 2013	September 26, 2013	0	BLOQ	NA
		0.3	0.297 ± 0.003	−0.9
		1	1.03 ± 0.00	3.0
		3	3.14 ± 0.02	4.6
		6	6.07 ± 0.01	1.2
October 18, 2013	October 21, 2013	0	BLOQ	NA
		0.3	0.293 ± 0.011	−2.2
		1	1.02 ± 0.00	2.0
		3	3.06 ± 0.05	2.1
		6	6.05 ± 0.09	0.8
Animal Room Samples				
August 5, 2013 (Drinking Water Bottle)	September 13, 2013	0	BLOQ	NA
		0.3	0.299 ± 0.001	−0.5
		1	1.03 ± 0.00	3.0
		3	2.96 ± 0.02	−1.2
		6	5.97 ± 0.03	−0.5
August 5, 2013 (Carboy)	September 13, 2013	0	BLOQ	NA
		0.3	0.301 ± 0.003	0.2
		1	1.04 ± 0.01	4.3
		3	3.01 ± 0.03	0.3
		6	5.86 ± 0.08	−2.4
September 25, 2013 (Drinking Water Bottle)	November 4, 2013	0	BLOQ	NA
		0.3	0.342 ± 0.002	14
		1	1.08 ± 0.01	7.7
		3	3.13 ± 0.02	4.4
		6	6.30 ± 0.02	5.0
September 25, 2013 (Carboy)	November 4, 2013	0	BLOQ	NA
		0.3	0.347 ± 0.001	15.8

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[a,b]	Difference from Target (%)
		1	1.06 ± 0.01	6.3
		3	3.15 ± 0.04	5.1
		6	6.31 ± 0.02	5.2
October 18, 2013 (Drinking Water Bottle)	November 22, 2013	0	BLOQ	NA
		0.3	0.317 ± 0.001	5.8
		1	1.09 ± 0.00	9.0
		3	3.18 ± 0.05	5.9
		6	6.57 ± 0.03	9.6
October 18, 2013 (Carboy)	November 22, 2013	0	BLOQ	NA
		0.3	0.321 ± 0.006	7.1
		1	1.11 ± 0.01	10.7
		3	3.24 ± 0.03	7.9
		6	6.55 ± 0.04	9.1

BLOQ = below the limit of quantification; NA = not applicable.
[a]Average and standard deviation of triplicate analysis.
[b]The limit of quantification of the analytical method, estimated as 10 times the standard deviation of the lowest vehicle standard, was 0.002 mg/mL.

Table A-13. Results of Analyses of Dose Formulations Administered to Male and Female Mice in the Three-month Drinking Water Study of 1-Butyl-1-Methylpyrrolidinium Chloride

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[a,b]	Difference from Target (%)
August 5, 2013	August 7, 2013	0	BLOQ	NA
		1	1.05 ± 0.01	5.3
		3	3.08 ± 0.01	2.5
		6	6.09 ± 0.08	1.5
		10	10.5 ± 0.2	5.3
September 25, 2013	September 26, 2013	0	BLOQ	NA
		1	1.03 ± 0.00	3.0
		3	3.14 ± 0.02	4.6
		6	6.07 ± 0.01	1.2
		10	10.7 ± 0.1	6.7
October 18, 2013	October 21, 2013	0	BLOQ	NA
		1	1.02 ± 0.00	2.0
		3	3.06 ± 0.05	2.1
		6	6.05 ± 0.09	0.8
		10	10.6 ± 0.1	6.3

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[a,b]	Difference from Target (%)
Animal Room Samples				
August 5, 2013 (Drinking Water Bottle)	September 13, 2013	0	BLOQ	NA
		1	1.02 ± 0.01	2.3
		3	2.97 ± 0.01	−1.0
		6	6.06 ± 0.07	0.9
		10	10.4 ± 0.0	4.0
August 5, 2013 (Carboy)	September 13, 2013	0	BLOQ	NA
		1	1.04 ± 0.01	4.3
		3	3.01 ± 0.03	0.3
		6	5.86 ± 0.08	−2.4
		10	10.2 ± 0.1	2.3
September 25, 2013 (Drinking Water Bottle)	November 4, 2013	0	BLOQ	NA
		1	1.08 ± 0.01	8.0
		3	3.15 ± 0.02	5.1
		6	6.19 ± 0.07	3.2
		10	11.2 ± 0.1	11.7
September 25, 2013 (Carboy)	November 4, 2013	0	BLOQ	NA
		1	1.06 ± 0.01	6.3
		3	3.15 ± 0.04	5.1
		6	6.31 ± 0.02	5.2
		10	11.4 ± 0.1	13.7
October 18, 2013 (Drinking Water Bottle)	November 22, 2013	0	BLOQ	NA
		1	1.07 ± 0.01	7.3
		3	3.11 ± 0.06	3.7
		6	6.36 ± 0.05	5.9
		10	11.2 ± 0.1	12.3
October 18, 2013 (Carboy)	November 22, 2013	0	BLOQ	NA
		1	1.11 ± 0.01	10.7
		3	3.24 ± 0.03	7.9
		6	6.55 ± 0.04	9.1
		10	11.4 ± 0.1	14.3

BLOQ = below the limit of quantification; NA = not applicable.
[a]Average and standard deviation of triplicate analysis.
[b]The limit of quantification of the analytical method, estimated as 10 times the standard deviation of the lowest vehicle standard, was 0.002 mg/mL.

Table A-14. Results of Analyses of Dose Formulations Administered to Male and Female Rats and Mice in the Two-week Drinking Water Studies of N-Butylpyridinium Chloride

Date Prepared	Date Analyzed[a]	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[b,c]	Difference from Target (%)
February 23, 2009	February 23, 2009	0	BLOQ	NA
		1	1.0 ± 0.0	1.6
		3	3.0 ± 0.0	−0.9
		6	6.0 ± 0.1	−0.3
Animal Room Samples (Rats)[c]				
February 23, 2009	April 2, 2009	0	BLOQ	NA
		1	1.0 ± 0.0	0.0
		3	3.0 ± 0.0	–0.3
		6	6.0 ± 0.0	0.0
Animal Room Samples (Mice)[d]				
February 23, 2009	April 2, 2009	0	BLOQ	NA
		1	1.0 ± 0.0	−0.4
		3	3.0 ± 0.0	–0.2
		6	6.0 ± 0.0	0.2

BLOQ = below the limit of quantification; NA = not applicable.
[a]Analysis dates assumed from laboratory report dates. Preadministration formulations were analyzed in triplicate within 1 week of preparation.
[b]Average and standard deviation of all sample measurements.
[c]The limit of quantification of the analytical method, estimated as 10 times the standard deviation of the lowest vehicle standard, was 0.03080 mg/mL.
[d]Preadministration samples were taken from carboys. Postadministration samples were taken from carboys and drinking water bottles.

Table A-15. Results of Analyses of Dose Formulations Administered to Male and Female Rats in the Three-month Drinking Water Study of N-Butylpyridinium Chloride

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[a,b]	Difference from Target (%)
September 3, 2013	September 3, 2013	0	BLOQ	NA
		0.3	0.297 ± 0.001	−1.0
		1	1.02 ± 0.01	1.7
		3	3.09 ± 0.02	2.9
October 11, 2013	October 14, 2013	0	BLOQ	NA
		0.3	0.310 ± 0.002	3.2
		1	1.07 ± 0.00	7.0
		3	3.04 ± 0.02	1.4

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[a,b]	Difference from Target (%)
November 25, 2013	December 2, 2013	0	BLOQ	NA
		0.3	0.300 ± 0.005	0.1
		1	1.01 ± 0.01	0.7
	December 3, 2013	3	3.04 ± 0.02	1.5
Animal Room Samples				
September 3, 2013 (Drinking Water Bottle)	October 4, 2013	0	BLOQ	NA
		0.3	0.305 ± 0.002	1.6
		1	1.02 ± 0.01	2.3
		3	3.06 ± 0.02	2.1
September 3, 2013 (Carboy)	October 4, 2013	0	BLOQ	NA
		0.3	0.306 ± 0.002	2.1
		1	1.03 ± 0.00	3.0
		3	3.07 ± 0.02	2.5
October 11, 2013 (Drinking Water Bottle)	November 6, 2013	0	BLOQ	NA
		0.3	0.300 ± 0.001	0.0
		1	1.03 ± 0.01	2.7
		3	3.07 ± 0.00	2.3
October 11, 2013 (Carboy)	November 6, 2013	0	BLOQ	NA
		0.3	0.301 ± 0.002	0.2
		1	1.05 ± 0.02	4.7
		3	3.09 ± 0.04	2.9
November 25, 2013 (Drinking Water Bottle)	December 19, 2013	0	BLOQ	NA
		0.3	0.328 ± 0.004	9.4
		1	1.03 ± 0.00	3.0
		3	3.07 ± 0.02	2.3
November 25, 2013 (Carboy)	December 19, 2013	0	BLOQ	NA
		0.3	0.339 ± 0.001	13.1
		1	1.03 ± 0.00	3.0
		3	3.05 ± 0.01	1.6

BLOQ = below the limit of quantification; NA = not applicable.
[a]Average and standard deviation of triplicate analysis.
[b]The limit of quantification of the analytical method, estimated as 10 times the standard deviation of the lowest vehicle standard, was 0.00130 mg/mL.

Table A-16. Results of Analyses of Dose Formulations Administered to Male and Female Mice in the Three-month Drinking Water Study of N-Butylpyridinium Chloride

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[a,b]	Difference from Target (%)
September 3, 2013	September 3, 2013	0	BLOQ	NA
		1	1.02 ± 0.01	1.7
		3	3.09 ± 0.02	2.9
		6	6.23 ± 0.03	3.8
October 11, 2013	October 14, 2013	0	BLOQ	NA
		1	1.07 ± 0.00	7.0
		3	3.04 ± 0.02	1.4
		6	6.06 ± 0.01	1.1
November 25, 2013	December 2, 2013	0	BLOQ	NA
		1	1.01 ± 0.01	0.7
	December 3, 2013	3	3.04 ± 0.02	1.5
		6	5.89 ± 0.25	−1.9
Animal Room Samples				
September 3, 2013 (Drinking Water Bottle)	October 4, 2013	0	BLOQ	NA
		1	1.02 ± 0.00	2.0
		3	3.03 ± 0.08	1.0
		6	6.21 ± 0.04	3.5
September 3, 2013 (Carboy)	October 4, 2013	0	BLOQ	NA
		1	1.03 ± 0.00	3.0
		3	3.07 ± 0.02	2.5
		6	6.27 ± 0.01	4.6
October 11, 2013 (Drinking Water Bottle)	November 6, 2013	0	BLOQ	NA
		1	1.03 ± 0.0l	3.3
		3	3.11 ± 0.02	3.8
		6	6.16 ± 0.03	2.7
October 11, 2013 (Carboy)	November 6, 2013	0	BLOQ	NA
		1	1.05 ± 0.02	4.7
		3	3.09 ± 0.04	2.9
		6	6.13 ± 0.04	2.2
November 25, 2013 (Drinking Water Bottle)	December 19, 2013	0	BLOQ	NA
		1	1.01 ± 0.00	1.0
		3	3.01 ± 0.01	0.4
		6	6.00 ± 0.03	−0.1

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)[a,b]	Difference from Target (%)
November 25, 2013 (Carboy)	December 19, 2013	0	BLOQ	NA
		1	1.03 ± 0.00	3.0
		3	3.05 ± 0.01	1.6
		6	6.03 ± 0.07	0.4

BLOQ = below the limit of quantification; NA = not applicable.
[a]Average and standard deviation of triplicate analysis.
[b]The limit of quantification of the analytical method, estimated as 10 times the standard deviation of the lowest vehicle standard, was 0.00130 mg/mL.

Table A-17. Results of pH Analyses of Dose Formulations Administered to Male and Female Rats and Mice in the Two-week Drinking Water Studies of 1-Ethyl-3-Methylimidazolium Chloride

Target Concentration (mg/mL)	Species	Precipitate Observed in One or More Samples	Average pH Across Measured Samples[a]
3	Rats	No	8.7 ± 0.3
10	Rats	No	8.1 ± 0.0
30	Rats	No	7.3 ± 0.4
100	Rats	No	7.1 ± 0.0
3	Mice	No	8.3 ± 0.3
10	Mice	No	7.9 ± 0.1
30	Mice	No	7.7 ± 0.1
100	Mice	No	7.1 ± 0.0

[a]Average and standard deviation of triplicate analysis.

Table A-18. Results of pH Analyses of Dose Formulations Administered to Male and Female Rats and Mice in the Two-week Drinking Water Studies of 1-Butyl-1-Methylpyrrolidinium Chloride

Target Concentration (mg/mL)	Species	Precipitate Observed in One or More Samples	Average pH Across Measured Samples[a]
1	Rats	No	9.1 ± 0.5
3	Rats	No	8.4 ± 0.1
6	Rats	No	8.5 ± 0.3
3	Mice	Yes	8.3 ± 0.1
6	Mice	Yes	8.5 ± 0.1
10	Mice	Yes	8.9 ± 0.3

[a]Average and standard deviation of triplicate analysis.

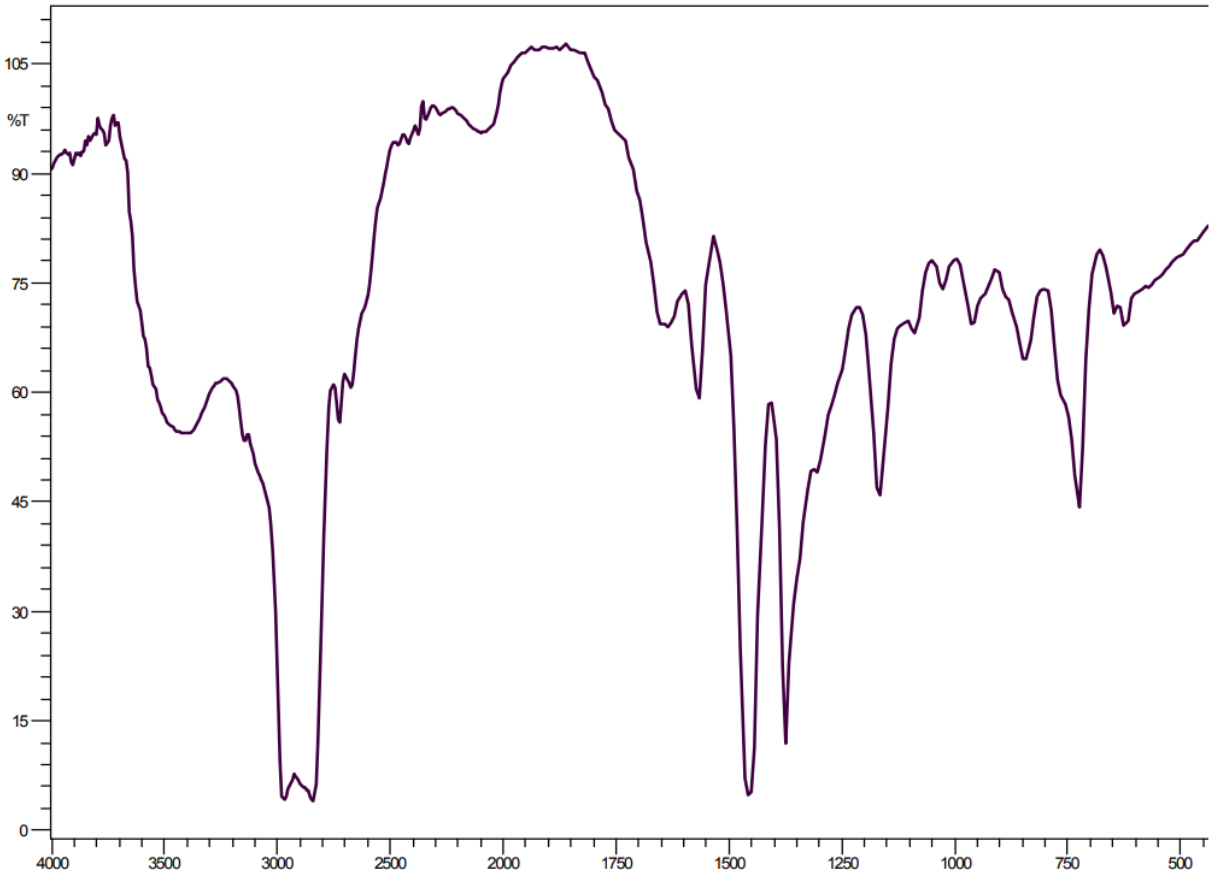


Figure A-1. Infrared Absorption Spectrum of 1-Ethyl-3-Methylimidazolium Chloride (Lot S37784), Nujol Mineral Oil

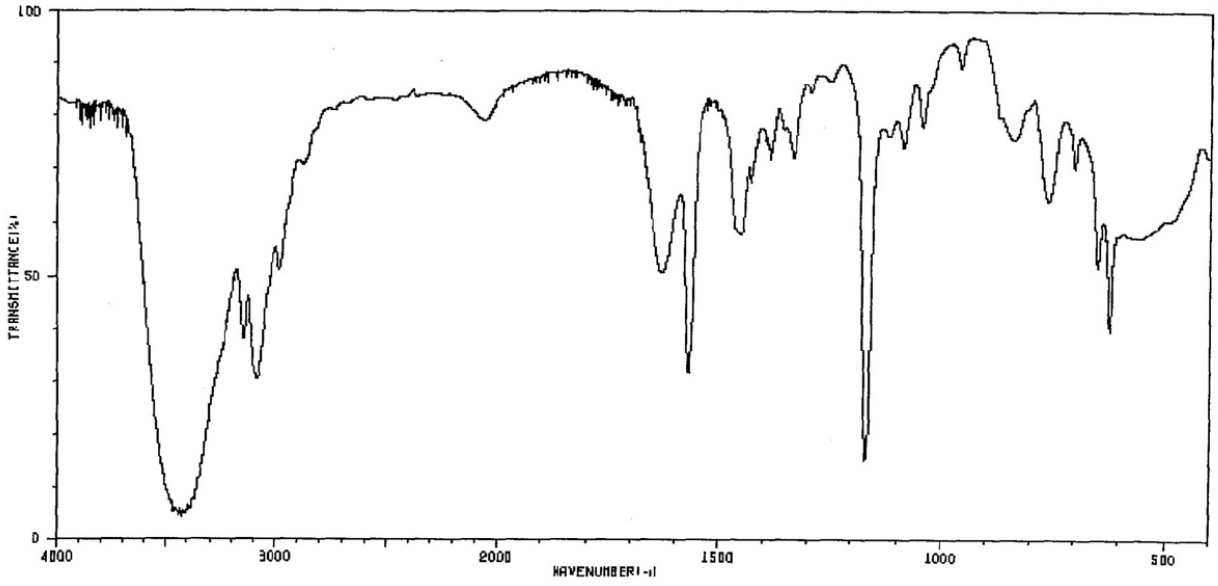


Figure A-2. Infrared Absorption Spectrum of 1-Ethyl-3-Methylimidazolium Chloride (Lot STBB3624), Neat

Assignments (δ ppm)		Multiplicity	Integration	
			Observed	Theoretical
(a)	1.54	triplet	3.24	3
(b)	3.93	singlet	3.24	3
(c)	4.27	quartet	2.17	2
(d)	7.47	singlet	1.87	2
(e)	7.54	singlet		
(f)	8.77	singlet	0.48	1

Note: The resonance observed at 4.8 δ ppm.

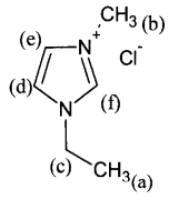


Figure A-3. ^{1}H Nuclear Magnetic Resonance Spectrum of 1-Ethyl-3-Methylimidazolium Chloride (Lot S37784)

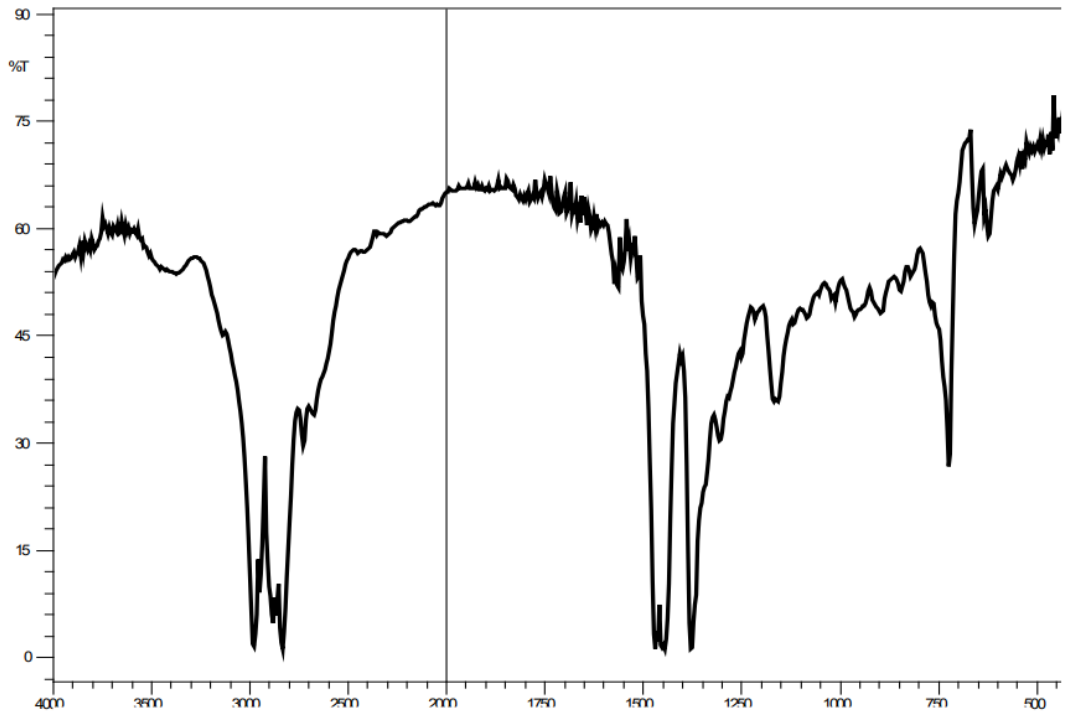


Figure A-4. Infrared Absorption Spectrum of 1-Butyl-3-Methylimidazolium Chloride (Lot STBB3444), Nujol Mineral Oil

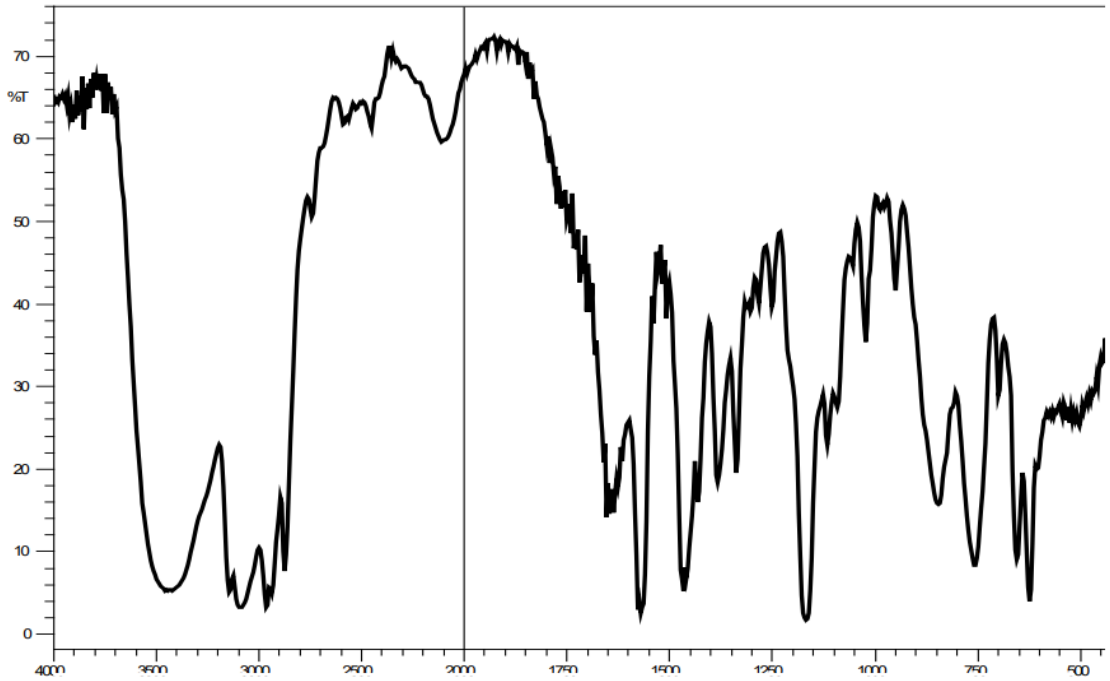
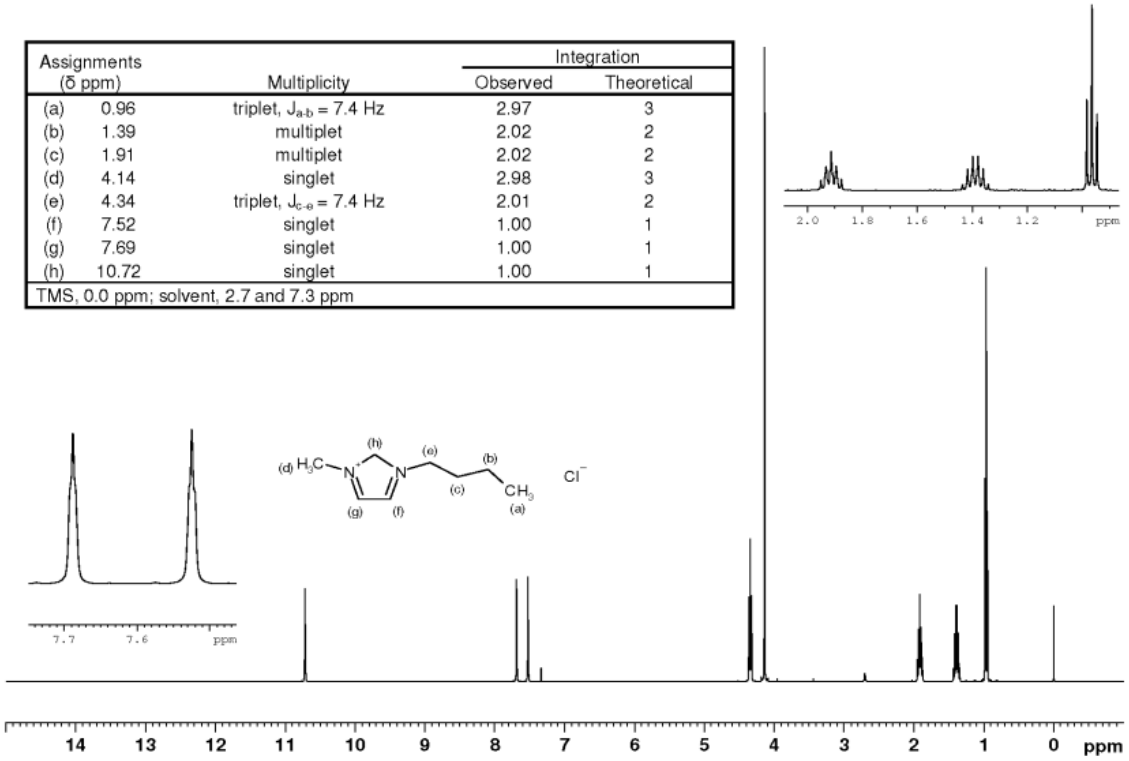


Figure A-5. Infrared Absorption Spectrum of 1-Butyl-3-Methylimidazolium Chloride (Lot STBB3444), Neat



Assignments (δ ppm)		Multiplicity	Integration Observed	Integration Theoretical
(a)	0.96	triplet, J_{a-b} = 7.4 Hz	2.97	3
(b)	1.39	multiplet	2.02	2
(c)	1.91	multiplet	2.02	2
(d)	4.14	singlet	2.98	3
(e)	4.34	triplet, J_{c-e} = 7.4 Hz	2.01	2
(f)	7.52	singlet	1.00	1
(g)	7.69	singlet	1.00	1
(h)	10.72	singlet	1.00	1

TMS, 0.0 ppm; solvent, 2.7 and 7.3 ppm

Figure A-6. ^{1}H Nuclear Magnetic Resonance Spectrum of 1-Butyl-3-Methylimidazolium Chloride (Lot STBB3444)

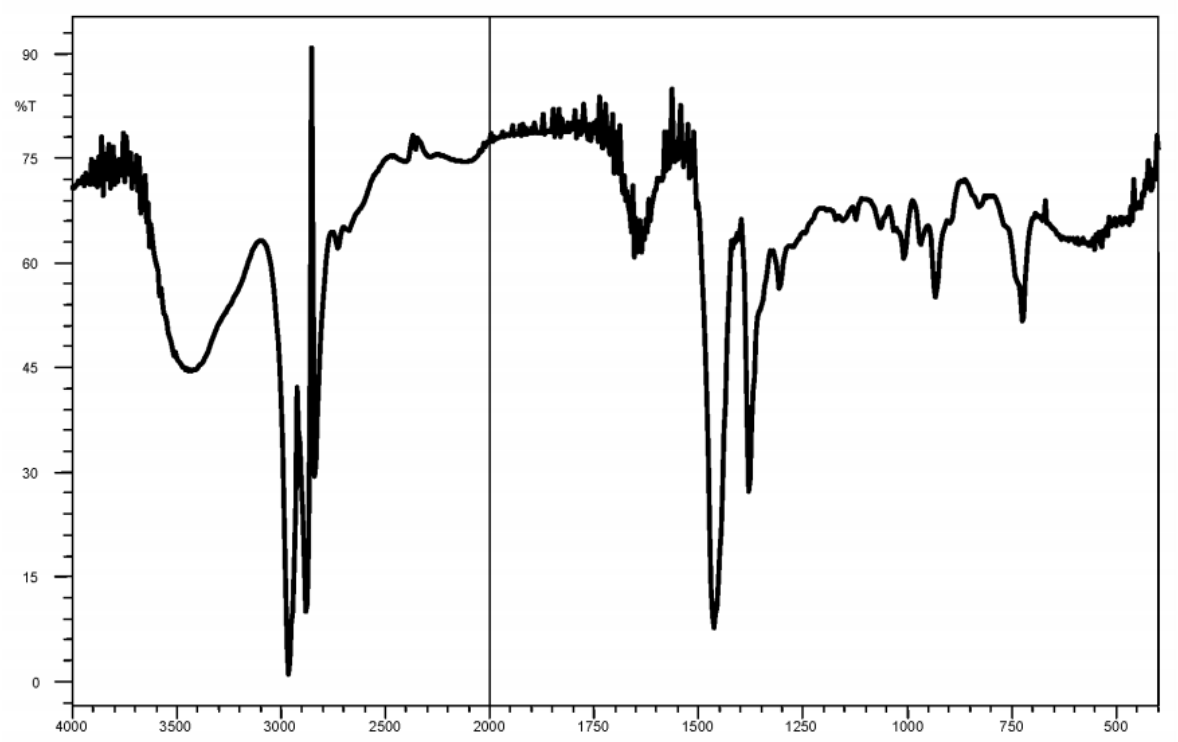


Figure A-7. Infrared Absorption Spectrum of 1-Butyl-1-Methylpyrrolidinium Chloride (Lot 20100610), Nujol Mineral Oil

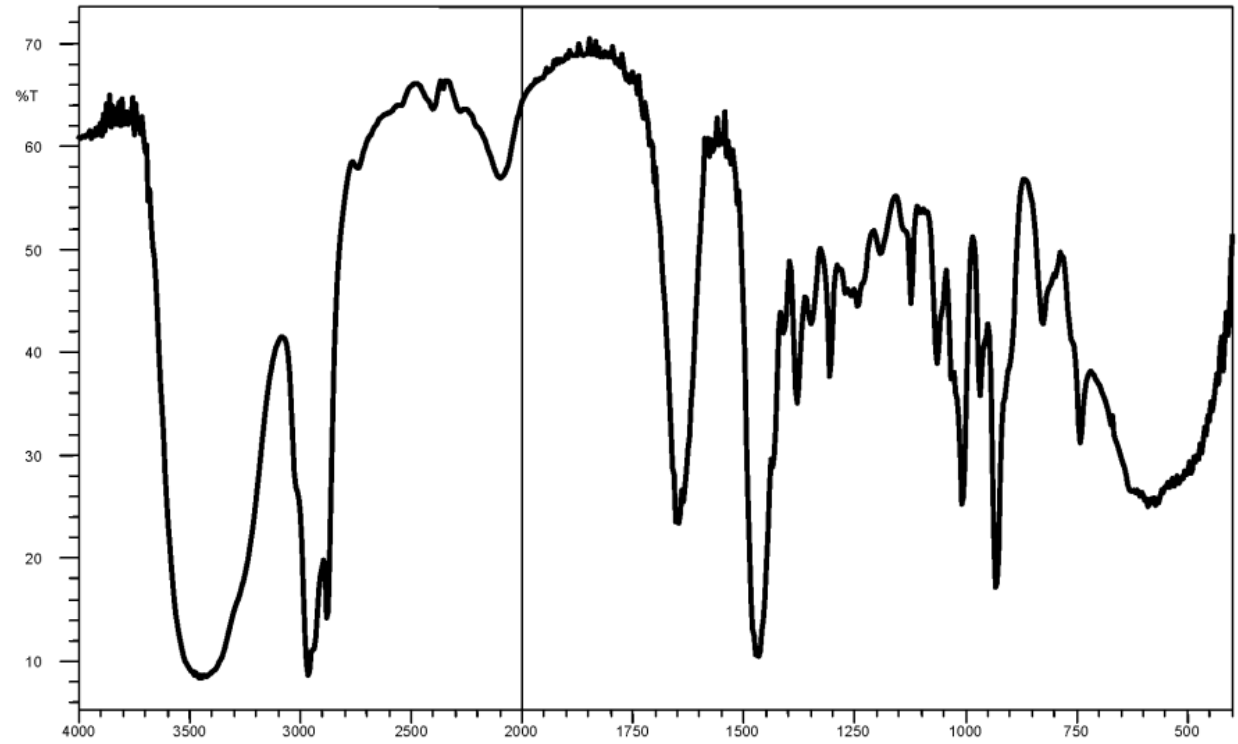


Figure A-8. Infrared Absorption Spectrum of 1-Butyl-1-Methylpyrrolidinium Chloride (Lot 20100610), Neat

Assignments (δ ppm)		Multiplicity	Integration Observed	Integration Theoretical
(a)	1.00	triplet, J_{a-b} = 7.4 Hz	2.96	3
(b)	1.46	multiplet	2.01	2
(c)	1.77	multiplet	2.00	2
(d)	2.30	multiplet	4.04	4
(e)	3.34	singlet	2.96	3
(f)	3.67	multiplet	1.98	2
(g)	3.86	multiplet	4.04	4
TMS, 0.0 ppm; solvent, 2.5 and 7.3 ppm				

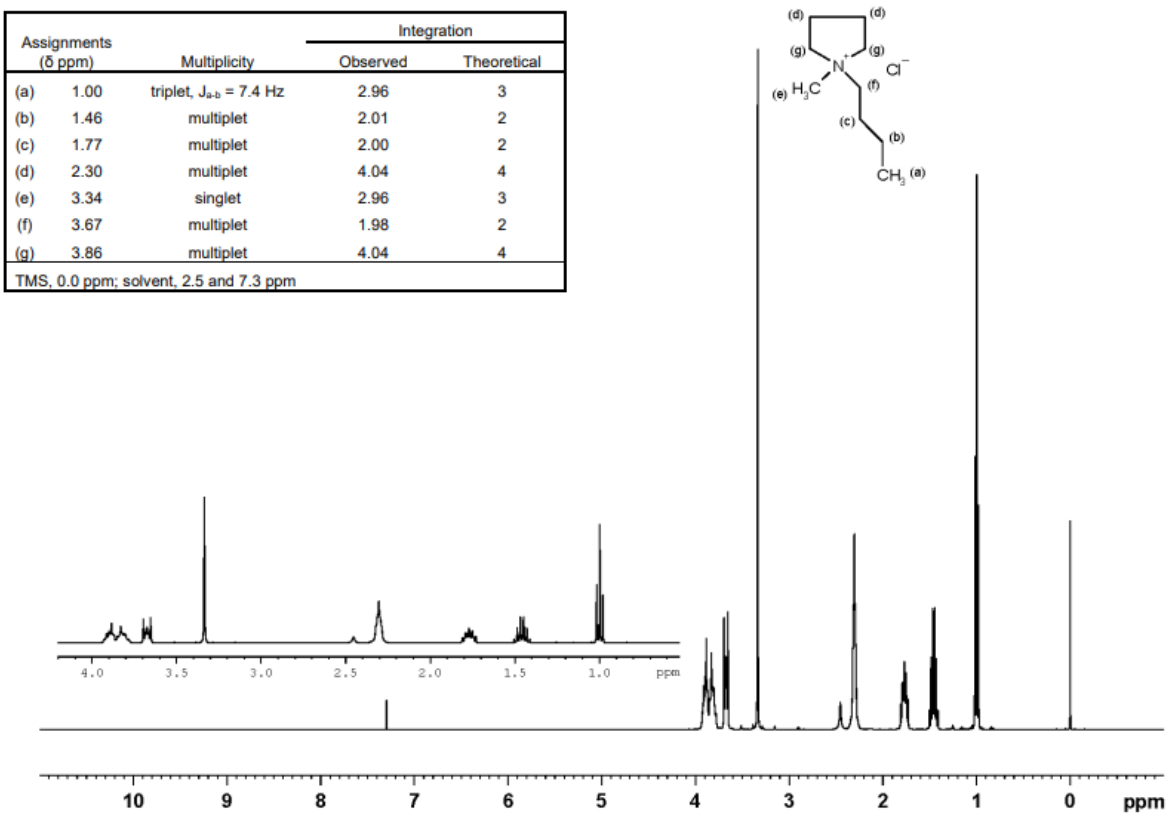


Figure A-9. ^{1}H Nuclear Magnetic Resonance Spectrum of 1-Butyl-1-Methylpyrrolidinium Chloride (Lot 20100610)

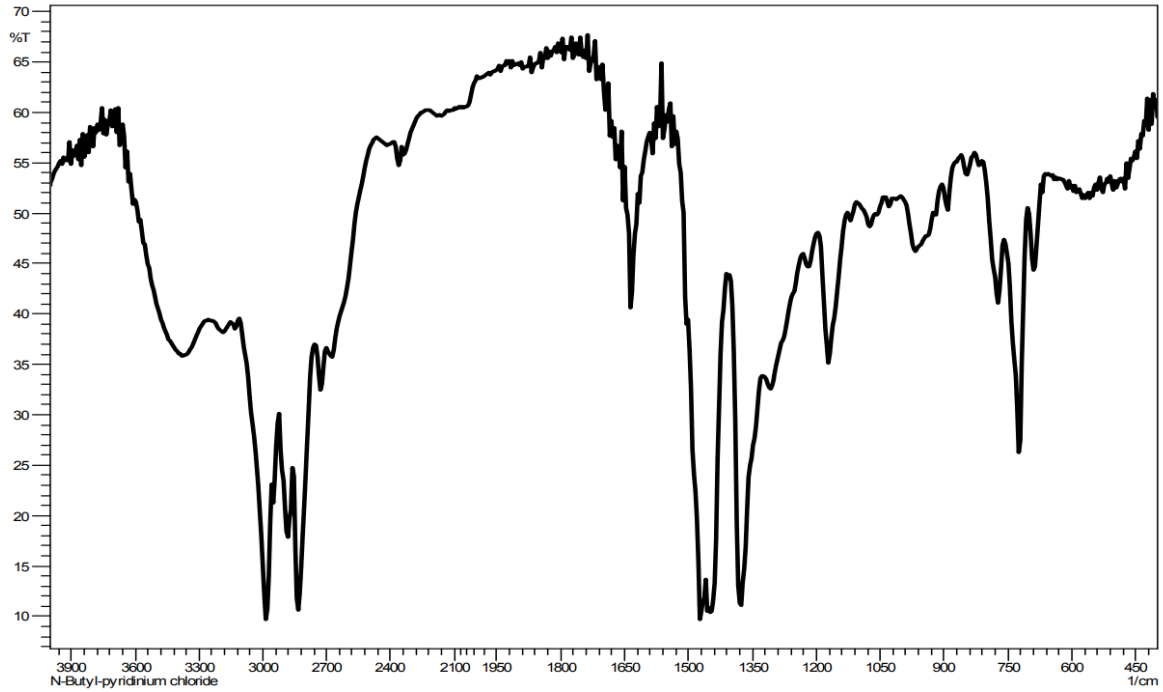


Figure A-10. Infrared Absorption Spectrum of N-Butylpyridinium Chloride (Lot 99/830), Nujol Mineral Oil

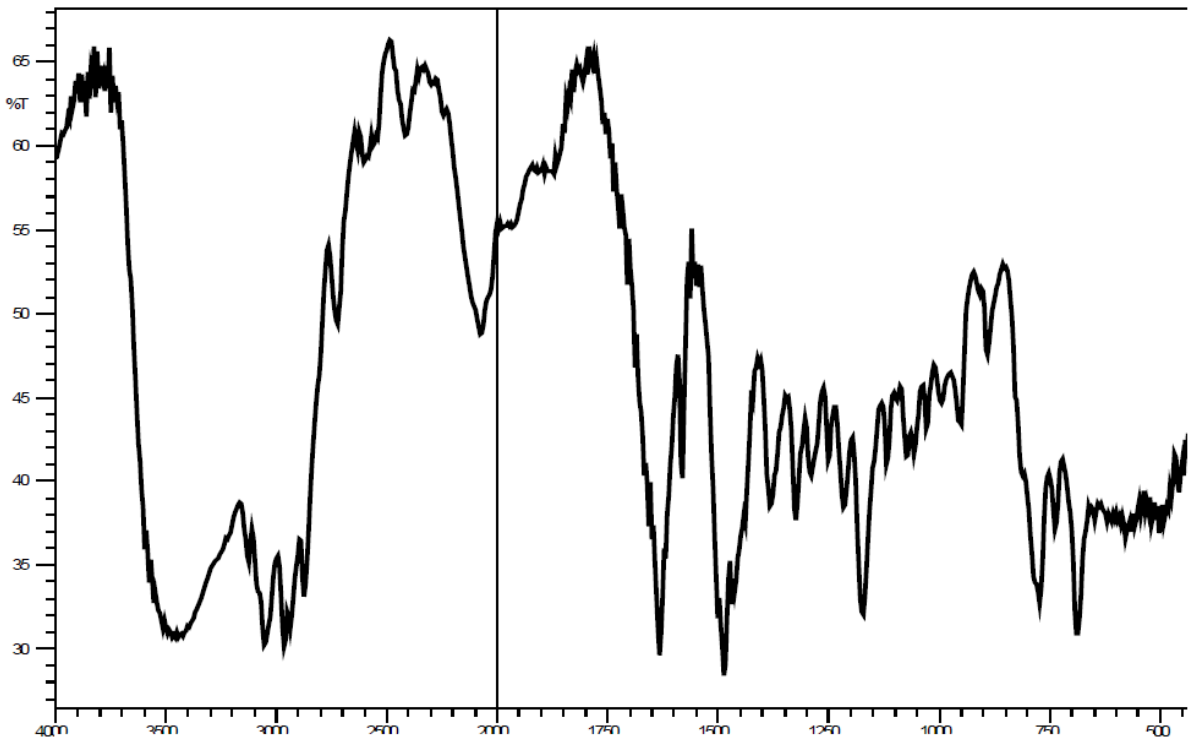


Figure A-11. Infrared Absorption Spectrum of N-Butylpyridinium Chloride (Lot 20100610), Neat

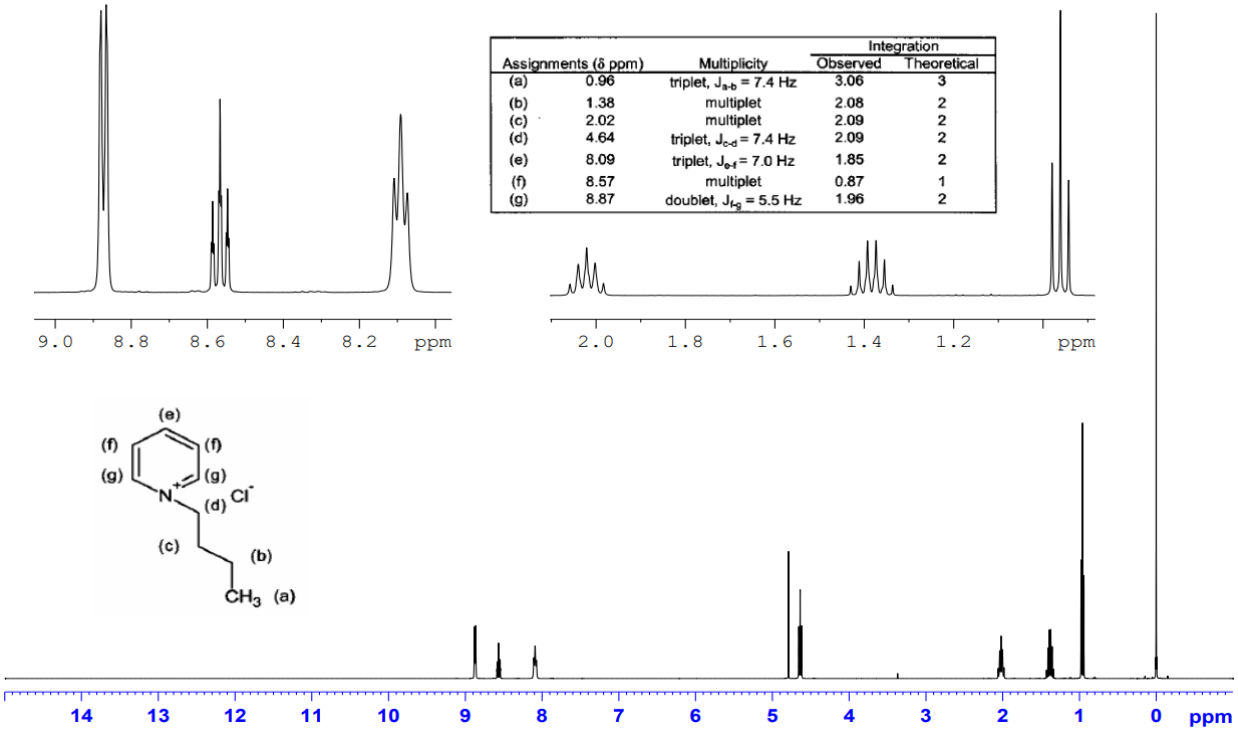


Figure A-12. ^{1}H Nuclear Magnetic Resonance Spectrum of N-Butylpyridinium Chloride (Lot 99/830)

Appendix B. Ingredients, Nutrient Composition, and Contaminant Levels in NTP-2000 Rat and Mouse Ration

Tables

Table B-1. Ingredients of NTP-2000 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground Hard Winter Wheat	23
Ground #2 Yellow Shelled Corn	22.44
Wheat Middlings	15
Oat Hulls	8.5
Alfalfa Meal (Dehydrated, 17% Protein)	7.5
Purified Cellulose	5.5
Soybean Meal (49% Protein)	4
Fish Meal (60% Protein)	4
Corn Oil (without Preservatives)	3
Soy Oil (without Preservatives)	3
Dried Brewer's Yeast	1
Calcium Carbonate (USP)	0.9
Vitamin Premix[a]	0.5
Mineral Premix[b]	0.5
Calcium Phosphate, Dibasic (USP)	0.4
Sodium Chloride	0.3
Choline Chloride (70% Choline)	0.26
Methionine	0.2

USP = United States Pharmacopeia.
[a]Wheat middlings as carrier.
[b]Calcium carbonate as carrier.

Table B-2. Vitamins and Minerals in NTP-2000 Rat and Mouse Ration

	Amount[a]	Source
Vitamins		
Vitamin A	4,000 IU	Stabilized vitamin A palmitate or acetate
Vitamin D	1,000 IU	D-activated animal sterol
Vitamin K	1.0 mg	Menadione sodium bisulfite complex
α-Tocopheryl Acetate	100 IU	–
Niacin	23 mg	–
Folic Acid	1.1 mg	–
d-Pantothenic Acid	10 mg	d-Calcium pantothenate
Riboflavin	3.3 mg	–
Thiamine	4 mg	Thiamine mononitrate
B_{12}	52 µg	–
Pyridoxine	6.3 mg	Pyridoxine hydrochloride

	Amount[a]	Source
Biotin	0.2 mg	d-Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

[a]Per kg of finished product.

Table B-3. Nutrient Composition of NTP-2000 Rat and Mouse Ration in the Two-week Studies

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by Weight)	13.74	–	1
Crude Fat (% by Weight)	7.8	–	1
Crude Fiber (% by Weight)	9.4	–	1
Ash (% by Weight)	5.2	–	1
Amino Acids (% of Total Diet)			
Arginine	0.805 ± 0.075	0.67–0.97	29
Cystine	0.220 ± 0.022	0.15–0.25	29
Glycine	0.702 ± 0.038	0.62–0.80	29
Histidine	0.342 ± 0.070	0.27–0.68	29
Isoleucine	0.549 ± 0.040	0.43–0.66	29
Leucine	1.097 ± 0.063	0.96–1.24	29
Lysine	0.700 ± 0.104	0.31–0.86	29
Methionine	0.409 ± 0.042	0.26–0.49	29
Phenylalanine	0.623 ± 0.047	0.47–0.72	29
Threonine	0.513 ± 0.041	0.43–0.61	29
Tryptophan	0.155 ± 0.027	0.11–0.20	29
Tyrosine	0.422 ± 0.066	0.28–0.54	29
Valine	0.666 ± 0.040	0.55–0.73	29
Essential Fatty Acids (% of Total Diet)			
Linoleic	3.945 ± 0.235	3.49–4.55	29
Linolenic	0.297 ± 0.064	0.005–0.368	29

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Vitamins			
Vitamin A (IU/kg)	5,120		1
Vitamin D (IU/kg)[a]	1,000	–	–
α-Tocopherol (ppm)	2,455 ± 12,817	13.6–69,100	29
Thiamine (ppm)[b]	6.5	–	1
Riboflavin (ppm)	8.17 ± 2.84	4.2–17.5	29
Niacin (ppm)	78.66 ± 8.11	66.4–98.2	29
Pantothenic Acid (ppm)	26.42 ± 11.05	17.4–81.0	29
Pyridoxine (ppm)[b]	9.75 ± 2.05	6.44–14.3	29
Folic Acid (ppm)	1.58 ± 0.43	1.15–3.27	29
Biotin (ppm)	0.323 ± 0.093	0.2–0.704	29
B_{12} (ppb)	50.41 ± 34.9	18.3–174	29
Choline (as Chloride) (ppm)	2,593 ± 633	1,160–3,790	29
Minerals			
Calcium (%)	0.887	–	1
Phosphorus (%)	0.544	–	1
Potassium (%)	0.668 ± 0.029	0.626–0.733	29
Chloride (%)	0.392 ± 0.044	0.3–0.517	29
Sodium (%)	0.195 ± 0.027	0.16–0.283	29
Magnesium (%)	0.217 ± 0.054	0.185–0.49	29
Iron (ppm)	191.6 ± 36.18	135–311	29
Manganese (ppm)	50.11 ± 9.42	21.0–73.1	29
Zinc (ppm)	57.3 ± 25.55	43.3–184.0	29
Copper (ppm)	7.57 ± 2.49	3.21–16.3	29
Iodine (ppm)	0.513 ± 0.221	0–0.972	29
Chromium (ppm)	1.017 ± 1.038	0.33–3.97	29
Cobalt (ppm)	0.222 ± 0.152	0.0857–0.864	29

[a]From formulation.
[b]As hydrochloride.

Table B-4. Nutrient Composition of NTP-2000 Rat and Mouse Ration in the Three-month Studies of 1-Ethyl-3-Methylimidazolium Chloride

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by Weight)	14.53 ± 0.435	13.9–14.9	4
Crude Fat (% by Weight)	8.4 ± 0.082	8.3–8.5	4
Crude Fiber (% by Weight)	9.34 ± 0.572	8.6–9.97	4
Ash (% by Weight)	4.93 ± 0.149	4.79–5.14	4
Amino Acids (% of Total Diet)			
Arginine	0.805 ± 0.075	0.67–0.97	29
Cystine	0.220 ± 0.022	0.15–0.25	29
Glycine	0.702 ± 0.038	0.62–0.80	29
Histidine	0.342 ± 0.070	0.27–0.68	29
Isoleucine	0.549 ± 0.040	0.43–0.66	29
Leucine	1.097 ± 0.063	0.96–1.24	29
Lysine	0.700 ± 0.104	0.31–0.86	29
Methionine	0.409 ± 0.042	0.26–0.49	29
Phenylalanine	0.623 ± 0.047	0.471–0.72	29
Threonine	0.513 ± 0.041	0.43–0.61	29
Tryptophan	0.155 ± 0.027	0.11–0.2	29
Tyrosine	0.422 ± 0.066	0.28–0.54	29
Valine	0.666 ± 0.040	0.55–0.73	29
Essential Fatty Acids (% of Total Diet)			
Linoleic	3.94 ± 0.235	3.49–4.55	29
Linolenic	0.297 ± 0.064	0.005–0.368	29
Vitamins			
Vitamin A (IU/kg)	3,620 ± 63.48	2,820–4,140	4
Vitamin D (IU/kg)	1,000[a]	–	–
α-Tocopherol (ppm)	2,455 ± 12,817	13.6–69,100	29
Thiamine (ppm)[b]	7.2 ± 0.141	7.0–7.3	4
Riboflavin (ppm)	8.17 ± 2.84	4.2–17.5	29
Niacin (ppm)	78.66 ± 8.11	66.4–98.2	29
Pantothenic Acid (ppm)	26.42 ± 11.05	17.4–81.0	29
Pyridoxine (ppm)[b]	9.75 ± 2.05	6.44–14.3	29
Folic Acid (ppm)	1.58 ± 0.43	1.15–3.27	29
Biotin (ppm)	0.323 ± 0.093	0.2–0.704	29
B_{12} (ppb)	50.41 ± 34.9	18.3–174	29
Choline (as Chloride) (ppm)	2,593 ± 633	1,160–3,790	29

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Minerals			
Calcium (%)	0.904 ± 0.033	0.867–0.947	4
Phosphorus (%)	0.539 ± 0.020	0.51–0.553	4
Potassium (%)	0.668 ± 0.029	0.626–0.733	29
Chloride (%)	0.392 ± 0.044	0.3–0.517	29
Sodium (%)	0.195 ± 0.027	0.16–0.283	29
Magnesium (%)	0.217 ± 0.054	0.185–0.49	29
Iron (ppm)	191.6 ± 36.18	135–311	29
Manganese (ppm)	50.11 ± 9.42	21–73.1	29
Zinc (ppm)	57.3 ± 25.55	43.3–184	29
Copper (ppm)	7.57 ± 2.49	3.21–16.3	29
Iodine (ppm)	0.513 ± 0.221	0–0.972	29
Chromium (ppm)	1.017 ± 1.038	0.33–3.97	29
Cobalt (ppm)	0.222 ± 0.152	0.0857–0.864	29

[a]From formulation.
[b]As hydrochloride.

Table B-5. Nutrient Composition of NTP-2000 Rat and Mouse Ration in the Three-month Studies of 1-Butyl-3-Methylimidazolium Chloride

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by Weight)	14.43 ± 0.457	13.9–14.9	4
Crude Fat (% by Weight)	8.38 ± 0.096	8.3–8.5	4
Crude Fiber (% by Weight)	9.36 ± 0.570	8.6–9.97	4
Ash (% by Weight)	4.99 ± 0.117	4.88–5.14	4
Amino Acids (% of Total Diet)			
Arginine	0.805 ± 0.075	0.6–0.97	29
Cystine	0.220 ± 0.022	0.15–0.25	29
Glycine	0.702 ± 0.038	0.62–0.80	29
Histidine	0.342 ± 0.070	0.27–0.68	29
Isoleucine	0.549 ± 0.040	0.43–0.66	29
Leucine	1.097 ± 0.063	0.96–1.24	29
Lysine	0.700 ± 0.104	0.31–0.86	29
Methionine	0.409 ± 0.042	0.26–0.49	29
Phenylalanine	0.623 ± 0.047	0.471–0.72	29
Threonine	0.513 ± 0.041	0.43–0.61	29
Tryptophan	0.155 ± 0.027	0.11–0.2	29

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Tyrosine	0.422 ± 0.066	0.28–0.54	29
Valine	0.666 ± 0.040	0.55–0.73	29
Essential Fatty Acids (% of Total Diet)			
Linoleic	3.94 ± 0.235	3.49–4.55	29
Linolenic	0.297 ± 0.064	0.005–0.368	29
Vitamins			
Vitamin A (IU/kg)	3,585 ± 654	2,820–4,140	4
Vitamin D (IU/kg)[a]	1,000	–	–
α-Tocopherol (ppm)	2,455 ± 12,817	13.6–69,100	29
Thiamine (ppm)[b]	7.3 ± 0.082	7.2–7.4	4
Riboflavin (ppm)	8.17 ± 2.84	4.2–17.5	29
Niacin (ppm)	78.66 ± 8.11	66.4–98.2	29
Pantothenic Acid (ppm)	26.42 ± 11.05	17.4–81.0	29
Pyridoxine (ppm)[b]	9.75 ± 2.05	6.44–14.3	29
Folic Acid (ppm)	1.58 ± 0.43	1.15–3.27	29
Biotin (ppm)	0.323 ± 0.093	0.2–0.704	29
B_{12} (ppb)	50.41 ± 34.9	18.3–174	29
Choline (as Chloride) (ppm)	2,593 ± 633	1,160–3,790	29
Minerals			
Calcium (%)	0.891 ± 0.017	0.867–0.905	4
Phosphorus (%)	0.535 ± 0.018	0.51–0.53	4
Potassium (%)	0.668 ± 0.029	0.626–0.733	29
Chloride (%)	0.392 ± 0.044	0.3–0.517	29
Sodium (%)	0.195 ± 0.027	0.16–0.283	29
Magnesium (%)	0.217 ± 0.054	0.185–0.49	29
Iron (ppm)	191.6 ± 36.18	135–311	29
Manganese (ppm)	50.11 ± 9.42	21–73.1	29
Zinc (ppm)	57.3 ± 25.55	43.3–184	29
Copper (ppm)	7.57 ± 2.49	3.21–16.3	29
Iodine (ppm)	0.513 ± 0.221	0–0.972	29
Chromium (ppm)	1.017 ± 1.038	0.33–3.97	29
Cobalt (ppm)	0.222 ± 0.152	0.0857–0.864	29

[a]From formulation.
[b]As hydrochloride.

Table B-6. Nutrient Composition of NTP-2000 Rat and Mouse Ration in the Three-month Studies of 1-Butyl-1-Methylpyrrolidinium Chloride

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by Weight)	14.55 ± 0.532	13.9–15.2	4
Crude Fat (% by Weight)	8.28 ± 0.320	7.8–8.5	4
Crude Fiber (% by Weight)	9.48 ± 0.337	9.12–9.83	4
Ash (% by Weight)	4.88 ± 0.132	4.79–5.07	4
Amino Acids (% of Total Diet)			
Arginine	0.805 ± 0.075	0.67–0.97	29
Cystine	0.220 ± 0.022	0.15–0.25	29
Glycine	0.702 ± 0.038	0.62–0.80	29
Histidine	0.342 ± 0.070	0.27–0.68	29
Isoleucine	0.549 ± 0.040	0.43–0.66	29
Leucine	1.097 ± 0.063	0.96–1.24	29
Lysine	0.700 ± 0.104	0.31–0.86	29
Methionine	0.409 ± 0.042	0.26–0.49	29
Phenylalanine	0.623 ± 0.047	0.471–0.72	29
Threonine	0.513 ± 0.041	0.43–0.61	29
Tryptophan	0.155 ± 0.027	0.11–0.2	29
Tyrosine	0.422 ± 0.066	0.28–0.54	29
Valine	0.666 ± 0.040	0.55–0.73	29
Essential Fatty Acids (% of Total Diet)			
Linoleic	3.94 ± 0.235	3.49–4.55	29
Linolenic	0.297 ± 0.064	0.005–0.368	29
Vitamins			
Vitamin A (IU/kg)	3,443 ± 30.23	3,040–3,740	4
Vitamin D (IU/kg)[a]	1,000	–	–
α-Tocopherol (ppm)	2,455 ± 12,817	13.6–69,100	29
Thiamine (ppm)[b]	7.55 ±0.580	7.0–8.3	4
Riboflavin (ppm)	8.17 ± 2.84	4.2–17.5	29
Niacin (ppm)	78.66 ± 8.11	66.4–98.2	29
Pantothenic Acid (ppm)	26.42 ± 11.05	17.4–81.0	29
Pyridoxine (ppm)[b]	9.75 ± 2.05	6.44–14.3	29
Folic Acid (ppm)	1.58 ± 0.43	1.15–3.27	29
Biotin (ppm)	0.323 ± 0.093	0.2–0.704	29
B_{12} (ppb)	50.41 ± 34.9	18.3–174	29
Choline (as Chloride) (ppm)	2,593 ± 633	1,160–3,790	29

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Minerals			
Calcium (%)	0.886 ± 0.138	0.697–1.02	4
Phosphorus (%)	0.570 ± 0.030	0.553–0.615	4
Potassium (%)	0.668 ± 0.029	0.626–0.733	29
Chloride (%)	0.392 ± 0.044	0.3–0.517	29
Sodium (%)	0.195 ± 0.027	0.16–0.283	29
Magnesium (%)	0.217 ± 0.054	0.185–0.49	29
Iron (ppm)	191.6 ± 36.18	135–311	29
Manganese (ppm)	50.11 ± 9.42	21–73.1	29
Zinc (ppm)	57.3 ± 25.55	43.3–184	29
Copper (ppm)	7.57 ± 2.49	3.21–16.3	29
Iodine (ppm)	0.513 ± 0.221	0–0.972	29
Chromium (ppm)	1.017 ± 1.038	0.33–3.97	29
Cobalt (ppm)	0.222 ± 0.152	0.0857–0.864	29

[a]From formulation.
[b]As hydrochloride.

Table B-7. Nutrient Composition of NTP-2000 Rat and Mouse Ration in the Three-month Studies of N-Butylpyridinium Chloride

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by Weight)	14.43 ± 0.574	13.9–15.2	4
Crude Fat (% by Weight)	8.1 ± 0.408	7.7–8.5	4
Crude Fiber (% by Weight)	9.56 ± 0.308	9.12–9.83	4
Ash (% by Weight)	4.91 ± 0.119	4.8–5.07	4
Amino Acids (% of Total Diet)			
Arginine	0.805 ± 0.075	0.67–0.97	29
Cystine	0.220 ± 0.022	0.15–0.25	29
Glycine	0.702 ± 0.038	0.62–0.80	29
Histidine	0.342 ± 0.070	0.27–0.68	29
Isoleucine	0.549 ± 0.040	0.43–0.66	29
Leucine	1.097 ± 0.063	0.96–1.24	29
Lysine	0.700 ± 0.104	0.31–0.86	29
Methionine	0.409 ± 0.042	0.26–0.49	29
Phenylalanine	0.623 ± 0.047	0.471–0.72	29
Threonine	0.513 ± 0.041	0.43–0.61	29
Tryptophan	0.155 ± 0.027	0.11–0.2	29

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Tyrosine	0.422 ± 0.066	0.28–0.54	29
Valine	0.666 ± 0.040	0.55–0.73	29
Essential Fatty Acids (% of Total Diet)			
Linoleic	3.94 ± 0.235	3.49–4.55	29
Linolenic	0.297 ± 0.064	0.005–0.368	29
Vitamins			
Vitamin A (IU/kg)	3,648 ± 48.61	3,040–4,220	4
Vitamin D (IU/kg)[a]	1,000	–	–
α-Tocopherol (ppm)	2,455 ± 12,817	13.6–69,100	29
Thiamine (ppm)[b]	7.5 ±0.648	6.8–8.3	4
Riboflavin (ppm)	8.17 ± 2.84	4.2–17.5	29
Niacin (ppm)	78.66 ± 8.11	66.4–98.2	29
Pantothenic Acid (ppm)	26.42 ± 11.05	17.4–81.0	29
Pyridoxine (ppm)[b]	9.75 ± 2.05	6.44–14.3	29
Folic Acid (ppm)	1.58 ± 0.43	1.15–3.27	29
Biotin (ppm)	0.323 ± 0.093	0.2–0.704	29
B_{12} (ppb)	50.41 ± 34.9	18.3–174	29
Choline (as Chloride) (ppm)	2,593 ± 633	1,160–3,790	29
Minerals			
Calcium (%)	0.902 ± 0.151	0.697–1.02	4
Phosphorus (%)	0.580 ± 0.030	0.553–0.615	4
Potassium (%)	0.668 ± 0.029	0.626–0.733	29
Chloride (%)	0.392 ± 0.044	0.3–0.517	29
Sodium (%)	0.195 ± 0.027	0.16–0.283	29
Magnesium (%)	0.217 ± 0.054	0.185–0.49	29
Iron (ppm)	191.6 ± 36.18	135–311	29
Manganese (ppm)	50.11 ± 9.42	21–73.1	29
Zinc (ppm)	57.3 ± 25.55	43.3–184	29
Copper (ppm)	7.57 ± 2.49	3.21–16.3	29
Iodine (ppm)	0.513 ± 0.221	0–0.972	29
Chromium (ppm)	1.017 ± 1.038	0.33–3.97	29
Cobalt (ppm)	0.222 ± 0.152	0.0857–0.864	29

[a]From formulation.
[b]As hydrochloride.

Table B-8. Contaminant Levels in NTP-2000 Rat and Mouse Ration in the Two-week Studies

	Level	Number of Samples
Contaminants		
Arsenic (ppm)	0.169	1
Cadmium (ppm)	0.068	1
Lead (ppm)	0.097	1
Mercury (ppm)	0.022	1
Selenium (ppm)	0.252	1
Aflatoxins (ppb)[a]	<5.0	1
Nitrate Nitrogen (ppm)[b]	10	1
Nitrite Nitrogen (ppm)[a,b]	<0.61	1
BHA (ppm)[a,c]	<1.00	1
BHT (ppm)[a,c]	<1.00	1
Aerobic Plate Count (CFU/g)	<10.0	1
Coliform (MPN/g)	<3.0	1
Escherichia coli (MPN/g)[a]	<10.0	1
Salmonella (MPN/g)	Negative	1
Total Nitrosamines (ppb)[d]	11.3	1
N-Ndimethylamine (ppb)[d]	1.1	1
N-Npyrrolidine (ppb)[d]	10.2	1
Pesticides (ppm)		
α-BHC[a]	<0.01	1
β-BHC[a]	<0.02	1
γ-BHC[a]	<0.01	1
δ-BHC[a]	<0.01	1
Heptachlor[a]	<0.01	1
Aldrin[a]	<0.01	1
Heptachlor Epoxide[a]	<0.01	1
DDE[a]	<0.01	1
DDD[a]	<0.01	1
DDT[a]	<0.01	1
HCB[a]	<0.01	1
Mirex[a]	<0.01	1
Methoxychlor[a]	<0.05	1
Dieldrin[a]	<0.01	1
Endrin[a]	<0.01	1
Telodrin[a]	<0.01	1

	Level	Number of Samples
Chlordane[a]	<0.05	1
Toxaphene[a]	<0.10	1
Estimated PCBs[a]	<0.20	1
Ronnel[a]	<0.01	1
Ethion[a]	<0.02	1
Trithion[a]	<0.05	1
Diazinon[a]	<0.10	1
Methyl Chlorpyrifos	0.18	1
Methyl Parathion[a]	<0.02	1
Ethyl Parathion[a]	<0.02	1
Malathion	0.0324	1
Endosulfan I[a]	<0.01	1
Endosulfan II[a]	<0.01	1
Endosulfane Sulfate[a]	<0.03	1

All samples were irradiated.
BHA = butylated hydroxyanisole; BHT = butylated hydroxytoluene; CFU = colony-forming units; MPN = most probable number; BHC = hexachlorocyclohexane or benzene hexachloride; DDE = dichlorodiphenyldichloroethylene; DDD = dichlorodiphenyldichloroethane; DDT = dichlorodiphenyltrichloroethane; HCB = hexachlorobenzene; PCB = polychlorinated biphenyl.
[a]All values were below the detection limit. The detection limit is given as the level.
[b]Sources of contamination include alfalfa, grains, and fish meal.
[c]Sources of contamination include soy oil and fish meal.
[d]All values were corrected for percent recovery.

Table B-9. Contaminant Levels in NTP-2000 Rat and Mouse Ration in the Three-month Studies of 1-Ethyl-3-Methylimidazolium Chloride

	Mean ± Standard Deviation	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.170 ± 0.023	0.143–0.192	4
Cadmium (ppm)	0.047 ± 0.006	0.043–0.055	4
Lead (ppm)	0.096 ± 0.030	0.066–0.122	4
Mercury (ppm)	0.010 ± 0.001	0.01–0.011	4
Selenium (ppm)	0.159 ± 0.003	0.155–0.162	4
Aflatoxins (ppb)[a]	<5.0	–	4
Nitrate Nitrogen (ppm)[b]	14.23 ± 5.57	10.0–22.0	4
Nitrite Nitrogen (ppm)[a,b]	<0.61	–	4
BHA (ppm)[a,c]	<1.00	–	4
BHT (ppm)[a,c]	<1.00	–	4
Aerobic Plate Count (CFU/g)	<10.0	–	4
Coliform (MPN/g)	<3.0	–	4
Escherichia coli (MPN/g)[a]	<10.0	–	4
Salmonella (MPN/g)	Negative	–	4

	Mean ± Standard Deviation	Range	Number of Samples
Total Nitrosamines (ppb)[d]	7.625 ± 2.082	5.4–9.04	4
N-Ndimethylamine (ppb)[d]	0.975 ± 1.127	0–2	4
N-Npyrrolidine (ppb)[d]	6.65 ± 0.995	5.4–7.5	4
Pesticides (ppm)			
α-BHC[a]	<0.01	–	4
β-BHC[a]	<0.02	–	4
γ-BHC[a]	<0.01	–	4
δ-BHC[a]	<0.01	–	4
Heptachlor[a]	<0.01	–	4
Aldrin[a]	<0.01	–	4
Heptachlor Epoxide[a]	<0.01	–	4
DDE[a]	<0.01	–	4
DDD[a]	<0.01	–	4
DDT[a]	<0.01	–	4
HCB[a]	<0.01	–	4
Mirex[a]	<0.01	–	4
Methoxychlor[a]	<0.05	–	4
Dieldrin[a]	<0.01	–	4
Endrin[a]	<0.01	–	4
Telodrin[a]	<0.01	–	4
Chlordane[a]	<0.05	–	4
Toxaphene[a]	<0.10	–	4
Estimated PCBs[a]	<0.20	–	4
Ronnel[a]	<0.01	–	4
Ethion[a]	<0.02	–	4
Trithion[a]	<0.05	–	4
Diazinon[a]	<0.10	–	4
Methyl Chlorpyrifos	0.074 ± 0.055	0.02–0.015	4
Methyl Parathion[a]	<0.02	–	4
Ethyl Parathion[a]	<0.02	–	4
Malathion	0.029 ± 0.014	0.02–0.05	4
Endosulfan I[a]	<0.01	–	4
Endosulfan II[a]	<0.01	–	4
Endosulfane Sulfate[a]	<0.03	–	4

All samples were irradiated.
BHA = butylated hydroxyanisole; BHT = butylated hydroxytoluene; CFU = colony-forming units; MPN = most probable number; BHC = hexachlorocyclohexane or benzene hexachloride; DDE = dichlorodiphenyldichloroethylene; DDD = dichlorodiphenyldichloroethane; DDT = dichlorodiphenyltrichloroethane; HCB = hexachlorobenzene; PCB = polychlorinated biphenyl.
[a]All values were below the detection limit. The detection limit is given as the mean.
[b]Sources of contamination include alfalfa, grains, and fish meal.
[c]Sources of contamination include soy oil and fish meal.
[d]All values were corrected for percent recovery.

Table B-10. Contaminant Levels in NTP-2000 Rat and Mouse Ration in the Three-month Studies of 1-Butyl-3-Methylimidazolium Chloride

	Mean ± Standard Deviation	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.163 ± 0.021	0.143–0.192	4
Cadmium (ppm)	0.046 ± 0.004	0.043–0.052	4
Lead (ppm)	0.084 ± 0.025	0.066–0.121	4
Mercury (ppm)	0.01	0.01–0.01	4
Selenium (ppm)	0.155 ± 0.005	0.147–0.159	4
Aflatoxins (ppb)[a]	<5.0	–	4
Nitrate Nitrogen (ppm)[b]	18.83 ± 8.29	10.0–28.8	4
Nitrite Nitrogen (ppm)[a,b]	<0.61	–	4
BHA (ppm)[a,c]	<1.00	–	4
BHT (ppm)[a,c]	<1.00	–	4
Aerobic Plate Count (CFU/g)	<10.0	–	4
Coliform (MPN/g)	<3.0	–	4
Escherichia coli (MPN/g)[a]	<10.0	–	4
Salmonella (MPN/g)	Negative	–	4
Total Nitrosamines (ppb)[d]	6.125 ± 2.497	3.4–9.4	4
N-Ndimethylamine (ppb)[d]	0.65 ± 0.896	0.0–1.9	4
N-Npyrrolidine (ppb)[d]	5.475 ± 2.040	2.7–7.5	4
Pesticides (ppm)			
α-BHC[a]	<0.01	–	4
β-BHC[a]	<0.02	–	4
γ-BHC[a]	<0.01	–	4
δ-BHC[a]	<0.01	–	4
Heptachlor[a]	<0.01	–	4
Aldrin[a]	<0.01	–	4
Heptachlor Epoxide[a]	<0.01	–	4
DDE[a]	<0.01	–	4
DDD[a]	<0.01	–	4
DDT[a]	<0.01	–	4
HCB[a]	<0.01	–	4
Mirex[a]	<0.01	–	4
Methoxychlor[a]	<0.05	–	4
Dieldrin[a]	<0.01	–	4
Endrin[a]	<0.01	–	4
Telodrin[a]	<0.01	–	4
Chlordane[a]	<0.05	–	4
Toxaphene[a]	<0.10	–	4
Estimated PCBs[a]	<0.20	–	4

	Mean ± Standard Deviation	Range	Number of Samples
Ronnel[a]	<0.01	–	4
Ethion[a]	<0.02	–	4
Trithion[a]	<0.05	–	4
Diazinon[a]	<0.10	–	4
Methyl Chlorpyrifos	0.065 ± 0.061	0.02–0.15	4
Methyl Parathion[a]	<0.02	–	4
Ethyl Parathion[a]	<0.02	–	4
Malathion	0.0213 ± 0.003	0.02–0.025	4
Endosulfan I[a]	<0.01	–	4
Endosulfan II[a]	<0.01	–	4
Endosulfane Sulfate[a]	<0.03	–	4

All samples were irradiated.
BHA = butylated hydroxyanisole; BHT = butylated hydroxytoluene; CFU = colony-forming units; MPN = most probable number; BHC = hexachlorocyclohexane or benzene hexachloride; DDE = dichlorodiphenyldichloroethylene; DDD = dichlorodiphenyldichloroethane; DDT = dichlorodiphenyltrichloroethane; HCB = hexachlorobenzene; PCB = polychlorinated biphenyl.
[a]All values were below the detection limit. The detection limit is given as the mean.
[b]Sources of contamination include alfalfa, grains, and fish meal.
[c]Sources of contamination include soy oil and fish meal.
[d]All values were corrected for percent recovery.

Table B-11. Contaminant Levels in NTP-2000 Rat and Mouse Ration in the Three-Month Studies of 1-Butyl-1-Methylpyrrolidinium Chloride

	Mean ± Standard Deviation	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.171 ± 0.023	0.149–0.196	4
Cadmium (ppm)	0.052 ± 0.005	0.046–0.058	4
Lead (ppm)	0.104 ± 0.020	0.078–0.122	4
Mercury (ppm)	0.011 ± 0.001	0.01–0.011	4
Selenium (ppm)	0.173 ± 0.01	0.162–0.186	4
Aflatoxins (ppb)[a]	<5.0	–	4
Nitrate Nitrogen (ppm)[b]	22.38 ± 16.84	10–45.9	4
Nitrite Nitrogen (ppm)[a,b]	<0.61	–	4
BHA (ppm)[a,c]	<1.00	–	4
BHT (ppm)[a,c]	<1.00	–	4
Aerobic Plate Count (CFU/g)	46.25 ± 44.98	10–110	4
Coliform (MPN/g)	<3.0	–	4
Escherichia coli (MPN/g)[a]	<10.0	–	4
Salmonella (MPN/g)	Negative	–	4
Total Nitrosamines (ppb)[d]	9.9 ± 2.23	7.4–12.8	4
N-Ndimethylamine (ppb)[d]	2.175 ± 1.978	0–4.8	4
N-Npyrrolidine (ppb)[d]	7.725 ± 0.378	7.4–8.1	4

	Mean ± Standard Deviation	Range	Number of Samples
Pesticides (ppm)			
α-BHC[a]	<0.01	–	4
β-BHC[a]	<0.02	–	4
γ-BHC[a]	<0.01	–	4
δ-BHC[a]	<0.01	–	4
Heptachlor[a]	<0.01	–	4
Aldrin[a]	<0.01	–	4
Heptachlor Epoxide[a]	<0.01	–	4
DDE[a]	<0.01	–	4
DDD[a]	<0.01	–	4
DDT[a]	<0.01	–	4
HCB[a]	<0.01	–	4
Mirex[a]	<0.01	–	4
Methoxychlor[a]	<0.05	–	4
Dieldrin[a]	<0.01	–	4
Endrin[a]	<0.01	–	4
Telodrin[a]	<0.01	–	4
Chlordane[a]	<0.05	–	4
Toxaphene[a]	<0.10	–	4
Estimated PCBs[a]	<0.20	–	4
Ronnel[a]	<0.01	–	4
Ethion[a]	<0.02	–	4
Trithion[a]	<0.05	–	4
Diazinon[a]	<0.10	–	4
Methyl Chlorpyrifos	0.058 ± 0.025	0.025–0.084	4
Methyl Parathion[a]	<0.02	–	4
Ethyl Parathion[a]	<0.02	–	4
Malathion	0.090 ± 0.067	0.02–0.169	4
Endosulfan I[a]	<0.01	–	4
Endosulfan II[a]	<0.01	–	4
Endosulfane Sulfate[a]	<0.03	–	4

All samples were irradiated.
BHA = butylated hydroxyanisole; BHT = butylated hydroxytoluene; CFU = colony-forming units; MPN = most probable number; BHC = hexachlorocyclohexane or benzene hexachloride; DDE = dichlorodiphenyldichloroethylene; DDD = dichlorodiphenyldichloroethane; DDT = dichlorodiphenyltrichloroethane; HCB = hexachlorobenzene; PCB = polychlorinated biphenyl.
[a]All values were below the detection limit. The detection limit is given as the mean.
[b]Sources of contamination include alfalfa, grains, and fish meal.
[c]Sources of contamination include soy oil and fish meal.
[d]All values were corrected for percent recovery.

Table B-12. Contaminant Levels in NTP-2000 Rat and Mouse Ration in the Three-month Studies of N-Butylpyridinium Chloride

	Mean ± Standard Deviation	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.179 ± 0.033	0.149–0.216	4
Cadmium (ppm)	0.051 ± 0.005	0.046–0.058	4
Lead (ppm)	0.101 ± 0.162	0.078–0.115	4
Mercury (ppm)	0.010 ± 0.001	0.01–0.011	4
Selenium (ppm)	0.171 ± 0.013	0.155–0.186	4
Aflatoxins (ppb)[a]	<5.0	–	4
Nitrate Nitrogen (ppm)[b]	26.625 ± 5.57	10.0–45.9	4
Nitrite Nitrogen (ppm)[a,b]	<0.61	–	4
BHA (ppm)[a,c]	<1.00	–	4
BHT (ppm)[a,c]	<1.00	–	4
Aerobic Plate Count (CFU/g)	46.25 ± 44.98	10–110	4
Coliform (MPN/g)	<3.0	–	4
Escherichia coli (MPN/g)[a]	<10.0	–	4
Salmonella (MPN/g)	Negative	–	4
Total Nitrosamines (ppb)[d]	9.75 ± 2.29	7.4–12.8	4
N-Ndimethylamine (ppb)[d]	1.975 ± 2.040	0–4.8	4
N-Npyrrolidine (ppb)[d]	7.775 ± 0.330	7.4–8.1	4
Pesticides (ppm)			
α-BHC[a]	<0.01	–	4
β-BHC[a]	<0.02	–	4
γ-BHC[a]	<0.01	–	4
δ-BHC[a]	<0.01	–	4
Heptachlor[a]	<0.01	–	4
Aldrin[a]	<0.01	–	4
Heptachlor Epoxide[a]	<0.01	–	4
DDE[a]	<0.01	–	4
DDD[a]	<0.01	–	4
DDT[a]	<0.01	–	4
HCB[a]	<0.01	–	4
Mirex[a]	<0.01	–	4
Methoxychlor[a]	<0.05	–	4
Dieldrin[a]	<0.01	–	4
Endrin[a]	<0.01	–	4

	Mean ± Standard Deviation	Range	Number of Samples
Telodrin[a]	<0.01	–	4
Chlordane[a]	<0.05	–	4
Toxaphene[a]	<0.10	–	4
Estimated PCBs[a]	<0.20	–	4
Ronnel[a]	<0.01	–	4
Ethion[a]	<0.02	–	4
Trithion[a]	<0.05	–	4
Diazinon[a]	<0.10	–	4
Methyl Chlorpyrifos	0.216 ± 0.315	0.025–0.686	4
Methyl Parathion[a]	<0.02	–	4
Ethyl Parathion[a]	<0.02	–	4
Malathion	0.082 ± 0.074	0.02–0.169	4
Endosulfan I[a]	<0.01	–	4
Endosulfan II[a]	<0.01	–	4
Endosulfane Sulfate[a]	<0.03	–	4

All samples were irradiated.
BHA = butylated hydroxyanisole; BHT = butylated hydroxytoluene; CFU = colony-forming units; MPN = most probable number; BHC = hexachlorocyclohexane or benzene hexachloride; DDE = dichlorodiphenyldichloroethylene; DDD = dichlorodiphenyldichloroethane; DDT = dichlorodiphenyltrichloroethane; HCB = hexachlorobenzene; PCB = polychlorinated biphenyl.
[a]All values were below the detection limit. The detection limit is given as the mean.
[b]Sources of contamination include alfalfa, grains, and fish meal.
[c]Sources of contamination include soy oil and fish meal.
[d]All values were corrected for percent recovery.

Appendix C. Sentinel Animal Program

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Tables

C.1. Methods

Rodents used in the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that might affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicological evaluation of test compounds. Under this program, the disease state of the rodents is monitored via sera or feces from extra (sentinel) or exposed animals in the study rooms. The sentinel animals and the study animals are subject to identical environmental conditions. Furthermore, the sentinel animals come from the same production source and weanling groups as the animals used for the studies of test compounds.

In these toxicity studies, blood samples were collected from each sentinel animal and allowed to clot, and the serum was separated. Additionally, fecal samples were collected and tested for endoparasites and *Helicobacter* species. All samples were processed appropriately with serology testing sent to IDEXX BioResearch (formerly Rodent Animal Diagnostic Laboratory [RADIL], University of Missouri), Columbia, MO, for determination of the presence of pathogens. Evaluation for endo- and ectoparasites was performed in-house by the testing laboratory.

The laboratory methods and agents for which testing was performed are tabulated below; the times at which samples were collected during the studies are also listed (Table C-1, Table C-2, Table C-3, Table C-4, Table C-5, Table C-6, Table C-7, Table C-8).

C.2. Results

C.2.1. 1-Ethyl-3-Methylimidazolium Chloride (Emim-Cl)

Rats: All test results were negative.

Mice: All test results were negative.

Table C-1. Methods and Results for Sentinel Animal Testing in Male and Female Rats in the Three-month Study of 1-Ethyl-3-Methylimidazolium Chloride

Collection Time Points	Quarantine	4 Weeks	Study Termination
Number Examined (Males/Females)	5/5	5/5	5/5
Method/Test			
Multiplex Fluorescent Immunoassay (MFI)			
Kilham rat virus (KRV)	–	–	–
Mycoplasma pulmonis	–	–	–
Parvo NS-1	–	–	–
Pneumonia virus of mice (PVM)	–	–	–
Rat coronavirus/sialodacryoadenitis virus (RCV/SDA)	–	–	–
Rat minute virus (RMV)	–	–	–
Rat parvo virus (RPV)	–	–	–
Rat theilovirus (RTV)	–	–	–

Collection Time Points	Quarantine	4 Weeks	Study Termination
Sendai	–	–	–
Theiler's murine encephalomyelitis virus (TMEV)	–	–	–
Toolan's H-1	–	–	–
Immunofluorescence Assay (IFA)			
Pneumocystis carinii	NT	NT	–
In-house Evaluation			
Endoparasites (evaluation of cecal content)	–	–	NT
Ectoparasites (evaluation of perianal surface)	–	–	NT

– = negative; NT = not tested.

Table C-2. Methods and Results for Sentinel Animal Testing in Male and Female Mice in the Three-month Study of 1-Ethyl-3-Methylimidazolium Chloride

Collection Time Points	Quarantine	4 Weeks	Study Termination
Number Examined (Males/Females)	5/5	5/5	5/5
Method/Test			
Multiplex Fluorescent Immunoassay (MFI)			
Ectromelia virus	–	–	–
Epizootic diarrhea of infant mice (EDIM)	–	–	–
Lymphocytic choriomeningitis virus	–	–	–
Mycoplasma pulmonis	–	–	–
Mouse hepatitis virus (MHV)	–	–	–
Mouse norovirus (MNV)	–	–	–
Parvo NS-1			
Mouse parvovirus (MPV)	–	–	–
Minute virus of mice (MVM)	–	–	–
Pneumonia virus of mice (PVM)	–	–	–
Reovirus (REO3)	–	–	–
Sendai	–	–	–
Theiler's murine encephalomyelitis virus (TMEV) GDVII	–	–	–
In-house Evaluation			
Endoparasites (evaluation of cecal content)	–	–	NT
Ectoparasites (evaluation of perianal surface)	–	–	NT

– = negative; NT = not tested.

C.2.2. 1-Butyl-3-Methylimidazolium Chloride (Bmim-Cl)

Rats: All test results were negative.

Mice: All test results were negative.

Table C-3. Methods and Results for Sentinel Animal Testing in Male and Female Rats in the Three-month Study of 1-Butyl-3-Methylimidazolium Chloride

Collection Time Points	Quarantine	4 Weeks	Study Termination
Number Examined (Males/Females)	5/5	5/5	5/5
Method/Test			
Multiplex Fluorescent Immunoassay (MFI)			
Kilham rat virus (KRV)	–	–	–
Mycoplasma pulmonis	–	–	–
Parvo NS-1	–	–	–
Pneumonia virus of mice (PVM)	–	–	–
Rat coronavirus/sialodacryoadenitis virus (RCV/SDA)	–	–	–
Rat minute virus (RMV)	–	–	–
Rat parvo virus (RPV)	–	–	–
Rat theilovirus (RTV)	–	–	–
Sendai	–	–	–
Theiler's murine encephalomyelitis virus (TMEV)	–	–	–
Toolan's H-1	–	–	–
Immunofluorescence Assay (IFA)			
Pneumocystis carinii	NT	NT	–
Rat coronavirus/sialodacryoadenitis virus (RCV/SDA)	NT	NT	–
In-house Evaluation			
Endoparasites (evaluation of cecal content)	–	–	NT
Ectoparasites (evaluation of perianal surface)	–	–	NT

– = negative; NT = not tested.

Table C-4. Methods and Results for Sentinel Animal Testing in Male and Female Mice in the Three-month Study of 1-Butyl-3-Methylimidazolium Chloride

Collection Time Points	Quarantine	4 Weeks	Study Termination
Number Examined (Males/Females)	5/5	5/5	5/5
Method/Test			
Multiplex Fluorescent Immunoassay (MFI)			
Ectromelia virus	–	–	–
Epizootic diarrhea of infant mice (EDIM)	–	–	–

Collection Time Points	Quarantine	4 Weeks	Study Termination
Lymphocytic choriomeningitis virus	–	–	–
Mycoplasma pulmonis	–	–	–
Mouse hepatitis virus (MHV)	–	–	–
Mouse norovirus (MNV)	–	–	–
Parvo NS-1	–	–	–
Mouse parvovirus (MPV)	–	–	–
Minute virus of mice (MVM)	–	–	–
Pneumonia virus of mice (PVM)	–	–	–
Reovirus (REO3)	–	–	–
Sendai	–	–	–
Theiler's murine encephalomyelitis virus (TMEV) GDVII	–	–	–
In-house Evaluation			
Endoparasites (evaluation of cecal content)	–	–	NT
Ectoparasites (evaluation of perianal surface)	–	–	NT

– = negative; NT = not tested.

C.2.3. 1-Butyl-1-Methylpyrrolidinium Chloride (Bmpy-Cl)

Rats: All test results were negative.

Mice: All test results were negative.

Table C-5. Methods and Results for Sentinel Animal Testing in Male and Female Rats in the Three-month Study of 1-Butyl-1-Methylpyrrolidinium Chloride

Collection Time Points	Quarantine	4 Weeks	Study Termination
Number Examined (Males/Females)	5/5	5/5	5/5
Method/Test			
Multiplex Fluorescent Immunoassay (MFI)			
Kilham rat virus (KRV)	–	–	–
Mycoplasma pulmonis	–	–	–
Parvo NS-1	–	–	–
Pneumonia virus of mice (PVM)	–	–	–
Rat coronavirus/sialodacryoadenitis virus (RCV/SDA)	–	–	–
Rat minute virus (RMV)	–	–	–
Rat parvo virus (RPV)	–	–	–
Rat theilovirus (RTV)	–	–	–
Sendai	–	–	–
Theiler's murine encephalomyelitis virus (TMEV)	–	–	–

Collection Time Points	Quarantine	4 Weeks	Study Termination
Toolan's H-1	–	–	–
Immunofluorescence Assay (IFA)			
Pneumocystis carinii	NT	NT	–
In-house Evaluation			
Endoparasites (evaluation of cecal content)	–	–	NT
Ectoparasites (evaluation of perianal surface)	–	–	NT

– = negative; NT = not tested.

Table C-6. Methods and Results for Sentinel Animal Testing in Male and Female Mice in the Three-month Study of 1-Butyl-1-Methylpyrrolidinium Chloride

Collection Time Points	Quarantine	4 Weeks	Study Termination
Number Examined (Males/Females)	5/5	5/5	5/5
Method/Test			
Multiplex Fluorescent Immunoassay (MFI)			
Ectromelia virus	–	–	–
Epizootic diarrhea of infant mice (EDIM)	–	–	–
Lymphocytic choriomeningitis virus	–	–	–
Mycoplasma pulmonis	–	–	–
Mouse hepatitis virus (MHV)	–	–	–
Mouse norovirus (MNV)	–	–	–
Parvo NS-1	–	–	–
Mouse parvovirus (MPV)	–	–	–
Minute virus of mice (MVM)	–	–	–
Pneumonia virus of mice (PVM)	–	–	–
Reovirus (REO3)	–	–	–
Sendai	–	–	–
Theiler's murine encephalomyelitis virus (TMEV) GDVII	–	–	–
In-house Evaluation			
Endoparasites (evaluation of cecal content)	–	–	NT
Ectoparasites (evaluation of perianal surface)	–	–	NT

– = negative; NT = not tested.

C.2.4. N-Butylpyridinium Chloride (NBuPy-Cl)

Rats: All test results were negative.

Mice: All test results were negative.

Table C-7. Methods and Results for Sentinel Animal Testing in Male and Female Rats in the Three-month Study of N-Butylpyridinium Chloride

Collection Time Points	Quarantine	4 Weeks	Study Termination
Number Examined (Males/Females)	5/5	5/5	5/5
Method/Test			
Multiplex Fluorescent Immunoassay (MFI)			
Kilham rat virus (KRV)	–	–	–
Mycoplasma pulmonis	–	–	–
Parvo NS-1	–	–	–
Pneumonia virus of mice (PVM)	–	–	–
Rat coronavirus/sialodacryoadenitis virus (RCV/SDA)	–	–	–
Rat minute virus (RMV)	–	–	–
Rat parvo virus (RPV)	–	–	–
Rat theilovirus (RTV)	–	–	–
Sendai	–	–	–
Theiler's murine encephalomyelitis virus (TMEV)	–	–	–
Toolan's H-1	–	–	–
Immunofluorescence Assay (IFA)			
Pneumocystis carinii	NT	NT	–
In-house Evaluation			
Endoparasites (evaluation of cecal content)	–	–	NT
Ectoparasites (evaluation of perianal surface)	–	–	NT

– = negative; NT = not tested.

Table C-8. Methods and Results for Sentinel Animal Testing in Male and Female Mice in the Three-month Study of N-Butylpyridinium Chloride

Collection Time Points	Quarantine	4 Weeks	Study Termination
Number Examined (Males/Females)	5/5	5/5	5/5
Method/Test			
Multiplex Fluorescent Immunoassay (MFI)			
Ectromelia virus	–	–	–
Epizootic diarrhea of infant mice (EDIM)	–	–	–
Lymphocytic choriomeningitis virus	–	–	–
Mycoplasma pulmonis	–	–	–
Mouse hepatitis virus (MHV)	–	–	–
Mouse norovirus (MNV)	–	–	–
Parvo NS-1	–	–	–
Mouse parvovirus (MPV)	–	–	–
Minute virus of mice (MVM)	–	–	–
Pneumonia virus of mice (PVM)	–	–	–
Reovirus (REO3)	–	–	–
Sendai	–	–	–
Theiler's murine encephalomyelitis virus (TMEV) GDVII	–	–	–
In-house Evaluation			
Endoparasites (evaluation of cecal content)	–	–	NT
Ectoparasites (evaluation of perianal surface)	–	–	NT

– = negative; NT = not tested.

Appendix D. Genetic Toxicology

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Tables

D.1. Evaluation Protocol

The National Toxicology Program (NTP) considers biological as well as statistical factors to determine an overall assay result. For an individual assay, the statistical procedures for data analysis are described in the following protocols. There have been instances, however, in which multiple samples of a chemical were tested in the same assay, and different results were obtained among these samples and/or among laboratories. In such cases, all the data are critically evaluated with attention given to possible protocol variations in determining the weight of evidence for an overall conclusion of chemical activity in an assay. For in vitro assays conducted with and without exogenous metabolic activation, results obtained in the absence of activation are analyzed separately from results obtained in the presence of activation. The summary table in the abstract of this toxicity report presents NTP's scientific judgment regarding the overall evidence for activity of the chemical in an assay.

D.2. Bacterial Mutagenicity

D.2.1. Bacterial Mutagenicity Test Protocol

Testing procedures were modified from those originally reported by Zeiger et al.[138] Coded samples of the same chemical lots used in the 3-month studies of ionic liquids (ILs; 1-ethyl-3-methylimidazolium chloride [Emim-Cl], 1-butyl-3-methylimidazolium chloride [Bmim-Cl], 1-butyl-1-methylpyrrolidinium chloride [Bmpy-Cl], or n-butylpyridinium chloride [NBuPy-Cl]) were incubated with the *Salmonella typhimurium* (TA98, TA100) or *Escherichia coli* WP2 *uvrA* (pKM101) tester strains, either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 125-induced male Sprague Dawley rat liver [Moltox, Boone, NC]) for 20 minutes at 37°C. Top agar supplemented with *L*-histidine (or tryptophan for the *E. coli* strain) and *d*-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine- or tryptophan-independent mutant colonies arising on these plates were counted after incubation for 2 days at 37°C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of each IL. The highest concentration tested was 10,000 μg/plate for each IL. All trials were repeated.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that is not dose-related, is not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed after chemical treatment. No minimum percentage or fold increase is required for a chemical to be judged positive or weakly positive, although positive calls are typically reserved for increases in mutant colonies that are at least twofold over background.

D.2.2. Results

The four ILs were tested for induction of gene mutations in the bacterial mutation (Ames) assay. All four ILs—Emim-Cl, Bmim-Cl, Bmpy-Cl, and NBuPy-Cl—were negative for induction of gene mutations in three strains of bacteria (*S. typhimurium* strains TA98 and TA100, and *E. coli*

strain WP2 *uvrA* [pKM101]), tested with and without induced rat liver S9 mix at doses up to 10,000 µg/plate (Table D-1, Table D-2, Table D-3, Table D-4).

Table D-1. Mutagenicity of 1-Ethyl-3-Methylimidazolium Chloride in Bacterial Tester Strains[a]

Strain	Concentration (µg/plate)	Without S9	Without S9	With 10% Rat S9	With 10% Rat S9
TA98					
	0	23 ± 2.0	24 ± 1.5	31 ± 2.4	30 ± 3.0
	1,000	18 ± 1.5	24 ± 2.3	24 ± 1.7	24 ± 2.2
	2,500	21 ± 1.9	26 ± 4.4	19 ± 2.7	23 ± 1.7
	5,000	21 ± 3.2	22 ± 4.2	29 ± 0.7	26 ± 1.5
	7,500	16 ± 0.6	23 ± 2.0	25 ± 6.4	30 ± 2.9
	10,000	20 ± 1.2	17 ± 2.6	17 ± 3.3	20 ± 3.5
Trial Summary		Negative	Negative	Negative	Negative
Positive Control[b]		420 ± 49.6	592 ± 13.9	1,119 ± 71.3	1,421 ± 43.9
TA100					
	0	104 ± 6.5	111 ± 1.3	115 ± 5.0	118 ± 1.9
	1,000	94 ± 1.5	110 ± 9.0	115 ± 10.4	128 ± 6.7
	2,500	95 ± 4.3	99 ± 4.5	120 ± 4.0	124 ± 5.7
	5,000	108 ± 1.8	110 ± 2.2	113 ± 12.0	118 ± 4.3
	7,500	93 ± 5.2	107 ± 5.5	112 ± 8.3	126 ± 11.0
	10,000	97 ± 10.4	110 ± 11.3	110 ± 4.8	127 ± 10.5
Trial Summary		Negative	Negative	Negative	Negative
Positive Control		445 ± 2.3	417 ± 15.5	1,347 ± 16.2	1,610 ± 125.3
***Escherichia coli* WP2 *uvrA* (pKM101)**					
	0	179 ± 4.7	220 ± 14.5	225 ± 15.9	226 ± 6.2
	1,000	181 ± 2.5	240 ± 6.4	256 ± 10.4	257 ± 23.1
	2,500	209 ± 14.3	290 ± 19.7	272 ± 10.2	254 ± 19.2
	5,000	191 ± 4.4	237 ± 15.2	238 ± 14.6	291 ± 3.7
	7,500	202 ± 15.7	257 ± 10.5	241 ± 3.1	241 ± 24.3
	10,000	259 ± 16.8	290 ± 18.2	262 ± 27.0	258 ± 18.9
Trial Summary		Negative	Negative	Negative	Negative
Positive Control		827 ± 13.1	2,339 ± 28.2	1,561 ± 63.3	1,156 ± 2.6

[a]Studies performed at Integrated Laboratory Systems, LLC. Data are presented as revertants/plate (mean ± standard error) from three plates; 0 µg/plate served as the solvent control.
[b]The positive controls in the absence of metabolic activation were 2-nitrofluorene (TA98), sodium azide (TA100), and methyl methanesulfonate (*E. coli*). The positive control for metabolic activation with all strains was 2-aminoanthracene.

Table D-2. Mutagenicity of 1-Butyl-3-Methylimidazolium Chloride in Bacterial Tester Strains[a]

Strain	Concentration (µg/plate)	Without S9	Without S9	With 10% Rat S9	With 10% Rat S9
TA98					
	0	22 ± 1.7	23 ± 2.6	36 ± 3.1	34 ± 2.4
	1,000	24 ± 2.9	25 ± 1.9	32 ± 3.2	33 ± 3.2
	2,500	21 ± 1.0	25 ± 2.4	28 ± 2.0	30 ± 1.9
	5,000	14 ± 2.0	23 ± 0.7	27 ± 2.7	25 ± 2.5
	7,500	19 ± 3.2	17 ± 2.3	28 ± 3.2	27 ± 3.2
	10,000	24 ± 1.9	15 ± 1.8	34 ± 1.9	20 ± 2.6
Trial Summary		Negative	Negative	Negative	Negative
Positive Control[b]		669 ± 27.2	347 ± 36.2	1,393 ± 104.8	1,280 ± 128.9
TA100					
	0	88 ± 3.5	104 ± 5.6	70 ± 6.5	104 ± 7.2
	1,000	105 ± 6.1	108 ± 5.5	70 ± 3.8	106 ± 5.1
	2,500	88 ± 7.4	108 ± 1.2	76 ± 2.6	113 ± 4.6
	5,000	102 ± 3.5	97 ± 4.9	80 ± 8.4	114 ± 2.2
	7,500	95 ± 7.5	90 ± 1.2	84 ± 4.4	114 ± 3.8
	10,000	105 ± 3.5	107 ± 14.2	81 ± 6.9	142 ± 18.4
Trial Summary		Negative	Negative	Negative	Negative
Positive Control		445 ± 32.3	374 ± 76.5	1,399 ± 55.7	1,682 ± 73.5
***Escherichia coli* WP2 *uvrA* (pKM101)**					
	0	167 ± 15.4	189 ± 4.6	193 ± 6.0	215 ± 5.9
	1,000	169 ± 6.1	168 ± 7.8	198 ± 10.3	203 ± 7.7
	2,500	162 ± 5.5	168 ± 3.2	191 ± 12.0	216 ± 5.9
	5,000	137 ± 5.2	163 ± 3.6	190 ± 10.4	223 ± 17.6
	7,500	137 ± 6.7	166 ± 7.8	198 ± 5.5	217 ± 3.7
	10,000	135 ± 2.0	189 ± 18.4	218 ± 27.4	259 ± 22.4
Trial Summary		Negative	Negative	Negative	Negative
Positive Control		1,921 ± 94.3	1,699 ± 90.8	854 ± 28.5	837 ± 34.9

[a]Studies performed at Integrated Laboratory Systems, LLC. Data are presented as revertants/plate (mean ± standard error) from three plates; 0 µg/plate served as the solvent control.
[b]The positive controls in the absence of metabolic activation were 2-nitrofluorene (TA98), sodium azide (TA100), and methyl methanesulfonate (*E. coli*). The positive control for metabolic activation with all strains was 2-aminoanthracene.

Table D-3. Mutagenicity of 1-Butyl-1-Methylpyrrolidinium Chloride in Bacterial Tester Strains[a]

Strain	Concentration (µg/plate)	Without S9	Without S9	With 10% Rat S9	With 10% Rat S9
TA98					
	0	27 ± 3.5	22 ± 1.0	33 ± 0.6	23 ± 1.5
	1,000	29 ± 1.3	22 ± 2.8	41 ± 6.9	25 ± 0.9
	2,500	30 ± 2.1	18 ± 5.7	28 ± 3.5	15 ± 0.3
	5,000	25 ± 2.2	18 ± 3.5	36 ± 1.7	20 ± 1.9
	7,500	26 ± 1.0	20 ± 3.5	34 ± 0.9	21 ± 1.5
	10,000	25 ± 2.8	18 ± 3.2	28 ± 4.2	18 ± 2.7
Trial Summary		Negative	Negative	Negative	Negative
Positive Control[b]		528 ± 63.5	640 ± 30.9	1,447 ± 15.4	1,666 ± 14.0
TA100					
	0	100 ± 1.2	81 ± 6.7	117 ± 3.1	110 ± 9.8
	1,000	104 ± 4.9	96 ± 6.4	117 ± 5.0	125 ± 4.0
	2,500	104 ± 3.9	82 ± 6.7	121 ± 9.0	134 ± 13.0
	5,000	96 ± 2.6	67 ± 5.9	119 ± 6.1	108 ± 5.6
	7,500	113 ± 2.0	90 ± 9.6	122 ± 5.7	121 ± 8.8
	10,000	106 ± 4.3	104 ± 6.3	145 ± 21.9	142 ± 21.9
Trial Summary		Negative	Negative	Negative	Negative
Positive Control		475 ± 10.5	491 ± 19.2	1,650 ± 25.5	1,054 ± 14.6
***Escherichia coli* WP2 *uvrA* (pKM101)**					
	0	182 ± 19.1	155 ± 3.9	261 ± 9.5	207 ± 12.7
	1,000	218 ± 1.2	168 ± 20.5	267 ± 0.7	235 ± 9.6
	2,500	220 ± 1.5	160 ± 4.7	259 ± 23.3	236 ± 11.8
	5,000	168 ± 6.4	141 ± 7.3	259 ± 13.0	186 ± 8.2
	7,500	147 ± 10.3	151 ± 5.8	265 ± 16.2	190 ± 4.5
	10,000	208 ± 33.9	198 ± 13.4	271 ± 36.5	215 ± 7.2
Trial Summary		Negative	Negative	Negative	Negative
Positive Control		2,009 ± 47.5	2,106 ± 119.4	941 ± 23.7	873 ± 67.7

[a]Studies performed at Integrated Laboratory Systems, LLC. Data are presented as revertants/plate (mean ± standard error) from three plates; 0 µg/plate served as the solvent control.
[b]The positive controls in the absence of metabolic activation were 2-nitrofluorene (TA98), sodium azide (TA100), and methyl methanesulfonate (*E. coli*). The positive control for metabolic activation with all strains was 2-aminoanthracene.

Table D-4. Mutagenicity of N-Butylpyridinium Chloride in Bacterial Tester Strains[a]

Strain	Concentration (µg/plate)	Without S9	Without S9	With 10% Rat S9	With 10% Rat S9
TA98					
	0	22 ± 1.7	20 ± 2.2	36 ± 3.1	27 ± 1.2
	1,000	18 ± 2.0	24 ± 3.1	30 ± 3.6	32 ± 1.2
	2,500	25 ± 1.7	17 ± 2.6	27 ± 3.8	28 ± 3.3
	5,000	17 ± 3.7	16 ± 0.9	24 ± 2.8	28 ± 5.8
	7,500	22 ± 2.3	12 ± 2.3	28 ± 2.8	23 ± 2.5
	10,000	21 ± 1.2	15 ± 0.9	33 ± 3.0	25 ± 1.2
Trial Summary		Negative	Negative	Negative	Negative
Positive Control[b]		669 ± 27.2	347 ± 36.2	1,393 ± 104.8	1,280 ± 128.9
TA100					
	0	88 ± 3.5	104 ± 5.6	70 ± 6.5	104 ± 7.2
	1,000	83 ± 7.0	104 ± 4.9	86 ± 1.5	99 ± 3.5
	2,500	105 ± 1.8	88 ± 7.0	89 ± 4.4	103 ± 2.6
	5,000	84 ± 5.4	98 ± 9.4	91 ± 1.2	109 ± 5.0
	7,500	86 ± 13.3	80 ± 2.2	95 ± 2.2	106 ± 0.7
	10,000	63 ± 9.0	69 ± 10.8	89 ± 4.2	153 ± 25.5
Trial Summary		Negative	Negative	Negative	Negative
Positive Control		445 ± 32.3	374 ± 76.5	1,399 ± 55.7	1,682 ± 73.5
***Escherichia coli* WP2 *uvrA* (pKM101)**					
	0	167 ± 15.4	189 ± 4.6	193 ± 6.0	215 ± 5.9
	1,000	173 ± 5.0	188 ± 6.2	203 ± 7.4	222 ± 11.0
	2,500	156 ± 5.7	175 ± 5.4	193 ± 7.2	218 ± 4.4
	5,000	173 ± 7.2	159 ± 4.9	209 ± 9.1	229 ± 5.5
	7,500	140 ± 6.0	156 ± 9.8	215 ± 9.2	249 ± 16.8
	10,000	159 ± 13.3	146 ± 11.4	225 ± 10.7	259 ± 10.4
Trial Summary		Negative	Negative	Negative	Negative
Positive Control		1,921 ± 94.3	1,699 ± 90.8	854 ± 28.5	837 ± 34.9

[a]Studies performed at Integrated Laboratory Systems, LLC. Data are presented as revertants/plate (mean ± standard error) from three plates; 0 µg/plate served as the solvent control.
[b]The positive controls in the absence of metabolic activation were 2-nitrofluorene (TA98), sodium azide (TA100), and methyl methanesulfonate (*E. coli*). The positive control for metabolic activation with all strains was 2-aminoanthracene.

D.3. Micronucleus Assay

D.3.1. Peripheral Blood Micronucleus Test Protocol

Upon termination of the 3-month toxicity studies of each of the four ILs (Emim-Cl, Bmim-Cl, Bmpy-Cl, and NBuPy-Cl), blood samples (approximately 200 μL) were collected from male and female rats and mice, placed in ethylenediaminetetraacetic acid (EDTA)-coated tubes, and shipped overnight to the testing laboratory. Upon arrival, blood samples were fixed in ultracold methanol using a MicroFlowPLUS Kit (Litron Laboratories, Rochester, NY) according to the manufacturer's instructions. Fixed samples were stored in a −80°C freezer until analysis. Thawed blood samples were analyzed for frequency of micronucleated immature erythrocytes (i.e., reticulocytes or polychromatic erythrocytes [PCEs]) and mature erythrocytes (i.e., normochromatic erythrocytes [NCEs]) using a flow cytometer[139]; both the mature and immature erythrocyte populations can be analyzed separately by employing special cell surface markers to differentiate the two cell types. Because the very young reticulocyte subpopulation (CD71+ cells) can be targeted using this technique, rat blood samples can be analyzed for damage that occurred in the bone marrow within the past 24–48 hours, before the rat spleen appreciably alters the percentage of PCEs in circulation.[140] In mice, both the mature and immature erythrocyte populations can be evaluated for micronucleus frequency because the mouse spleen does not sequester and eliminate damaged erythrocytes. Damaged erythrocytes achieve steady state in the peripheral blood of mice after 4 weeks of continuous exposure. Approximately 20,000 PCEs and 1×10^6 NCEs were analyzed per animal for the frequency of micronucleated cells, and the percentage of immature erythrocytes (% PCE) was calculated as a measure of bone marrow toxicity resulting from chemical exposure.

Prior experience with the large number of cells scored using flow cytometric scoring techniques[141] suggests it is reasonable to assume that the proportion of micronucleated reticulocytes is approximately normally distributed. The statistical tests selected for trend and for pairwise comparisons with the control group depend on whether the variances among the groups are equal. The Levene test at $\alpha = 0.05$ is used to test for equal variances. In the case of equal variances, linear regression is used to test for a linear trend with exposure concentration and the Williams test is used to test for pairwise differences between each exposed group and the control group. In the case of unequal variances, the Jonckheere test is used to test for linear trend and the Dunn test is used for pairwise comparisons of each exposed group with the control group. To correct for multiple pairwise comparisons, the p value for each comparison with the control group is multiplied by the number of comparisons made. In the event that this product is >1.00, it is replaced with 1.00. Trend tests and pairwise comparisons with the controls are considered statistically significant at $p \leq 0.025$.

In the micronucleus test, it is preferable to base a positive result on the presence of both a positive trend as well as at least one significantly elevated exposed group compared to the corresponding control group. In addition, historical control data are used to evaluate the biological significance of any observed response. Both statistical significance and biological significance are considered when arriving at a call. The presence of either a positive trend or a single significant exposed group generally results in an equivocal call. The absence of both a trend and any significant differences between exposed groups and the control group results in a negative call. Ultimately, the scientific staff determines the final call after considering the results

of statistical analyses, reproducibility of any effects observed (in acute studies), and the magnitudes of those effects.

D.3.2. Results

The four ILs were tested for induction of structural or numerical chromosomal damage in the rodent peripheral blood erythrocyte micronucleus assay following 3 months of exposure via dosed water.

Emim-Cl did not increase the frequency of micronucleated erythrocytes in male or female rats or in male mice exposed via dosed water for 3 months (Table D-5, Table D-9). In female mice, the micronucleus assay response was judged to be negative, although a small, significant increase was observed in micronucleated NCEs; this response was downgraded because it was not considered of sufficient magnitude to be biologically relevant and was well within the laboratory historical control range for female B6C3F1 mice.

Bmim-Cl did not increase the frequency of micronucleated erythrocytes in male or female rats or mice exposed via dosed water for 3 months (Table D-6, Table D-10).

Bmpy-Cl was positive in the peripheral blood micronucleus test in male rats following 3 months of exposure via dosed water, although the observed increases in micronucleated PCEs were within the laboratory historical control 95% confidence interval. Bmpy-Cl did not induce an increase in micronucleated erythrocytes in female rats or in male or female mice (Table D-7, Table D-11).

A clearly significant increase in micronucleated NCEs was observed in male rats exposed to NBuPy-Cl for 3 months via dosed water, whereas no increase was seen in micronucleated PCEs in these same animals (Table D-8). The response was judged to be equivocal because (a) this response pattern is in direct contrast to what would be expected in rats for a truly positive compound, (b) the increases observed were within the laboratory 95% confidence interval for male rats, and (c) there is no biological explanation for the observation (such as splenic malfunction). NBuPy-Cl was negative in female rats and male and female mice in the micronucleus assay (Table D-8, Table D-12).

Occasional small increases in % PCE were observed in some of the animal groups, but none was associated with an effect on micronucleus frequencies and all were within the laboratory historical control 95% confidence interval. They do not suggest an effect on erythropoiesis.

Table D-5. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Male and Female Rats in the Three-month Drinking Water Study of 1-Ethyl-3-Methylimidazolium Chloride[a]

	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs (%)[b]	P Value[c]
Male							
Exposure Concentration (mg/mL)							
0	5	0.540 ± 0.076		0.068 ± 0.015		0.850 ± 0.021	
1	5	0.610 ± 0.080	0.3751	0.051 ± 0.013	0.7411	1.019 ± 0.039	0.1222
3	5	0.540 ± 0.043	0.4445	0.066 ± 0.007	0.8218	0.955 ± 0.039	0.1447
10	5	0.657 ± 0.092	0.1836	0.047 ± 0.006	0.8529	0.881 ± 0.061	0.1510
Trend[d]		p = 0.1559		p = 0.8595		p = 0.4519	
Female							
Exposure Concentration (mg/mL)							
0	5	0.780 ± 0.109		0.099 ± 0.014		0.811 ± 0.070	
1	5	0.560 ± 0.120	0.7612	0.065 ± 0.018	0.8421	0.936 ± 0.079	0.3839
3	5	0.670 ± 0.066	0.8393	0.082 ± 0.008	0.9050	1.030 ± 0.136	0.2406
10	5	0.630 ± 0.157	0.8682	0.076 ± 0.008	0.9268	1.160 ± 0.125	0.0546
Trend		p = 0.6555		p = 0.6985		p = 0.0494	

PCE = polychromatic erythrocyte; NCE = normochromatic erythrocyte.
[a]Study was performed at Integrated Laboratory Systems, LLC.
[b]Data are presented as mean ± standard error.
[c]Pairwise comparisons with the control group performed using the Williams or Dunn test ($p \leq 0.025$).
[d]Exposure concentration-related trends evaluated by linear regression or the Jonckheere test ($p \leq 0.025$).

Table D-6. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Male and Female Rats in the Three-month Drinking Water Study of 1-Butyl-3-Methylimidazolium Chloride[a]

	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs (%)[b]	P Value[c]
Male							
Exposure Concentration (mg/mL)							
0	5	0.420 ± 0.082		0.175 ± 0.037		0.892 ± 0.031	
0.1	5	0.400 ± 0.085	0.5367	0.137 ± 0.008	1.0000	0.835 ± 0.013	0.6568
0.3	5	0.440 ± 0.087	0.5311	0.191 ± 0.037	0.9457	0.948 ± 0.024	0.9295
1	5	0.430 ± 0.073	0.5627	0.089 ± 0.015	1.0000	1.053 ± 0.045	0.1267
Trend[d]		p = 0.4322		p = 0.9468		p = 0.0058	

	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs (%)[b]	P Value[c]
Female							
Exposure Concentration (mg/mL)							
0	5	0.660 ± 0.071		0.105 ± 0.027		0.829 ± 0.043	
0.1	5	0.550 ± 0.074	0.8557	0.074 ± 0.009	0.6790	0.806 ± 0.100	1.0000
0.3	5	0.410 ± 0.010	0.9157	0.099 ± 0.020	0.6686	1.114 ± 0.062	0.0226
1	5	0.580 ± 0.116	0.8697	0.116 ± 0.032	0.4791	1.048 ± 0.053	0.0975
Trend		p = 0.5711		p = 0.2058		p = 0.0087	

PCE = polychromatic erythrocyte; NCE = normochromatic erythrocyte.
*Statistically significant pairwise or trend test.
[a]Study was performed at Integrated Laboratory Systems, LLC.
[b]Data are presented as mean ± standard error.
[c]Pairwise comparisons with the control group performed using the Williams or Dunn test ($p \leq 0.025$).
[d]Exposure concentration-related trends evaluated by linear regression or the Jonckheere test ($p \leq 0.025$).

Table D-7. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Male and Female Rats in the Three-month Drinking Water Study of 1-Butyl-1-Methylpyrrolidinium Chloride[a]

	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs (%)[b]	P Value[c]
Male							
Exposure Concentration (mg/mL)							
0	5	0.440 ± 0.062		0.037 ± 0.005		0.984 ± 0.038	
0.3	5	0.810 ± 0.102	0.0023	0.018 ± 0.003	0.9602	0.867 ± 0.020	0.6756
1	5	0.890 ± 0.099	<0.001	0.016 ± 0.003	0.9838	1.024 ± 0.071	0.7961
3	5	1.000 ± 0.035	<0.001	0.022 ± 0.008	0.9893	0.985 ± 0.062	0.8386
Trend[d]		p = 0.0020		p = 0.8110		p = 0.5253	
Female							
Exposure Concentration (mg/mL)							
0	5	0.580 ± 0.064		0.048 ± 0.016		0.917 ± 0.103	
1	5	0.280 ± 0.051	0.9643	0.037 ± 0.005	0.6221	1.061 ± 0.183	0.7850
3	5	0.430 ± 0.046	0.9857	0.077 ± 0.020	0.3393	1.121 ± 0.087	0.3264
6	5	0.380 ± 0.072	0.9908	0.043 ± 0.007	0.3618	1.019 ± 0.064	1.0000
Trend		p = 0.7863		p = 0.5396		p = 0.5010	

PCE = polychromatic erythrocyte; NCE = normochromatic erythrocyte.
[a]Study was performed at Integrated Laboratory Systems, LLC.
[b]Data are presented as mean ± standard error.
[c]Pairwise comparisons with the control group performed using the Williams or Dunn test ($p \leq 0.025$).
[d]Exposure concentration-related trends evaluated by linear regression or the Jonckheere test ($p \leq 0.025$).

Table D-8. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Male and Female Rats in the Three-month Drinking Water Study of N-Butylpyridinium Chloride[a]

	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs (%)[b]	P Value[c]
Male							
Exposure Concentration (mg/mL)							
0	5	0.740 ± 0.094		0.049 ± 0.005		0.891 ± 0.033	
0.3	5	0.590 ± 0.053	0.7173	0.063 ± 0.014	0.6340	0.938 ± 0.078	0.7054
1	5	0.900 ± 0.097	0.4202	0.212 ± 0.043	0.0029	0.905 ± 0.024	0.8261
3	5	0.680 ± 0.109	0.4480	0.141 ± 0.024	0.0242	0.914 ± 0.037	0.8682
Trend[d]		p = 0.5187		p = 0.0019		p = 0.8940	
Female							
Exposure Concentration (mg/mL)							
0	5	0.690 ± 0.099		0.054 ± 0.016		0.931 ± 0.105	
0.3	5	1.094 ± 0.171	0.2413	0.197 ± 0.109	0.2991	1.000 ± 0.070	0.6851
1	5	0.730 ± 0.106	0.2907	0.066 ± 0.016	1.0000	0.948 ± 0.109	0.8053
3	5	0.600 ± 0.057	0.3093	0.030 ± 0.006	1.0000	1.328 ± 0.093	0.0150
Trend		p = 0.9356		p = 0.9306		p = 0.0080	

PCE = polychromatic erythrocyte; NCE = normochromatic erythrocyte.
[a]Study was performed at Integrated Laboratory Systems, LLC.
[b]Data are presented as mean ± standard error.
[c]Pairwise comparisons with the control group performed using the Williams or Dunn test ($p \leq 0.025$).
[d]Exposure concentration-related trends evaluated by linear regression or the Jonckheere test ($p \leq 0.025$).

Table D-9. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Male and Female Mice in the Three-month Drinking Water Study of 1-Ethyl-3-Methylimidazolium Chloride[a]

	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs (%)[b]	P Value[c]
Male							
Exposure Concentration (mg/mL)							
0	5	3.110 ± 0.245		1.653 ± 0.032		1.145 ± 0.088	
3	5	3.120 ± 0.248	0.5978	1.663 ± 0.042	0.4547	1.064 ± 0.057	1.0000
10	5	3.320 ± 0.312	0.6854	1.766 ± 0.076	0.2848	1.266 ± 0.079	0.2610
30	5	2.560 ± 0.076	0.7213	1.656 ± 0.060	0.3025	1.387 ± 0.016	0.0285
Trend[d]		p = 0.9672		p = 0.5300		p = 0.0049	
Female							
Exposure Concentration (mg/mL)							
0	5	1.520 ± 0.179		0.924 ± 0.032		1.066 ± 0.228	
3	5	1.720 ± 0.131	0.1868	0.967 ± 0.013	0.1077	1.352 ± 0.181	1.0000
10	5	1.850 ± 0.161	0.1331	1.027 ± 0.021	0.0039	1.533 ± 0.087	0.2615

	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs (%)[b]	P Value[c]
30	5	1.750 ± 0.143	0.1405	1.053 ± 0.023	<0.001	1.424 ± 0.118	0.8551
Trend		p = 0.2376		p = 0.0011		p = 0.2258	

PCE = polychromatic erythrocyte; NCE = normochromatic erythrocyte.
[a]Study was performed at Integrated Laboratory Systems, LLC.
[b]Data are presented as mean ± standard error.
[c]Pairwise comparisons with the control group performed using the Williams or Dunn test ($p \leq 0.025$).
[d]Exposure concentration-related trends evaluated by linear regression or the Jonckheere test ($p \leq 0.025$).

Table D-10. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Male and Female Mice in the Three-month Drinking Water Study of 1-Butyl-3-Methylimidazolium Chloride[a]

	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs (%)[b]	P Value[c]
Male							
Exposure Concentration (mg/mL)							
0	5	3.890 ± 0.375		1.736 ± 0.080		1.211 ± 0.030	
0.3	5	3.170 ± 0.135	0.9053	1.617 ± 0.054	0.8356	1.251 ± 0.032	1.0000
1	5	3.020 ± 0.225	0.9515	1.593 ± 0.047	0.9001	1.150 ± 0.021	0.6568
3	5	3.560 ± 0.296	0.8969	1.659 ± 0.061	0.9018	1.215 ± 0.057	1.0000
Trend[d]		p = 0.5067		p = 0.6191		p = 0.5448	
Female							
Exposure Concentration (mg/mL)							
0	5	2.590 ± 0.288		1.133 ± 0.041		1.495 ± 0.426	
0.3	5	3.680 ± 0.476	0.1105	1.177 ± 0.018	0.5453	1.490 ± 0.263	1.0000
1	5	2.970 ± 0.363	0.1343	1.152 ± 0.014	1.0000	1.085 ± 0.162	0.8403
3	5	3.030 ± 0.241	0.1420	1.167 ± 0.057	1.0000	1.365 ± 0.134	0.8810
Trend		p = 0.5116		p = 0.6317		p = 0.9722	

PCE = polychromatic erythrocyte; NCE = normochromatic erythrocyte.
[a]Study was performed at Integrated Laboratory Systems, LLC.
[b]Data are presented as mean ± standard error.
[c]Pairwise comparisons with the control group performed using the Williams or Dunn test ($p \leq 0.025$).
[d]Exposure concentration-related trends evaluated by linear regression or the Jonckheere test ($p \leq 0.025$).

Table D-11. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Male and Female Mice in the Three-month Drinking Water Study of 1-Butyl-1-Methylpyrrolidinium Chloride[a]

	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs (%)[b]	P Value[c]
Male							
Exposure Concentration (mg/mL)							
0	5	3.180 ± 0.255		1.573 ± 0.053		1.409 ± 0.060	
1	5	2.900 ± 0.299	0.7707	1.524 ± 0.040	0.6877	1.533 ± 0.032	0.2376
3	5	3.000 ± 0.267	0.8476	1.527 ± 0.037	0.7739	1.531 ± 0.066	0.2855
10	5	2.580 ± 0.147	0.8752	1.607 ± 0.048	0.3880	1.427 ± 0.045	0.3022
Trend[d]		p = 0.9380		p = 0.2116		p = 0.8299	
Female							
Exposure Concentration (mg/mL)							
0	5	2.600 ± 0.287		1.080 ± 0.034		1.312 ± 0.240	
1	5	1.861 ± 0.082	0.9663	1.057 ± 0.016	0.7957	1.105 ± 0.100	0.8426
3	5	1.790 ± 0.189	0.9866	0.990 ± 0.038	0.8687	1.193 ± 0.075	0.9445
6	5	2.380 ± 0.112	0.8929	1.027 ± 0.044	0.8941	1.365 ± 0.093	0.7231
Trend		p = 0.5239		p = 0.8812		p = 0.3302	

PCE = polychromatic erythrocyte; NCE = normochromatic erythrocyte.
[a]Study was performed at Integrated Laboratory Systems, LLC.
[b]Data are presented as mean ± standard error.
[c]Pairwise comparisons with the control group performed using the Williams or Dunn test ($p \leq 0.025$).
[d]Exposure concentration-related trends evaluated by linear regression or the Jonckheere test ($p \leq 0.025$).

Table D-12. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Male and Female Mice in the Three-month Drinking Water Study of N-Butylpyridinium Chloride[a]

	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs (%)[b]	P Value[c]
Male							
Exposure Concentration (mg/mL)							
0	5	4.240 ± 0.493		1.586 ± 0.042		1.250 ± 0.049	
1	5	4.380 ± 0.228	0.5725	1.741 ± 0.072	0.0763	1.137 ± 0.052	0.3546
3	5	4.220 ± 0.269	0.6602	1.669 ± 0.033	0.0918	1.157 ± 0.072	0.4291
6	5	3.770 ± 0.311	0.6964	1.757 ± 0.067	0.0292	1.191 ± 0.101	0.4565
Trend[d]		p = 0.8866		p = 0.0722		p = 0.6975	

	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs (%)[b]	P Value[c]
Female							
Exposure Concentration (mg/mL)							
0	5	2.380 ± 0.352		1.009 ± 0.020		1.381 ± 0.194	
1	5	3.009 ± 0.370	0.2360	1.038 ± 0.022	0.2999	1.004 ± 0.098	0.3159
3	5	2.717 ± 0.272	0.2850	1.032 ± 0.051	0.3595	0.993 ± 0.135	0.3804
6	5	2.510 ± 0.390	0.3028	1.092 ± 0.032	0.0612	1.424 ± 0.048	0.4062
Trend		p = 0.5786		p = 0.0468		p = 0.4197	

PCE = polychromatic erythrocyte; NCE = normochromatic erythrocyte.
[a]Study was performed at Integrated Laboratory Systems, LLC.
[b]Data are presented as mean ± standard error.
[c]Pairwise comparisons with the control group performed using the Williams or Dunn test ($p \leq 0.025$).
[d]Exposure concentration-related trends evaluated by linear regression or the Jonckheere test ($p \leq 0.025$).

Appendix E. Supplemental Data

Tables with supplemental data can be found here: https://doi.org/10.22427/NTP-DATA-TOX-103

E.1. 1-Ethyl-3-Methylimidazolium Chloride (Emim-Cl)

E.1.1. Two-week Study Tables – Rats

E03 – Growth Curves
0504903_EMIM_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
0504903_EMIM_E04_Mean_Body_Weights_and_Survival_Table.pdf

E05 – Clinical Observations Summary
0504903_EMIM_E05_Clinical_Observations_Summary.pdf

E07 – Mean Water Consumption by Treatment Group
0504903_EMIM_E07_Mean_Water_Consumption_by_Treatment_Group.pdf

E08 – Water and Compound Consumption Table
0504903_EMIM_E08_Water_and_Compound_Consumption_Table.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
0504903_EMIM_P03_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site.pdf

P09 – Non-Neoplastic Lesions by Individual Animal
0504903_EMIM_P09_Nonneoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions
0504903_EMIM_P10_Statistical_Analysis_of_Nonneoplastic_Lesions.pdf

P14 – Individual Animal Pathology Data
0504903_EMIM_P14_Individual_Animal_0504903_EMIM_PAthology_Data.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades
0504903_EMIM_P18_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

PA06 – Organ Weights Summary
0504903_EMIM_PA06_Organ_Weights_Summary.pdf

E.1.2. Two-week Individual Animal Data – Rats

Female Individual Animal Body Weight Data All Animals
0504903_EMIM_Female_Individual_Animal_Body_Weight_Data_All_Animals.xls

Female Individual Animal Clinical Observations
0504903_EMIM_Female_Individual_Animal_Clinical_Observations.xls

Female Individual Animal Neoplastic Pathology Data
0504903_EMIM_Female_Individual_Animal_Neoplastic_Pathology_Data.xls

Female Individual Animal Non-Neoplastic Pathology Data
0504903_EMIM_Female_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Female Individual Animal Survival Data
0504903_EMIM_Female_Individual_Animal_Survival_Data.xls

Female Individual Animal Terminal Body Weight Data
0504903_EMIM_Female_Individual_Animal_Terminal_Body_Weight_Data.xls

Male Individual Animal Body Weight Data All Animals
0504903_EMIM_Male_Individual_Animal_Body_Weight_Data_All_Animals.xls

Male Individual Animal Clinical Observations
0504903_EMIM_Male_Individual_Animal_Clinical_Observations.xls

Male Individual Animal Non-Neoplastic Pathology Data
0504903_EMIM_Male_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Male Individual Animal Survival Data
0504903_EMIM_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data
0504903_EMIM_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

Individual Animal Organ Weight Data
0504903_EMIM_Individual_Animal_Organ_Weight_Data.xlsx

E.1.3. Two-week Study Tables – Mice

E03 – Growth Curves
0504904_EMIM_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
0504904_EMIM_E04_Mean_Body_Weights_and_Survival_Table.pdf

E05 – Clinical Observations Summary
0504904_EMIM_E05_Clinical_Observations_Summary.pdf

E07 – Mean Water Consumption by Treatment Group
0504904_EMIM_E07_Mean_Water_Consumption_by_Treatment_Group.pdf

E08 – Water and Compound Consumption Table
0504904_EMIM_E08_Water_and_Compound_Consumption_Table.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
0504904_EMIM_P03_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site.pdf

P09 – Non-Neoplastic Lesions by Individual Animal
0504904_EMIM_P09_Nonneoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions
0504904_EMIM_P10_Statistical_Analysis_of_Nonneoplastic_Lesions.pdf

P14 – Individual Animal Pathology Data
0504904_EMIM_P14_Individual_Animal_0504904_EMIM_PAthology_Data.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades
0504904_EMIM_P18_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

PA06 – Organ Weights Summary
0504904_EMIM_PA06_Organ_Weights_Summary.pdf

E.1.4. Two-week Individual Animal Data – Mice

Female Individual Animal Body Weight Data All Animals
0504904_EMIM_Female_Individual_Animal_Body_Weight_Data_All_Animals.xls

Female Individual Animal Clinical Observations
0504904_EMIM_Female_Individual_Animal_Clinical_Observations.xls

Female Individual Animal Non-Neoplastic Pathology Data
0504904_EMIM_Female_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Female Individual Animal Survival Data
0504904_EMIM_Female_Individual_Animal_Survival_Data.xls

Female Individual Animal Terminal Body Weight Data
0504904_EMIM_Female_Individual_Animal_Terminal_Body_Weight_Data.xls

Male Individual Animal Body Weight Data All Animals
0504904_EMIM_Male_Individual_Animal_Body_Weight_Data_All_Animals.xls

Male Individual Animal Clinical Observations
0504904_EMIM_Male_Individual_Animal_Clinical_Observations.xls

Male Individual Animal Non-Neoplastic Pathology Data
0504904_EMIM_Male_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Male Individual Animal Survival Data
0504904_EMIM_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data
0504904_EMIM_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

Individual Animal Organ Weight Data
0504904_EMIM_Individual_Animal_Organ_Weight_Data.xlsx

E.1.5. Three-month Study Tables – Rats

E03 – Growth Curves
0701801_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
0701801_E04_Mean_Body_Weights_and_Survival_Table.pdf

E05 – Clinical Observations Summary
0701801_E05_Clinical_Observations_Summary.pdf

E07 – Mean Water Consumption by Treatment Group
0701801_E07_Mean_Water_Consumption_by_Treatment_Group.pdf

E08 – Feed Water and Compound Consumption Table
0701801_E08_Water_and_Compound_Consumption_Table.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
0701801_P03_Incidence_Rates_of_Nonn+H10eoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal
0701801_P04_Neoplasms_by_Individual_Animal.pdf

P09 – Non-Neoplastic Lesions by Individual Animal
0701801_P09_Nonneoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions
0701801_P10_Statistical_Analysis_of_Nonneoplastic_Lesions.pdf

P11 – Statistical Analysis of Survival Data
0701801_P11_Statistical_Analysis_of_Survival_Data.pdf

P14 – Individual Animal Pathology Data
0701801_P14_Individual_Animal_Pathology_Data.pdf

P17 – Neoplasms by Individual Animal (Systemic Lesions Abridged)
0701801_P17_Neoplasms_by_Individual_Animal_(Systemic_Lesions_Abridged).pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades
0701801_P18_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

P40 – Survival Curves
0701801_P40_Survival_Curves.pdf

PA06 – Organ Weights Summary
0701801_PA06_Organ_Weights_Summary.pdf

PA41 – Clinical Chemistry Summary
0701801_PA41_Clinical_Chemistry_Summary.pdf

PA43 – Hematology Summary
0701801_PA43_Hematology_Summary.pdf

Vaginal Cytology Markov Model
0701801_Vaginal_Cytology_Markov_Model.pdf

Vaginal Cytology Plots
0701801_Vaginal_Cytology_Plots.pdf

Vaginal Cytology Summary
C07018_Rats_Vaginal_Cytology_Summary.pdf

E.1.6. Three-month Individual Animal Data – Rats

Female Individual Animal Body Weight Data All Animals
0701801_Female_Individual_Animal_Body_Weight_Data_All_Animals.xls

Female Individual Animal Non-Neoplastic Pathology Data
0701801_Female_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Female Individual Animal Survival Data
0701801_Female_Individual_Animal_Survival_Data.xls

Female Individual Animal Terminal Body Weight Data
0701801_Female_Individual_Animal_Terminal_Body_Weight_Data.xls

Male Individual Animal Body Weight Data All Animals
0701801_Male_Individual_Animal_Body_Weight_Data_All_Animals.xls

Male Individual Animal Clinical Observations
0701801_Male_Individual_Animal_Clinical_Observations.xls

Male Individual Animal Non-Neoplastic Pathology Data
0701801_Male_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Male Individual Animal Survival Data
0701801_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data
0701801_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

Individual Animal Clinical Chemistry Data
0701801_Individual_Animal_Clinical_Chemistry_Data.xlsx

Individual Animal Hematology Data
0701801_Individual_Animal_Hematology_Data.xlsx

Individual Animal Organ Weight Data
0701801_Individual_Animal_Organ_Weight_Data.xlsx

E.1.7. Three-month Study Tables – Mice

E03 – Growth Curves
0701802_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
0701802_E04_Mean_Body_Weights_and_Survival_Table.pdf

E07 – Mean Water Consumption by Treatment Group
0701802_E07_Mean_Water_Consumption_by_Treatment_Group.pdf

E08 – Feed Water and Compound Consumption Table
0701802_E08_Water_and_Compound_Consumption_Table.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
0701802_P03_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal
0701802_P04_Neoplasms_by_Individual_Animal.pdf

P09 – Non-Neoplastic Lesions by Individual Animal
0701802_P09_Nonneoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions
0701802_P10_Statistical_Analysis_of_Nonneoplastic_Lesions.pdf

P11 – Statistical Analysis of Survival Data
0701802_P11_Statistical_Analysis_of_Survival_Data.pdf

P14 – Individual Animal Pathology Data
0701802_P14_Individual_Animal_Pathology_Data.pdf

P17 – Neoplasms by Individual Animal (Systemic Lesions Abridged)
0701802_P17_Neoplasms_by_Individual_Animal_(Systemic_Lesions_Abridged).pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades
0701802_P18_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

P40 – Survival Curves
0701802_P40_Survival_Curves.pdf

PA06 – Organ Weights Summary
0701802_PA06_Organ_Weights_Summary.pdf

PA43 – Hematology Summary
0701802_PA43_Hematology_Summary.pdf

Vaginal Cytology Markov Model
0701802_Vaginal_Cytology_Markov_Model.pdf

Vaginal Cytology Plots
0701802_Vaginal_Cytology_Plots.pdf

Vaginal Cytology Summary
0701802_Vaginal_Cytology_Summary.pdf

E.1.8. Three-month Individual Animal Data – Mice

Female Individual Animal Body Weight Data All Animals
0701802_Female_Individual_Animal_Body_Weight_Data_All_Animals.xls

Female Individual Animal Non-Neoplastic Pathology Data
0701802_Female_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Female Individual Animal Survival Data
0701802_Female_Individual_Animal_Survival_Data.xls

Female Individual Animal Terminal Body Weight Data
0701802_Female_Individual_Animal_Terminal_Body_Weight_Data.xls

Male Individual Animal Body Weight Data All Animals
0701802_Male_Individual_Animal_Body_Weight_Data_All_Animals.xls

Male Individual Animal Non-Neoplastic Pathology Data
0701802_Male_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Male Individual Animal Survival Data
0701802_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data
0701802_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

Individual Animal Hematology Data
0701802_Individual_Animal_Hematology_Data.xlsx

Individual Animal Organ Weight Data
0701802_Individual_Animal_Organ_Weight_Data.xlsx

E.2. 1-Butyl-3-Methylimidazolium Chloride (Bmim-Cl)

E.2.1. Two-week Study Tables – Rats

E03 – Growth Curves
0504903_BMIM_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
0504903_BMIM_E04_Mean_Body_Weights_and_Survival_Table.pdf

E05 – Clinical Observations Summary
0504903_BMIM_E05_Clinical_Observations_Summary.pdf

E07 – Mean Water Consumption by Treatment Group
0504903_BMIM_E07_Mean_Water_Consumption_by_Treatment_Group.pdf

E08 – Water and Compound Consumption Table
0504903_BMIM_E08_Water_and_Compound_Consumption_Table.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
0504903_BMIM_P03_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site.pdf

P09 – Non-Neoplastic Lesions by Individual Animal
0504903_BMIM_P09_Nonneoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions
0504903_BMIM_P10_Statistical_Analysis_of_Nonneoplastic_Lesions.pdf

P14 – Individual Animal Pathology Data
0504903_BMIM_P14_Individual_Animal_0504903_BMIM_PAthology_Data.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades
0504903_BMIM_P18_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

PA06 – Organ Weights Summary
0504903_BMIM_PA06_Organ_Weights_Summary.pdf

E.2.2. Two-week Individual Animal Data – Rats

Female Individual Animal Body Weight Data All Animals
0504903_BMIM_Female_Individual_Animal_Body_Weight_Data_All_Animals.xls

Female Individual Animal Clinical Observations
0504903_BMIM_Female_Individual_Animal_Clinical_Observations.xls

Female Individual Animal Non-Neoplastic Pathology Data
0504903_BMIM_Female_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Female Individual Animal Survival Data
0504903_BMIM_Female_Individual_Animal_Survival_Data.xls

Female Individual Animal Terminal Body Weight Data
0504903_BMIM_Female_Individual_Animal_Terminal_Body_Weight_Data.xls

Male Individual Animal Body Weight Data All Animals
0504903_BMIM_Male_Individual_Animal_Body_Weight_Data_All_Animals.xls

Male Individual Animal Non-Neoplastic Pathology Data
0504903_BMIM_Male_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Male Individual Animal Survival Data
0504903_BMIM_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data
0504903_BMIM_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

Individual Animal Organ Weight Data
0504903_BMIM_Individual_Animal_Organ_Weight_Data.xlsx

E.2.3. Two-week Study Tables – Mice

E03 – Growth Curves
0504904_BMIM_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
0504904_BMIM_E04_Mean_Body_Weights_and_Survival_Table.pdf

E05 – Clinical Observations Summary
0504904_BMIM_E05_Clinical_Observations_Summary.pdf

E07 – Mean Water Consumption by Treatment Group
0504904_BMIM_E07_Mean_Water_Consumption_by_Treatment_Group.pdf

E08 – Water and Compound Consumption Table
0504904_BMIM_E08_Water_and_Compound_Consumption_Table.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
0504904_BMIM_P03_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site.pdf

P09 – Non-Neoplastic Lesions by Individual Animal
0504904_BMIM_P09_Nonneoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions
0504904_BMIM_P10_Statistical_Analysis_of_Nonneoplastic_Lesions.pdf

P14 – Individual Animal Pathology Data
0504904_BMIM_P14_Individual_Animal_0504904_BMIM_PAthology_Data.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades
0504904_BMIM_P18_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

PA06 – Organ Weights Summary
0504904_BMIM_PA06_Organ_Weights_Summary.pdf

E.2.4. Two-week Individual Animal Data – Mice

Female Individual Animal Body Weight Data All Animals
0504904_BMIM_Female_Individual_Animal_Body_Weight_Data_All_Animals.xls

Female Individual Animal Survival Data
0504904_BMIM_Female_Individual_Animal_Survival_Data.xls

Female Individual Animal Terminal Body Weight Data
0504904_BMIM_Female_Individual_Animal_Terminal_Body_Weight_Data.xls

Male Individual Animal Body Weight Data All Animals
0504904_BMIM_Male_Individual_Animal_Body_Weight_Data_All_Animals.xls

Male Individual Animal Clinical Observations
0504904_BMIM_Male_Individual_Animal_Clinical_Observations.xls

Male Individual Animal Non-Neoplastic Pathology Data
0504904_BMIM_Male_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Male Individual Animal Survival Data
0504904_BMIM_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data
0504904_BMIM_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

Individual Animal Organ Weight Data
0504904_BMIM_Individual_Animal_Organ_Weight_Data.xlsx

E.2.5. Three-month Study Tables – Rats

E03 – Growth Curves
5005001_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
5005001_E04_Mean_Body_Weights_and_Survival_Table.pdf

E05 – Clinical Observations Summary
5005001_E05_Clinical_Observations_Summary.pdf

E07 – Mean Water Consumption by Treatment Group
5005001_E07_Mean_Water_Consumption_by_Treatment_Group.pdf

E08 – Feed Water and Compound Consumption Table
5005001_E08_Water_and_Compound_Consumption_Table.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
5005001_P03_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal
5005001_P04_Neoplasms_by_Individual_Animal.pdf

P09 – Non-Neoplastic Lesions by Individual Animal
5005001_P09_Nonneoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions
5005001_P10_Statistical_Analysis_of_Nonneoplastic_Lesions.pdf

P11 – Statistical Analysis of Survival Data
5005001_P11_Statistical_Analysis_of_Survival_Data.pdf

P14 – Individual Animal Pathology Data
5005001_P14_Individual_Animal_Pathology_Data.pdf

P17 – Neoplasms by Individual Animal (Systemic Lesions Abridged)
5005001_P17_Neoplasms_by_Individual_Animal_(Systemic_Lesions_Abridged).pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades
5005001_P18_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

P40 – Survival Curves
5005001_P40_Survival_Curves.pdf

PA06 – Organ Weights Summary
5005001_PA06_Organ_Weights_Summary.pdf

PA41 – Clinical Chemistry Summary
5005001_PA41_Clinical_Chemistry_Summary.pdf

PA43 – Hematology Summary
5005001_PA43_Hematology_Summary.pdf

Vaginal Cytology Markov Model
5005001_Vaginal_Cytology_Markov_Model.pdf

Vaginal Cytology Plots
5005001_Vaginal_Cytology_Plots.pdf

Vaginal Cytology Summary
C50050_Rats_Vaginal_Cytology_Summary.pdf

E.2.6. Three-month Individual Animal Data – Rats

Female Individual Animal Body Weight Data All Animals
5005001_Female_Individual_Animal_Body_Weight_Data_All_Animals.xls

Female Individual Animal Non-Neoplastic Pathology Data
5005001_Female_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Female Individual Animal Survival Data
5005001_Female_Individual_Animal_Survival_Data.xls

Female Individual Animal Terminal Body Weight Data
5005001_Female_Individual_Animal_Terminal_Body_Weight_Data.xls

Male Individual Animal Body Weight Data All Animals
5005001_Male_Individual_Animal_Body_Weight_Data_All_Animals.xls

Male Individual Animal Clinical Observations
5005001_Male_Individual_Animal_Clinical_Observations.xls

Male Individual Animal Non-Neoplastic Pathology Data
5005001_Male_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Male Individual Animal Survival Data
5005001_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data
5005001_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

Individual Animal Clinical Chemistry Data
5005001_Individual_Animal_Clinical_Chemistry_Data.xlsx

Individual Animal Hematology Data
5005001_Individual_Animal_Hematology_Data.xlsx

Individual Animal Organ Weight Data
5005001_Individual_Animal_Organ_Weight_Data.xlsx

E.2.7. Three-month Study Tables – Mice

E03 – Growth Curves
5005002_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
5005002_E04_Mean_Body_Weights_and_Survival_Table.pdf

E07 – Mean Water Consumption by Treatment Group
5005002_E07_Mean_Water_Consumption_by_Treatment_Group.pdf

E08 – Feed Water and Compound Consumption Table
5005002_E08_Water_and_Compound_Consumption_Table.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
5005002_P03_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal
5005002_P04_Neoplasms_by_Individual_Animal.pdf

P09 – Non-Neoplastic Lesions by Individual Animal
5005002_P09_Nonneoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions
5005002_P10_Statistical_Analysis_of_Nonneoplastic_Lesions.pdf

P11 – Statistical Analysis of Survival Data
5005002_P11_Statistical_Analysis_of_Survival_Data.pdf

P14 – Individual Animal Pathology Data
5005002_P14_Individual_Animal_Pathology_Data.pdf

P17 – Neoplasms by Individual Animal (Systemic Lesions Abridged)
5005002_P17_Neoplasms_by_Individual_Animal_(Systemic_Lesions_Abridged).pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades
5005002_P18_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

P40 – Survival Curves
5005002_P40_Survival_Curves.pdf

PA06 – Organ Weights Summary
5005002_PA06_Organ_Weights_Summary.pdf

PA43 – Hematology Summary
5005002_PA43_Hematology_Summary.pdf

Vaginal Cytology Markov Model
5005002_Vaginal_Cytology_Markov_Model.pdf

Vaginal Cytology Plots
5005002_Vaginal_Cytology_Plots.pdf

Vaginal Cytology Summary
C50050_Mice_Vaginal_Cytology_Summary.pdf

E.2.8. Three-month Individual Animal Data – Mice

Female Individual Animal Body Weight Data All Animals
5005002_Female_Individual_Animal_Body_Weight_Data_All_Animals.xls

Female Individual Animal Non-Neoplastic Pathology Data
5005002_Female_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Female Individual Animal Survival Data
5005002_Female_Individual_Animal_Survival_Data.xls

Female Individual Animal Terminal Body Weight Data
5005002_Female_Individual_Animal_Terminal_Body_Weight_Data.xls

Male Individual Animal Body Weight Data All Animals
5005002_Male_Individual_Animal_Body_Weight_Data_All_Animals.xls

Male Individual Animal Non-Neoplastic Pathology Data
5005002_Male_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Male Individual Animal Survival Data
5005002_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data
5005002_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

Individual Animal Hematology Data
5005002_Individual_Animal_Hematology_Data.xlsx

Individual Animal Organ Weight Data
5005002_Individual_Animal_Organ_Weight_Data.xlsx

E.3. 1-Butyl-1-Methylpyrrolidinium Chloride (Bmpy-Cl)

E.3.1. Two-week Study Tables – Rats

E03 – Growth Curves
0504903_BMPY_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
0504903_BMPY_E04_Mean_Body_Weights_and_Survival_Table.pdf

E07 – Mean Water Consumption by Treatment Group
0504903_BMPY_E07_Mean_Water_Consumption_by_Treatment_Group.pdf

E08 – Water and Compound Consumption Table
0504903_BMPY_E08_Water_and_Compound_Consumption_Table.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
0504903_BMPY_P03_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site.pdf

P09 – Non-Neoplastic Lesions by Individual Animal
0504903_BMPY_P09_Nonneoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions
0504903_BMPY_P10_Statistical_Analysis_of_Nonneoplastic_Lesions.pdf

P14 – Individual Animal Pathology Data
0504903_BMPY_P14_Individual_Animal_0504903_BMPY_PAthology_Data.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades
0504903_BMPY_P18_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

PA06 – Organ Weights Summary
0504903_BMPY_PA06_Organ_Weights_Summary.pdf

E.3.2. Two-week Individual Animal Data – Rats

Female Individual Animal Body Weight Data All Animals
0504903_BMPY_Female_Individual_Animal_Body_Weight_Data_All_Animals.xls

Female Individual Animal Non-Neoplastic Pathology Data
0504903_BMPY_Female_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Female Individual Animal Survival Data
0504903_BMPY_Female_Individual_Animal_Survival_Data.xls

Female Individual Animal Terminal Body Weight Data
0504903_BMPY_Female_Individual_Animal_Terminal_Body_Weight_Data.xls

Male Individual Animal Body Weight Data All Animals
0504903_BMPY_Male_Individual_Animal_Body_Weight_Data_All_Animals.xls

Male Individual Animal Non-Neoplastic Pathology Data
0504903_BMPY_Male_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Male Individual Animal Survival Data
0504903_BMPY_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data
0504903_BMPY_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

Individual Animal Organ Weight Data
0504903_BMPY_Individual_Animal_Organ_Weight_Data.xlsx

E.3.3. Two-week Study Tables – Mice

E03 – Growth Curves
0504904_BMPY_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
0504904_BMPY_E04_Mean_Body_Weights_and_Survival_Table.pdf

E05 – Clinical Observations Summary
0504904_BMPY_E05_Clinical_Observations_Summary.pdf

E07 – Mean Water Consumption by Treatment Group
0504904_BMPY_E07_Mean_Water_Consumption_by_Treatment_Group.pdf

E08 – Water and Compound Consumption Table
0504904_BMPY_E08_Water_and_Compound_Consumption_Table.pdf

P14 – Individual Animal Pathology Data
0504904_BMPY_P14_Individual_Animal_Pathology_Data.pdf

PA06 – Organ Weights Summary
0504904_BMPY_PA06_Organ_Weights_Summary.pdf

E.3.4. Two-week Individual Animal Data – Mice

Female Individual Animal Body Weight Data All Animals
0504904_BMPY_Female_Individual_Animal_Body_Weight_Data_All_Animals.xls

Female Individual Animal Survival Data
0504904_BMPY_Female_Individual_Animal_Survival_Data.xls

Female Individual Animal Terminal Body Weight Data
0504904_BMPY_Female_Individual_Animal_Terminal_Body_Weight_Data.xls

Male Individual Animal Body Weight Data All Animals
0504904_BMPY_Male_Individual_Animal_Body_Weight_Data_All_Animals.xls

Male Individual Animal Clinical Observations
0504904_BMPY_Male_Individual_Animal_Clinical_Observations.xls

Male Individual Animal Survival Data
0504904_BMPY_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data
0504904_BMPY_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

Individual Animal Organ Weight Data
0504904_BMPY_Individual_Animal_Organ_Weight_Data.xlsx

E.3.5. Three-month Study Tables – Rats

E03 – Growth Curves
5005101_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
5005101_E04_Mean_Body_Weights_and_Survival_Table.pdf

E05 – Clinical Observations Summary
5005101_E05_Clinical_Observations_Summary.pdf

E07 – Mean Water Consumption by Treatment Group
5005101_E07_Mean_Water_Consumption_by_Treatment_Group.pdf

E08 – Feed Water and Compound Consumption Table
5005101_E08_Water_and_Compound_Consumption_Table.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
5005101_P03_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal
5005101_P04_Neoplasms_by_Individual_Animal.pdf

P09 – Non-Neoplastic Lesions by Individual Animal
5005101_P09_Nonneoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions
5005101_P10_Statistical_Analysis_of_Nonneoplastic_Lesions.pdf

P11 – Statistical Analysis of Survival Data
5005101_P11_Statistical_Analysis_of_Survival_Data.pdf

P14 – Individual Animal Pathology Data
5005101_P14_Individual_Animal_Pathology_Data.pdf

P17 – Neoplasms by Individual Animal (Systemic Lesions Abridged)
5005101_P17_Neoplasms_by_Individual_Animal_(Systemic_Lesions_Abridged).pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades
5005101_P18_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

P40 – Survival Curves
5005101_P40_Survival_Curves.pdf

PA06 – Organ Weights Summary
5005101_PA06_Organ_Weights_Summary.pdf

PA41 – Clinical Chemistry Summary
5005101_PA41_Clinical_Chemistry_Summary.pdf

PA43 – Hematology Summary
5005101_PA43_Hematology_Summary.pdf

Vaginal Cytology Markov Model
0505101_Vaginal_Cytology_Markov_Model.pdf

Vaginal Cytology Plots
0505101_Vaginal_Cytology_Plots.pdf

Vaginal Cytology Summary
C05051_Rats_Vaginal_Cytology_Summary.pdf

E.3.6. Three-month Individual Animal Data – Rats

Female Individual Animal Body Weight Data All Animals
5005101_Female_Individual_Animal_Body_Weight_Data_All_Animals.xls

Female Individual Animal Clinical Observations
5005101_Female_Individual_Animal_Clinical_Observations.xls

Female Individual Animal Non-Neoplastic Pathology Data
5005101_Female_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Female Individual Animal Survival Data
5005101_Female_Individual_Animal_Survival_Data.xls

Female Individual Animal Terminal Body Weight Data
5005101_Female_Individual_Animal_Terminal_Body_Weight_Data.xls

Male Individual Animal Body Weight Data All Animals
5005101_Male_Individual_Animal_Body_Weight_Data_All_Animals.xls

Male Individual Animal Clinical Observations
5005101_Male_Individual_Animal_Clinical_Observations.xls

Male Individual Animal Non-Neoplastic Pathology Data
5005101_Male_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Male Individual Animal Survival Data
5005101_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data
5005101_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

Individual Animal Clinical Chemistry Data
5005101_Individual_Animal_Clinical_Chemistry_Data.xlsx

Individual Animal Hematology Data
5005101_Individual_Animal_Hematology_Data.xlsx

Individual Animal Organ Weight Data
5005101_Individual_Animal_Organ_Weight_Data.xlsx

E.3.7. Three-month Study Tables – Mice

E03 – Growth Curves
5005102_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
5005102_E04_Mean_Body_Weights_and_Survival_Table.pdf

E07 – Mean Water Consumption by Treatment Group
5005102_E07_Mean_Water_Consumption_by_Treatment_Group.pdf

E08 – Feed Water and Compound Consumption Table
5005102_E08_Water_and_Compound_Consumption_Table.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
5005102_P03_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal
5005102_P04_Neoplasms_by_Individual_Animal.pdf

P05 – Incidence Rates of Neoplasms by Anatomic Site (Systemic Lesions Abridged)
5005102_P05_Incidence_Rates_of_Neoplasms_by_Anatomic_Site_(Systemic_Lesions_Abridged).pdf

P09 – Non-Neoplastic Lesions by Individual Animal
5005102_P09_Nonneoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions
5005102_P10_Statistical_Analysis_of_Nonneoplastic_Lesions.pdf

P11 – Statistical Analysis of Survival Data
5005102_P11_Statistical_Analysis_of_Survival_Data.pdf

P14 – Individual Animal Pathology Data
5005102_P14_Individual_Animal_Pathology_Data.pdf

P17 – Neoplasms by Individual Animal (Systemic Lesions Abridged)
5005102_P17_Neoplasms_by_Individual_Animal_(Systemic_Lesions_Abridged).pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades
5005102_P18_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

P40 – Survival Curves
5005102_P40_Survival_Curves.pdf

PA06 – Organ Weights Summary
5005102_PA06_Organ_Weights_Summary.pdf

PA43 – Hematology Summary
5005102_PA43_Hematology_Summary.pdf

Vaginal Cytology Markov Model
0505102_Vaginal_Cytology_Markov_Model.pdf

Vaginal Cytology Plots
0505102_Vaginal_Cytology_Plots.pdf

Vaginal Cytology Summary
C05051_Mice_Vaginal_Cytology_Summary.pdf

E.3.8. Three-month Individual Animal Data – Mice

Female Individual Animal Body Weight Data All Animals
5005102_Female_Individual_Animal_Body_Weight_Data_All_Animals.xls

Female Individual Animal Non-Neoplastic Pathology Data
5005102_Female_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Female Individual Animal Survival Data
5005102_Female_Individual_Animal_Survival_Data.xls

Female Individual Animal Terminal Body Weight Data
5005102_Female_Individual_Animal_Terminal_Body_Weight_Data.xls

Male Individual Animal Body Weight Data All Animals
5005102_Male_Individual_Animal_Body_Weight_Data_All_Animals.xls

Male Individual Animal Neoplastic Pathology Data
5005102_Male_Individual_Animal_Neoplastic_Pathology_Data.xls

Male Individual Animal Non-Neoplastic Pathology Data
5005102_Male_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Male Individual Animal Survival Data
5005102_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data
5005102_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

Individual Animal Hematology Data
5005102_Individual_Animal_Hematology_Data.xlsx

Individual Animal Organ Weight Data
5005102_Individual_Animal_Organ_Weight_Data.xlsx

E.4. N-Butylpyridinium Chloride (NBuPy-Cl)

E.4.1. Two-week Study Tables – Rats

E03 – Growth Curves
0504903_NBUPY_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
0504903_NBUPY_E04_Mean_Body_Weights_and_Survival_Table.pdf

E05 – Clinical Observations Summary
0504903_NBUPY_E05_Clinical_Observations_Summary.pdf

E07 – Mean Water Consumption by Treatment Group
0504903_NBUPY_E07_Mean_Water_Consumption_by_Treatment_Group.pdf

E08 – Water and Compound Consumption Table
0504903_NBUPY_E08_Water_and_Compound_Consumption_Table.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
0504903_NBUPY_P03_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site.pdf

P09 – Non-Neoplastic Lesions by Individual Animal
0504903_NBUPY_P09_Nonneoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions
0504903_NBUPY_P10_Statistical_Analysis_of_Nonneoplastic_Lesions.pdf

P14 – Individual Animal Pathology Data
0504903_NBUPY_P14_Individual_Animal_Pathology_Data.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades
0504903_NBUPY_P18_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

PA06 – Organ Weights Summary
0504903_NBUPY_PA06_Organ_Weights_Summary.pdf

E.4.2. Two-week Individual Animal Data – Rats

Female Individual Animal Body Weight Data All Animals
0504903_NBUPY_Female_Individual_Animal_Body_Weight_Data_All_Animals.xls

Female Individual Animal Clinical Observations
0504903_NBUPY_Female_Individual_Animal_Clinical_Observations.xls

Female Individual Animal Non-Neoplastic Pathology Data
0504903_NBUPY_Female_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Female Individual Animal Survival Data
0504903_NBUPY_Female_Individual_Animal_Survival_Data.xls

Female Individual Animal Terminal Body Weight Data
0504903_NBUPY_Female_Individual_Animal_Terminal_Body_Weight_Data.xls

Male Individual Animal Body Weight Data All Animals
0504903_NBUPY_Male_Individual_Animal_Body_Weight_Data_All_Animals.xls

Male Individual Animal Clinical Observations
0504903_NBUPY_Male_Individual_Animal_Clinical_Observations.xls

Male Individual Animal Non-Neoplastic Pathology Data
0504903_NBUPY_Male_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Male Individual Animal Survival Data
0504903_NBUPY_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data
0504903_NBUPY_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

Individual Animal Organ Weight Data
0504903_NBUPY_Individual_Animal_Organ_Weight_Data.xlsx

E.4.3. Two-week Study Tables – Mice

E03 – Growth Curves
0504904_NBUPY_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
0504904_NBUPY_E04_Mean_Body_Weights_and_Survival_Table.pdf

E05 – Clinical Observations Summary
0504904_NBUPY_E05_Clinical_Observations_Summary.pdf

E07 – Mean Water Consumption by Treatment Group
0504904_NBUPY_E07_Mean_Water_Consumption_by_Treatment_Group.pdf

E08 – Water and Compound Consumption Table
0504904_NBUPY_E08_Water_and_Compound_Consumption_Table.pdf

P14 – Individual Animal Pathology Data
0504904_NBUPY_P14_Individual_Animal_Pathology_Data.pdf

PA06 – Organ Weights Summary
0504904_NBUPY_PA06_Organ_Weights_Summary.pdf

E.4.4. Two-week Individual Animal Data – Mice

Female Individual Animal Body Weight Data All Animals
0504904_NBUPY_Female_Individual_Animal_Body_Weight_Data_All_Animals.xls

Female Individual Animal Clinical Observations
0504904_NBUPY_Female_Individual_Animal_Clinical_Observations.xls

Female Individual Animal Survival Data
0504904_NBUPY_Female_Individual_Animal_Survival_Data.xls

Female Individual Animal Terminal Body Weight Data
0504904_NBUPY_Female_Individual_Animal_Terminal_Body_Weight_Data.xls

Male Individual Animal Body Weight Data All Animals
0504904_NBUPY_Male_Individual_Animal_Body_Weight_Data_All_Animals.xls

Male Individual Animal Clinical Observations
0504904_NBUPY_Male_Individual_Animal_Clinical_Observations.xls

Male Individual Animal Survival Data
0504904_NBUPY_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data
0504904_NBUPY_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

Individual Animal Organ Weight Data
0504904_NBUPY_Individual_Animal_Organ_Weight_Data.xlsx

E.4.5. Three-month Study Tables – Rats

E03 – Growth Curves
5005201_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
5005201_E04_Mean_Body_Weights_and_Survival_Table.pdf

E05 – Clinical Observations Summary
5005201_E05_Clinical_Observations_Summary.pdf

E07 – Mean Water Consumption by Treatment Group
5005201_E07_Mean_Water_Consumption_by_Treatment_Group.pdf

E08 – Feed Water and Compound Consumption Table
5005201_E08_Water_and_Compound_Consumption_Table.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
5005201_P03_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal
5005201_P04_Neoplasms_by_Individual_Animal.pdf

P09 – Non-Neoplastic Lesions by Individual Animal
5005201_P09_Nonneoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions
5005201_P10_Statistical_Analysis_of_Nonneoplastic_Lesions.pdf

P11 – Statistical Analysis of Survival Data
5005201_P11_Statistical_Analysis_of_Survival_Data.pdf

P14 – Individual Animal Pathology Data
5005201_P14_Individual_Animal_Pathology_Data.pdf

P17 – Neoplasms by Individual Animal (Systemic Lesions Abridged)
5005201_P17_Neoplasms_by_Individual_Animal_(Systemic_Lesions_Abridged).pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades
5005201_P18_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

P40 – Survival Curves
5005201_P40_Survival_Curves.pdf

PA06 – Organ Weights Summary
5005201_PA06_Organ_Weights_Summary.pdf

PA41 – Clinical Chemistry Summary
5005201_PA41_Clinical_Chemistry_Summary.pdf

PA43 – Hematology Summary
5005201_PA43_Hematology_Summary.pdf

Vaginal Cytology Markov Model
0505201_Vaginal_Cytology_Markov_Model.pdf

Vaginal Cytology Plots
0505201_Vaginal_Cytology_Plots.pdf

Vaginal Cytology Summary
5005201_Vaginal_Cytology_Summary.pdf

E.4.6. Three-month Individual Animal Data – Rats

Female Individual Animal Body Weight Data All Animals
5005201_Female_Individual_Animal_Body_Weight_Data_All_Animals.xls

Female Individual Animal Clinical Observations
5005201_Female_Individual_Animal_Clinical_Observations.xls

Female Individual Animal Non-Neoplastic Pathology Data
5005201_Female_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Female Individual Animal Survival Data
5005201_Female_Individual_Animal_Survival_Data.xls

Female Individual Animal Terminal Body Weight Data
5005201_Female_Individual_Animal_Terminal_Body_Weight_Data.xls

Male Individual Animal Body Weight Data All Animals
5005201_Male_Individual_Animal_Body_Weight_Data_All_Animals.xls

Male Individual Animal Clinical Observations
5005201_Male_Individual_Animal_Clinical_Observations.xls

Male Individual Animal Non-Neoplastic Pathology Data
5005201_Male_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Male Individual Animal Survival Data
5005201_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data
5005201_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

Individual Animal Clinical Chemistry Data
5005201_Individual_Animal_Clinical_Chemistry_Data.xlsx

Individual Animal Hematology Data
5005201_Individual_Animal_Hematology_Data.xlsx

Individual Animal Organ Weight Data
5005201_Individual_Animal_Organ_Weight_Data.xlsx

E.4.7. Three-month Study Tables – Mice

E03 – Growth Curves
5005202_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
5005202_E04_Mean_Body_Weights_and_Survival_Table.pdf

E07 – Mean Water Consumption by Treatment Group
5005202_E07_Mean_Water_Consumption_by_Treatment_Group.pdf

E08 – Feed Water and Compound Consumption Table
5005202_E08_Water_and_Compound_Consumption_Table.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
5005202_P03_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal
5005202_P04_Neoplasms_by_Individual_Animal.pdf

P05 – Incidence Rates of Neoplasms by Anatomic Site (Systemic Lesions Abridged)
5005202_P05_Incidence_Rates_of_Neoplasms_by_Anatomic_Site_(Systemic_Lesions_Abridged).pdf

P09 – Non-Neoplastic Lesions by Individual Animal
5005202_P09_Nonneoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions
5005202_P10_Statistical_Analysis_of_Nonneoplastic_Lesions.pdf

P11 – Statistical Analysis of Survival Data
5005202_P11_Statistical_Analysis_of_Survival_Data.pdf

P14 – Individual Animal Pathology Data
5005202_P14_Individual_Animal_Pathology_Data.pdf

P17 – Neoplasms by Individual Animal (Systemic Lesions Abridged)
5005202_P17_Neoplasms_by_Individual_Animal_(Systemic_Lesions_Abridged).pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades
5005202_P18_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

P40 – Survival Curves
5005202_P40_Survival_Curves.pdf

PA06 – Organ Weight Summary
5005202_PA06_Organ_Weight_Summary.pdf

PA43 – Hematology Summary
5005202_PA43_Hematology_Summary.pdf

Vaginal Cytology Markov Model
0505202_Vaginal_Cytology_Markov_Model.pdf

Vaginal Cytology Plots
0505202_Vaginal_Cytology_Plots.pdf

Vaginal Cytology Summary
5005202_Vaginal_Cytology_Summary.pdf

E.4.8. Three-month Individual Animal Data – Mice

Female Individual Animal Body Weight Data All Animals
5005202_Female_Individual_Animal_Body_Weight_Data_All_Animals.xls

Female Individual Animal Non-Neoplastic Pathology Data
5005202_Female_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Female Individual Animal Survival Data
5005202_Female_Individual_Animal_Survival_Data.xls

Female Individual Animal Terminal Body Weight Data
5005202_Female_Individual_Animal_Terminal_Body_Weight_Data.xls

Male Individual Animal Body Weight Data All Animals
5005202_Male_Individual_Animal_Body_Weight_Data_All_Animals.xls

Male Individual Animal Non-Neoplastic Pathology Data
5005202_Male_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Male Individual Animal Survival Data
5005202_Male_Individual_Animal_Survival_Data.xls

Male Individual Animal Terminal Body Weight Data
5005202_Male_Individual_Animal_Terminal_Body_Weight_Data.xls

Individual Animal Hematology Data
5005202_Individual_Animal_Hematology_Data.xlsx

Individual Animal Organ Weight Data
5005202_Individual_Animal_Organ_Weight_Data.xlsx

E.5. Genetic Toxicology

E.5.1. 1-Ethyl-3-Methylimidazolium Chloride Micronucleus Study in Sprague Dawley Rats

G04 In Vivo Micronucleus Summary Data
G07018_G04_In_Vivo_Micronucleus_Summary_Data.pdf

Individual Animal In Vivo Micronucleus Data
G07018_Individual_Animal_In_Vivo_Micronucleus_Data.xlsx

E.5.2. 1-Ethyl-3-Methylimidazolium Chloride Micronucleus Study in B6C3F1 Mice

G04 In Vivo Micronucleus Summary Data
G07018B_G04_In_Vivo_Micronucleus_Summary_Data.pdf

Individual Animal In Vivo Micronucleus Data
G07018B_Individual_Animal_In_Vivo_Micronucleus_Data.xlsx

E.5.3. 1-Ethyl-3-Methylimidazolium Chloride Ames Test

G06 Ames Summary Data
A09944_G06_Ames_Summary_Data.pdf

E.5.4. 1-Butyl-3-Methylimidazolium Chloride Micronucleus Study in Sprague Dawley Rats

G04 In Vivo Micronucleus Summary Data
G05050_G04_In_Vivo_Micronucleus_Summary_Data.pdf

Individual Animal In Vivo Micronucleus Data
G05050_Individual_Animal_In_Vivo_Micronucleus_Data.xlsx

E.5.5. 1-Butyl-3-Methylimidazolium Chloride Micronucleus Study in B6C3F1 Mice

G04 In Vivo Micronucleus Summary Data
G05050B_G04_In_Vivo_Micronucleus_Summary_Data.pdf

Individual Animal In Vivo Micronucleus Data
G05050B_Individual_Animal_In_Vivo_Micronucleus_Data.xlsx

E.5.6. 1-Butyl-3-Methylimidazolium Chloride Ames Test

G06 Ames Summary Data
A33096_G06_Ames_Summary_Data.pdf

E.5.7. 1-Butyl-1-Methylpyrrolidinium Chloride Micronucleus Study in Sprague Dawley Rats

G04 In Vivo Micronucleus Summary Data
G03021_G04_In_Vivo_Micronucleus_Summary_Data.pdf

Individual Animal In Vivo Micronucleus Data
G03021_Individual_Animal_In_Vivo_Micronucleus_Data.xlsx

E.5.8. 1-Butyl-1-Methylpyrrolidinium Chloride Micronucleus Study in B6C3F1 Mice

G04 In Vivo Micronucleus Summary Data
G03021B_G04_In_Vivo_Micronucleus_Summary_Data.pdf

Individual Animal In Vivo Micronucleus Data
G03021B_Individual_Animal_In_Vivo_Micronucleus_Data.xlsx

E.5.9. 1-Butyl-1-Methylpyrrolidinium Chloride Ames Test

G06 Ames Summary Data
A74446_G06_Ames_Summary_Data.pdf

E.5.10. N-Butylpyridinium Chloride Micronucleus Study in Sprague Dawley Rats

G04 In Vivo Micronucleus Summary Data
G03020_G04_In_Vivo_Micronucleus_Summary_Data.pdf

Individual Animal In Vivo Micronucleus Data
G03020_Individual_Animal_In_Vivo_Micronucleus_Data.xlsx

E.5.11. N-Butylpyridinium Chloride Micronucleus Study in B6C3F1 Mice

G04 In Vivo Micronucleus Summary Data
G03020B_G04_In_Vivo_Micronucleus_Summary_Data.pdf

Individual Animal In Vivo Micronucleus Data
G03020B_Individual_Animal_In_Vivo_Micronucleus_Data.xlsx

E.5.12. N-Butylpyridinium Chloride Ames Test

G06 Ames Summary Data
A75013_G06_Ames_Summary_Data.pdf

National Toxicology Program
National Institute of Environmental Health Sciences
National Institutes of Health
P.O. Box 12233, MD K2-05
Durham, NC 27709
Tel: 984-287-3211
ntpwebrequest@niehs.nih.gov

https://ntp.niehs.nih.gov

ISSN 2378-8992