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MINIMAL URANIUM IMMUNOTOXICITY FOLLOWING A 60-DAY DRINKING WATER EXPOSURE TO URANYL ACETATE IN MALE AND FEMALE C57BL/6J MICE

Alicia M. Bolt^{a,b,*}, Sebastian Medina^{a,b}, Fredine T. Lauer^a, Ke Jian Liu^a, and Scott W. Burchiel^a

^aThe University of New Mexico College of Pharmacy, Department of Pharmaceutical Sciences, Albuquerque, NM 87131

^bCo-first authors

Abstract

Historical uranium (U) mining in the Southwestern United States resulted in significant environmental contamination throughout this region and presents a significant risk of chronic metal exposure and toxicity for communities living in close proximity to mine waste sites. Uranium exposure is associated with numerous deleterious health effects including immune dysfunction; however, its effects on the immune system have yet to be fully characterized. We recently published that drinking water exposure to U, in the form of uranyl acetate (UA), results in overall low tissue retention of U (<0.01%), with very little accumulation in immune organs (blood, bone marrow, spleen, and thymus) of male and female mice. In the present study we characterized the immunotoxicity of U, in the form of UA, following a 60-day drinking water exposure to 5 and 50 ppm in male and female C57BL/6J mice. The following immunotoxicity endpoints were evaluated: hematology, immune tissue weights and total cell recoveries, immunophenotyping of the spleen and thymus, and immune cell function (lymphocyte mitogenesis and T-dependent antibody response). Uranium exposure had subtle impacts on the immune endpoints evaluated, likely due to low U accumulation at these sites. The only significant alterations were a slight decrease in the percentages of splenic natural killer T-cells and macrophages in exposed male mice. Despite minimal immunological effects, this study highlights the importance of investigating toxicological endpoints in both sexes and developing accurate animal models that model epidemiological exposures in the future.

Keywords

uranium; drinking water exposure; immunotoxicity; humoral immunity; innate immunity

*To whom correspondence should be addressed. Alicia M. Bolt, Department of Pharmaceutical Science, College of Pharmacy, MSC09 5360, 1 University of New Mexico, Albuquerque, NM 87131. ambolt@salud.unm.edu.

Conflict of interest statement

We declare that we have no conflicts of interest.

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INTRODUCTION

Historical mining operations in the Southwestern United States have resulted in a legacy of uranium (U) and other heavy metal contamination of water, soil, and air (Orescanin et al., 2011; Blake et al., 2015; Hoover et al., 2017). This is particularly concerning for many Native American communities living in close proximity to the more than 500 abandoned U mines located throughout the Navajo Nation (Hoover et al., 2017). Many of these rural communities rely on unregulated drinking water sources (i.e., well water) that exceed the United States Environmental Protection Agency and World Health Organization maximum contaminant level for U of 30 ppb (US EPA 2006; WHO 2012; deLemos et al., 2009; Hoover et al., 2017). As a result, many people in this region are chronically exposed to U at levels that have been linked to a multitude of detrimental health outcomes, including immune dysfunction (Lu-Fritts et al., 2014; Lourenço et al., 2013; and Erdei et al., 2019). In particular, data from Native American populations living in close proximity to abandoned mine waste sites indicate a correlation between uranium and other metal exposures and the presence of serological indicators of autoimmunity (i.e., increased prevalence of anti-nuclear antibodies) (Erdei et al., 2019; Ong et al., 2014).

Despite the documented associations between U and mixed mine waste exposures with immunological alterations in human populations, the effects of U on the immune system have not been fully characterized. Importantly, there has only been one previous study evaluating the immunotoxicity of U in mice (Hao et al., 2013), which reported significant immune dysregulation associated with chronic dietary U intake in male mice.

However, no *in vivo* studies have investigated the chronic effects of drinking water exposure to U at environmentally relevant concentrations, which is a primary route of U exposure in humans (Hoover et al., 2017). Nor, have any such studies been performed using both male and female mice. The goal of the present study was to characterize the immunotoxicity of U, in the form of uranyl acetate (UA), following a 60-day drinking water exposure to 5 and 50 ppm in male and female C57BL/6J mice.

METHODS

Uranium

Uranyl acetate (98% purity, $\text{UO}_2(\text{OCOCH}_3)_2 \cdot 2\text{H}_2\text{O}$; U^{235} depleted, U^{238} – 99.9%, U^{235} – 0.1%) was purchased from Electron Microscopy Services, Hatfield, PA. The specific radioactivity of UA is 51 $\mu\text{Ci/g}$. Uranium is toxic and radioactive and was handled in accordance with regulations and safety procedures established by the Radiation Safety Office at the University of New Mexico.

Mouse Drinking Water Exposures

All animal experiments were performed in accordance with protocols approved by the Institutional Animal Use and Care Committee at the University of New Mexico Health Sciences Center (UNM HSC). Male and Female, wild-type C57BL/6J mice were purchased at 6 wks of age from Jackson Laboratory (Bar Harbor, MA). Mice were provided food and

water *ad libitum* and were acclimated in the UNM HSC Animal Resource Facility for 1 wk prior to the onset of exposures. For drinking water exposures, mice were divided into 3 groups with 7 mice per group: control (tap water), 5 or 50 ppm U. To model environmental exposures from abandoned U mine waste, we used uranyl acetate (UA), which is U²³⁵ depleted. Stock solutions of UA were prepared in sterile water at the initiation of the experiment and used throughout the duration of the study. Concentration of stock UA solution was confirmed by ICP-MS analysis. UA was diluted into mouse drinking pouches to yield final dosing concentrations of 5 or 50 ppm U. The concentration of U used for dosing in our study represent elemental U, as opposed to the dehydrate compound ($1.8 \text{ g UO}_2(\text{OCOCH}_3)_2 \cdot 2\text{H}_2\text{O} = 1 \text{ g U}$). The volume of stock UA added to each water pouch was determined by weighing the water pouch and estimating the volume of water based on the weight (1 g = 1 mL water). All U doses were prepared fresh weekly. Water bags were collected and weighed at the end of each week and the change in weight was used to estimate water consumption by mice in each cage.

Blood collection and hematological analysis

Following the 60-day exposure, mice were by CO₂ asphyxiation. Blood was collected from each mouse by cardiac puncture using a 1 mL syringe and 25-G needle and transferred into a EDTA and heparin coated 250 µL tubes (Greiner bio-one, Monroe, NC) for complete blood count and biochemical analyses, respectively. Complete blood counts with differential was performed using an Abaxis VetScan HM5 hematology analyzer (Abaxis, Union City, CA). Blood (100 µL) was transferred to a Comprehensive Diagnostic Profile reagent rotor (VetScan) for biochemical analysis using an Abaxis VetScan VS2.

Isolation of Thymus and Spleen Cells

Spleen and thymus cells were isolated as described by Xu et al., (2016). In summary, the spleen and thymus were harvested from each mouse and were homogenized between the frosted ends of two sterile microscope slides (Fisher Scientific, Pittsburgh, PA) in a dish containing 5 mL of cold mouse medium (500 ml RPMI 1640 with 10% FBS, 2 mM L-glutamine, and 100 U/mL Pen/Strep). The cell suspensions were transferred to a 15 mL centrifuge tube, centrifuged at $200 \times g$ for 10 mins, and resuspended in 10 mL of cold mouse media medium. Cell viabilities and concentrations were determined using AO/PI staining and a Nexcelom Cellometer® Auto 2000 (Nexcelom Bioscience, Manchester, UK).

Flow cytometry

Immune cell subsets were evaluated in single cell suspensions of spleen and thymus tissue based on surface marker phenotypes. 1×10^6 cells from the thymus or spleen of each mouse were transferred to 12×75 mm tubes and stained in 100 µL of flow stain/wash buffer (Dulbecco's phosphate-buffered saline (DPBS) without calcium and magnesium with 2% heat inactivated FBS and 0.09% sodium azide) with 0.5 µg of surface marker specific, fluorochrome conjugated monoclonal antibodies for 30 mins at RT. The following fluorochrome conjugated monoclonal antibodies (purchased from BD Biosciences, San Jose, CA) were used to identify immune cell subsets in the spleen and thymus: rat anti-mouse CD45-APC, CD3e-PerCP Cy5.5, CD4-PE, CD8a-FITC, F4/80-Alexa647, and Nk1.1-PE; and thymus: rat anti-mouse CD8a-FITC, CD4-APC, and CD3e-PECy7. In the spleen, T-cell

subsets were identified as CD45⁺, CD3e⁺, CD4^{+/-} and CD8^{+/-}; B-cells as CD45⁺, CD19⁺; macrophage as CD45⁺, F4/80⁺ and natural killer (NK) as CD45⁺, CD3e⁻, NK1.1⁺ and NK T-cells as CD45⁺, CD3e⁺, NK1.1⁺. T-cell subsets in the thymus were identified based on CD3e⁺, CD4^{+/-}, and CD8a^{+/-}. After staining, samples were washed twice and resuspended in 0.5 mL flow stain/wash buffer. Samples were analyzed using an Accuri™ C6 flow cytometer (BD Biosciences, San Jose, CA).

Ex Vivo Lymphocyte Mitogenesis

Splenocyte mitogenesis was performed following procedures described in detail by Burchiel et al., 2009. Spleen cells were plated at 2×10^5 cells (in replicates of 6/mouse) in wells of a 96-well U-bottom plate. Splenocytes were stimulated with either 1 µg/mL concanavalin-A (Con-A; T-cell mitogen; Sigma Aldrich) or 10 µg/mL lipopolysaccharide (LPS; B-cell mitogen; Enzo Life Sciences) for 48 h. After 48 h simulation, cells were pulsed with 1 µCi tritiated thymidine (Perkin Elmer) and incubated for 18 h. Cells from each well were then harvested onto glass fiber filters (Brandel, Gaithersburg, MD) using a Brandel Model M-96T cell harvester (Brandel, Gaithersburg, MD) and lysed with a 0.05% (v/v) Tween-20 solution. Dried filter paper samples were then transferred into liquid scintillation vials containing 3 mL ScintiVerse scintillation fluid (Fisher Scientific) and allowed to sit at RT for 30 mins. Tritiated thymidine incorporation was then assessed by liquid scintillation counting using a Beckman Coulter LS 6500 Multi-Purpose Scintillation Counter (Beckman Coulter, Indianapolis, IN).

Ex Vivo T-Dependent Antibody Response Assay

Ex vivo T-dependent antibody response assay was performed as described by Li et al., 2010. Spleen cells were plated at 4×10^6 cells (in replicates of 6/mouse) and immunized *ex vivo* in a humidified incubator at 37°C for 4 days using a 10% (v/v) solution of sheep red blood cells (SRBC; Colorado Serum, Denver Colorado) prepared in plaque-forming cell medium (RPMI, 10 % heat inactivated FBS, 2 mM L-Glutamine, 100 U/mL Penicillin with 100 µg/mL streptomycin sulfate, 1 mM sodium pyruvate, 50 µg/mL gentamycin, 50 µM β-mercaptoethanol). Following the 4 day immunization, cells were collected, washed, and 2×10^6 cells were added to a 0.8% (w/v) Sea Plaque agarose (Lonza, Rockland, ME) solution in RPMI media containing 50 µL of 10% SRBC. The cell/agarose solution was poured and spread evenly across the surface of a 0.15% agarose-coated glass microscope slide. Slides were incubated for 1.5 h at 37°C without CO₂. After 1.5 h, the slides were flooded with a 1:20 (v/v) solution of guinea pig complement (Colorado Serum, Denver, Colorado) in DPBS with calcium and magnesium and placed back into incubator for 2 h at 37°C without CO₂. Slides were then immersed in 0.85% (w/v) sodium chloride. The number of anti-SRBC plaque-forming cells were counted based on visible plaque formation in the agarose using a low power dissecting microscope and an indirect light source.

Statistics

Data were analyzed with Excel 2010 and Sigma Plot version 12.5 software. Differences between control and treatment groups were determined using a one-way analysis of variance (ANOVA) and Dunnett's post-hoc test. In cases that data was not normally distributed, a Kruskal-Wallis ANOVA was performed to evaluate differences between control and

treatment groups. Statistically significant differences between control and treatment groups were identified as $p < 0.05$.

RESULTS

Uranium exposure does not significantly alter mouse body weights, water intake, or spleen and thymus tissue weight, cell recoveries or cell viabilities

The objective of this study was to characterize the immunotoxicity of U in male and female C57BL/6J mice following a drinking water exposure to UA (5 and 50 ppm U) for 60 days. Over the course of the 60-day exposure, there were no significant differences in daily water intake for either sex or exposure groups. Additionally, there were no changes in total body weights between control and U exposed groups in male or female mice. There were also no overt signs of immune toxicity, including differences in immune tissue weights, total number of cells recovered, or cell viabilities (Table 1).

In vivo uranium exposure does not significantly alter hematology or blood biochemical parameters

To determine if U alters immune cells in the blood, a complete blood count and differential was performed on each mouse. There were no significant differences in red blood cell (RBC) parameters (RBC counts, hemoglobin levels, mean corpuscular hemoglobin (MCH), MCH concentration, and hematocrit) or white blood cell (WBC) parameters (WBC counts, lymphocyte, monocyte, or neutrophil counts) in either sex (Table 2). Additionally, as a verification that U did not produce any significant kidney or liver injury, we evaluated blood biochemical parameters (Table 2). No significant changes in blood biochemical indicators of kidney and liver function (i.e., blood urea nitrogen, creatinine, total protein, globulin, alanine aminotransferase, or alkaline phosphatase) were found in male or female mice at either the 5 or 50 ppm U dose (Table 2). These findings indicate that exposure to U at concentrations as high as 50 ppm, did not induce hematological toxicity or overt liver or kidney injury in this study.

Uranium decreases macrophages and NK T-Cells in the spleen of male mice

In an effort to understand whether U exposure disrupts immune cell differentiation or induces cell-specific cytotoxicity, immune cell subsets were evaluated in the spleen and thymus based on cell surface marker phenotypes. In the spleen, we found subtle, yet statistically significant reductions in the percentage of macrophages and NK T-cells at 5 and 50 ppm in U exposed males, but not females (Fig. 1A and B). No changes in the number of total splenic B-cells or T-cell subsets (T helper, CD3e⁺, CD4⁺, CD8a⁻; cytotoxic T cells, CD3e⁺, CD4⁻, CD8a⁺) were identified following U exposure in male or female mice (Fig. 1C and D). Additionally, no significant differences in thymic T-cell subsets (double negative, CD3⁺, CD4⁻, CD8⁻; double positive, CD3⁺, CD4⁺, CD8⁺; T helper CD3e⁺, CD4⁺, CD8⁻; cytotoxic CD3e⁺, CD4⁻, CD8a⁺) were identified in male or female mice (Fig. 2 A and B). Collectively, these findings show that U produced a modest sex-specific reduction of innate immune cells in male mice, but did not significantly alter the proportions of adaptive immune cells in the spleen or thymus of either sex.

Uranium does not alter T-dependent antibody response to sheep red blood cells or B- and T-cell mitogenesis

To determine whether U exposure disrupts the function of mature lymphocytes in the spleen, we performed two independent assays to measure the *ex vivo* immune response of B- and T-cells. To measure T-dependent antibody response, splenocytes were collected from each mouse and immunized *ex vivo* with SRBC and the numbers of anti-SRBC plaque forming cells was determined. There were no significant differences in the number of plaques formed following U exposure in males or females (Fig. 3A and B), indicating that U did not alter SRBC-induced T-dependent antibody production. Additionally, we measured splenocyte mitogenesis based on tritiated thymidine incorporation following *ex vivo* stimulation of splenocytes with a B- cell mitogen (LPS) or T cell mitogen (Con-A). No significant differences in mitogen-induced B- or T-cell proliferation were detected following U exposure in either sex (Figure 3C and D) Taken together, these results provide evidence that U does not significantly disrupt the function of mature immune cells in the spleen following a 60-day drinking water U exposure.

DISCUSSION

The goal of the current study was to evaluate the immunotoxicity of U in male and female mice following a 60-day drinking water exposure to environmentally relevant levels of U (Bolt et al., 2018), using a set of conventional immunotoxicity assays (Burchiel et al., 2009; Boverhof et al., 2014). To our knowledge, this is the first study to evaluate the immunotoxicity of U (in the form of UA) following a chronic drinking water exposure in both male and female mice. In this study, we identified minimal immunotoxic effects of U. Subtle changes in percentages of macrophages and NK T-cells in male mice, suggesting potential sex-specific differences in the toxicity of U on certain innate immune cell populations.

In an effort to better understand the minimal immunotoxicity observed in this study, we also evaluated the distribution of U in immune tissues of male and female mice following a 60-day drinking water exposure to 5 and 50 ppm U (Bolt et al., 2018). Not surprisingly, we found very little U accumulation in the spleen and thymus. The lack of U exposure at these sites is likely responsible for the minimal immunotoxicity observed in this study. Interestingly, we did observe a slight accumulation of U in the blood and bone marrow of male, but not female mice, which might explain the slight sex-specific differences observed. Bone and bone marrow were found to be sites of significant U accumulation (Bolt et al., 2018) and as a result, immune cell populations in the bone marrow will be a focus of our future immunotoxicological investigations.

Multiple studies in human populations report evidence of immunotoxicity in human populations chronically exposed to U. Exposure to elevated levels of U was found to be associated with increased incidence of systemic lupus erythematosus in a cohort of individuals living near a U processing plant (Lu-Fritts et al., 2014). Recent findings from Erdei et al., (2019), indicate elevation of serological autoantibodies in Native Americans living in close proximity to abandoned uranium mine wastes sites. Additionally, individuals living near abandoned U mine sites had decreased NK and T-cell counts in peripheral blood

(Lourengo et al., 2013). These findings were consistent with our results showing that oral U exposure in male mice decreased the percentage of NK T-cells in the spleen. Sex-specific increases in susceptibility to autoimmunity have been reported in populations exposed to heavy metals (Nielsen and Hultman 2002; Ong et al., 2014; Lu-Fritts et al., 2014). These studies indicate sex-specific differences in immune disorders and highlight the importance of performing animal studies using both sexes. Consistent with these studies, in our study, we identified a subtle male-specific reduction of innate immune cell subsets in the spleen, which suggests differential sensitivities between males and females to U-induced immunotoxicity.

To date, very few rodent studies have been conducted to evaluate the immunotoxicity of U, and to our knowledge, no studies have included females or evaluated the immunotoxicity of U following oral drinking water exposures. At exposure levels similar to those used in the present study (i.e., 3 and 30 ppm), Hao et al., (2013), found that male mice exposed to uranyl nitrate for 120 days showed significant immune dysregulation induced by chronic dietary U intake. This was concluded to result in a shift in the balance of T helper (Th) 1 and Th2 cells (in favor of Th2), promoting a phenotype that may increase susceptibility to autoimmune diseases and infections.

The discrepancy between our findings and those reported by Hao et al., (2013) may be attributed to a number of factors, including the strain of mice used (Kunming vs. C57BL/6J), duration of exposure (120 vs. 60 days), and form of U used (uranyl nitrate vs. UA). Another study, by Dublineau et al., (2014), did not evaluate immune endpoints directly, but found that male rats exposed to U (in the form of uranyl nitrate) at levels as high as 120 ppm for 9 months did not have significant alterations in immune parameters in the blood (WBC, lymphocyte, monocyte, granulocyte, and neutrophil counts), which is consistent with results from the present study. Interestingly, they did find increased inflammatory cytokine gene expression in the intestines without consistent increases in protein expression (Dublineau et al., 2014). While this is evidence of immune modulation, these findings somewhat contrasts the findings presented by Hao et al., (2013), which observed an increase in Th2 cytokines (IL-4 and IL-10) and a decrease in Th1 cytokines (IFN- γ and TNF- α). Similarly, Zychowski et al., (2018) reported an increase in inflammatory cytokines in lung bronchoalveolar lavage fluid following acute U exposure. Future studies investigating cytokine production in immune and other tissues following drinking water U exposure will be important for understanding the immunotoxicity of U. Additionally, discrepancies between these studies highlight the importance of determining which routes and forms of U are most appropriate for use in animal models to accurately model human exposures to U.

This study has some limitations that should be considered when interpreting the data and designing experiments in the future. In the current study, we evaluated oral drinking water exposure to U because it is a major route of exposure for humans, however people are exposed through other routes, in particular inhalation of contaminated dust is another potential source of exposure. As such, alternative routes of U exposure should be evaluated for immunological endpoints in future studies. In addition, utilizing uranium salts such as UA or uranyl nitrate as model compounds for environmental U exposures are not completely reflective of human exposures to mine wastes due to differences in chemical forms of U present as well as the presence of other co-occurring metals, which could impact

toxicological investigations. Identification of proper animal models for understanding the immune dysregulation observed in human populations, including identifying appropriate routes of exposure and chemical forms of uranium, are needed to accurately assess the immunotoxicity of U.

Additionally, in both humans and rodents, the overall gastrointestinal absorption of U is low; however, studies report the percentage of U absorption is higher in humans (~0.2–6%) than rodents (~0.04–0.8%) (Sullivan, 1980; Harrison and Stather 1981; Sullivan, et al., 1986; Wrenn et al., 1989; Bhattacharyya et al., 1989; Zamora et al., 2003). Humans are exposed to U via multiple routes (e.g., inhalation, food, etc.), whereas mice in this study were only exposed to U through drinking water. The major sites of U deposition are similar between humans and rodents, with the bone and kidneys being the major sites of U accumulation. The patterns of U deposition are largely the same, but the exposure burden at these sites is likely greater among humans as result of increased absorption and longer duration of exposures. Differences in exposure routes, absorption, along with the chronic nature of human exposures may account for the differential immunotoxicity of U in humans compared to mice.

Results from this study show that drinking water exposure to U did not produce significant immunotoxicological alterations in male or female mice, likely resulting from limited U accumulation in immune organs (Bolt et al., 2018). We found subtle, albeit important sex-specific differences in U immunotoxicity in male and female mice. Innate immune cell populations (macrophages and NK T-cells) in the spleen of male mice may be sensitive to U-induced toxicity. As a result, this may increase the susceptibility of males to U-induced immune dysregulation. These findings highlight the necessity of performing toxicological investigations in both sexes and for developing animal models that accurately model human exposures.

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REFERENCES

- Bhattacharyya MH, et al. (1989). Gastrointestinal absorption of plutonium and uranium in feed and fasted adult baboons and mice: application to humans. *Radiat. Prot. Dosimetry* 26, 159–165.
- Blake JM, et al. (2015). Elevated concentrations of U and co-occurring metals in abandoned mine wastes in a northeastern Arizona Native American community. *Environ. Sci. Technol* 49(14), 8506–8514. [PubMed: 26158204]
- Bolt AM, et al. (2018). Minimal uranium accumulation in lymphoid tissues following an oral 60-day uranyl acetate exposure in male and female C57BL/6J mice. *PLOS One*. 13(10), e0205211. [PubMed: 30356336]

- Boverhof DR, et al. (2014). Approaches and considerations for the assessment of immunotoxicity for environmental chemicals: A workshop summary. *Regul. Toxicol. Pharmacol* 68, 96–107 [PubMed: 24280359]
- Burchiel SW, et al. (2009). Immunotoxicity and biodistribution analysis of arsenic trioxide in C57Bl/6 mice following a 2-week inhalation exposure. *Toxicol. Appl. Pharmacol* 241(3), 253–259. [PubMed: 19800901]
- deLemos JL, et al. (2009). Development of risk maps to minimize uranium exposures in the Navajo Churchrock mining district. *Environ. Health* 8(1), 29. [PubMed: 19589163]
- Dublineau I, et al. (2014). Unexpected lack of deleterious effects of uranium on physiological systems following a chronic oral intake in adult rat. *Biomed. Res. Int* 2014, 181989. [PubMed: 24693537]
- Erdei E, et al. (2019). Elevated autoimmunity in residents living near abandoned uranium mine sites on the Navajo Nation. *J. Autoimmunity* 3 13.
- Hao Y, et al. (2013). Immunological changes of chronic oral exposure to depleted uranium in mice. *Toxicology*. 309, 81–90. [PubMed: 23659960]
- Harrison JD, Stather JW (1981). The gastrointestinal absorption of protactinium, uranium, and neptunium in the hamster. *Radiat. Res* 88, 47–55. [PubMed: 7302131]
- Hoover J, et al. (2017). Elevated arsenic and uranium concentrations in unregulated water sources on the Navajo Nation, USA. *Exposure and Health*. 9(2), 113–124. [PubMed: 28553666]
- Lourenço J, et al. (2013). Biomonitoring a human population inhabiting nearby a deactivated uranium mine. *Toxicology*. 305, 89–98. [PubMed: 23370006]
- Lu-Fritts PY, et al. (2014). Association of systemic lupus erythematosus with uranium exposure in a community living near a uranium-processing plant: a nested case-control study. *Arthritis Rheumatol*. 66(11), 3105–3112. [PubMed: 25103365]
- Nielsen JB, Hultman P (2002). Mercury-induced autoimmunity in mice. *Environ. Health Perspect* 5, 877–81.
- Ong J, et al. (2014). Mercury, autoimmunity, and environmental factors on Cheyenne River Sioux Tribal lands. *Autoimmune Dis*. 2014, 325461. [PubMed: 24864198]
- Orescanin V, et al. (2011). Characterization and treatment of water used for human consumption from six sources located in the Cameron/Tuba City abandoned uranium mining area. *J. Environ. Sci. Health A* 46(6), 627–635.
- Sullivan FM (1980). Absorption of actinide elements from the gastrointestinal tract of rats, guinea pigs and dogs. *Health Phys*. 38, 159–171. [PubMed: 6246038]
- Sullivan MF, et al. (1986). Influence of oxidizing or reducing Agents on gastrointestinal absorption of U, Am, Cm and Pm by rats. *Health Phys*. 50, 223–232. [PubMed: 3005196]
- U.S. Environmental Protection Agency. Basic information about radionuclides in drinking water. Washington DC 2006; Available from: <https://safewater.zendesk.com/hc/en-us/sections/202346157>
- World Health Organization. Uranium in drinking-water. Geneva Switzerland: WHO Press 2012; 1–21. Available from http://www.who.int/water_sanitation_health/publications/2012/background_uranium.pdf
- Wrenn ME, et al. (1989). Gastrointestinal absorption of soluble uranium from drinking water by man. *Radiat. Prot. Dosimetry* 26, 119–122.
- Xu H, et al. (2016). Environmentally-relevant concentrations of arsenite induce dose-dependent differential genotoxicity through Poly(ADP-ribose) polymerase (PARP) inhibition and oxidative stress in mouse thymus cells. *Toxicol. Sci* 149, 31–41. [PubMed: 26443841]
- Zamora ML, et al. (2003). Uranium gastrointestinal absorption: the F1 factor in humans. *Radiat. Prot. Dosimetry* 105, 55–60. [PubMed: 14526927]
- Zychowski KE, et al. (2018). Respirable uranyl-vanadate-containing particulate matter derived from a legacy uranium mine site exhibits potentiated cardiopulmonary toxicity. *Toxicol. Sci* 164(1), 101–114. [PubMed: 29660078]

HIGHLIGHTS

- Drinking water uranium exposure (uranyl acetate) produced minimal immunotoxicity in male or female C57BL/6J mice.
- Innate immune cell subsets were slightly reduced in male, but not female mice.
- Mature splenic lymphocyte function was not significantly altered in male or female mice.

Figure 1.

Immunophenotyping in the spleen of male and female mice following oral exposure to 0, 5, or 50 ppm uranium (in the form of uranyl acetate) for 60 days. Percentage of B-cells ($CD45^+$, $CD19^+$), macrophages ($CD45^+$, $F4/80^+$), natural killer (NK) cells ($CD45^+$, $CD3e^-$, $NK1.1^+$), and NK T-cells ($CD45^+$, $CD3e^+$, $NK1.1^+$) in the spleen of **A.** male and **B.** female mice. Percentage of T-cell subsets ($CD45^+$, $CD3e^+$, $CD4^{+/-}$ and $CD8^{+/-}$) in the spleen of **C.** male and **D.** female mice. Data are expressed as percentage of total spleen cells (mean $\pm$ SD). $n = 7$ mice/group; *Statistically significant difference compared to control ($p < 0.05$).

Figure 2.

Immunophenotyping in the thymus of male and female mice following oral exposure to 0, 5, or 50 ppm uranium (in the form of uranyl acetate) for 60 days. Percentage of T-cell subsets ($CD3e^+$, $CD4^{+/-}$, and $CD8^{+/-}$) in the thymus of **A.** male and **B.** female mice. DN = double negative cells, $CD4^-/CD8^-$. DP = double positive cells, $CD4^+/CD8^+$. Data are expressed as percentage of total thymus cells (mean $\pm$ SD); $n = 7$ mice/group. *Statistically significant difference compared to control ($p < 0.05$).

Figure 3.

Immune function of mature splenic lymphocytes of male and female mice following oral exposure to 0, 5, or 50 ppm uranium (in the form of uranyl acetate) for 60 days. T-dependent antibody response to sheep red blood cells in **A.** male and **B.** female mice. Data are expressed as the number of plaques per culture (4×10^6 cells) $\pm$ SD; $n = 7$ mice/Group. B- and T-cell proliferation following stimulation with LPS (B cell mitogen) and Con-A (T cell mitogen) measured by tritiated thymidine incorporation using liquid scintillation counting in **C.** male and **D.** female mice. Data are expressed as mean counts per minute (CPM) $\pm$ SD; $n = 7$ mice/group. *Statistically significant difference compared to control ($p < 0.05$).

Table 1.

Mouse body weights, spleen and thymus weights, cell recoveries and viabilities.^{a,b}

Sex	Exposure	Body Wt. (g)	Spleen Wt. (mg)	Spleen Cell Recovery ($\times 10^6$ cells)	Spleen Cell Viability (%)	Thymus Wt. (mg)	Thymus Cell Recovery ($\times 10^6$ cells)	Thymus Cell Viability (%)
Male	Control	30.08 $\pm$ 1.80	91.86 $\pm$ 9.75	131.33 $\pm$ 11.57	76.74 $\pm$ 2.90	57.30 $\pm$ 10.80	50.83 $\pm$ 19.22	89.03 $\pm$ 2.36
	5 ppm U	30.76 $\pm$ 2.22	86.29 $\pm$ 11.28	133.06 $\pm$ 13.03	74.00 $\pm$ 3.17	74.10 $\pm$ 7.72	68.13 $\pm$ 15.41	88.40 $\pm$ 1.48
	50ppm U	31.65 $\pm$ 3.11	86.00 $\pm$ 2.24	124.26 $\pm$ 18.17	72.86 $\pm$ 1.98	72.87 $\pm$ 20.02	65.07 $\pm$ 21.68	88.30 $\pm$ 1.95
Female	Control	23.90 $\pm$ 2.48	99.10 $\pm$ 9.48	141.22 $\pm$ 23.44	74.63 $\pm$ 5.45	56.00 $\pm$ 10.80	84.71 $\pm$ 22.62	91.30 $\pm$ 1.57
	5 ppm U	24.32 $\pm$ 2.53	92.00 $\pm$ 7.59	126.40 $\pm$ 14.86	74.36 $\pm$ 3.96	47.90 $\pm$ 20.55	73.60 $\pm$ 16.71	89.86 $\pm$ 1.56
	50ppm U	25.20 $\pm$ 2.17	99.60 $\pm$ 15.31	140.73 $\pm$ 26.24	75.81 $\pm$ 6.24	52.40 $\pm$ 7.84	75.93 $\pm$ 17.90	90.06 $\pm$ 1.84

^aData expressed as mean $\pm$ SD of each group (control, 5 and 50 ppm uranium).

^bStatistical significance was determined using a one-way ANOVA with a Dunnett's post-hoc test ($p < 0.05$) compared to control values for each category.

Hematology and blood biochemical parameters in male and female mice following a 60-day drinking water exposure to uranium.^{a,b,c}

Table 2.

Sex	Exposure	RBC (×10 ¹² /L)	Hgb (g/dL)	MCH (pg)	HCT (%)	WBC (×10 ⁹ /L)	Lym. (×10 ⁹ /L)	Mon. (×10 ⁹ /L)	Neut. (×10 ⁹ /L)	BUN (g/dL)	TP (g/dL)	GLOB (g/dL)	CRE (g/dL)	ALT (U/L)	ALP (U/L)
Male	Control	10.30 ± 0.90	15.0 ± 0.90	14.70 ± 1.10	44.9 ± 3.8	9.90 ± 1.00	7.50 ± 0.70	0.40 ± 0.20	2.00 ± 0.80	2.60 ± 0.30	6.00 ± 0.30	2.10 ± 0.30	0.04 ± 0.02	50.80 ± 19.20	41.00 ± 23.00
	5 ppm U	10.50 ± 0.40	15.30 ± 0.60	14.60 ± 0.80	45.90 ± 1.50	9.10 ± 2.70	7.30 ± 2.10	0.40 ± 0.20	1.40 ± 0.80	2.90 ± 0.50	6.30 ± 0.20	2.00 ± 0.20	0.03 ± 0.02	35.70 ± 1.50	42.20 ± 13.60
	50ppm U	10.50 ± 0.20	15.20 ± 0.30	14.40 ± 0.40	46.20 ± 1.20	8.80 ± 1.10	7.10 ± 1.10	0.40 ± 0.10	1.20 ± 0.30	2.70 ± 0.30	6.50 ± 0.50	2.00 ± 0.20	0.03 ± 0.01	45.00 ± 11.50	39.00 ± 19.30
Female															
	Control	9.68 ± 0.59	13.70 ± 1.08	14.10 ± 0.38	43.00 ± 2.70	6.62 ± 1.86	5.63 ± 1.55	0.27 ± 0.15	0.71 ± 0.38	2.50 ± 0.50	6.00 ± 0.30	1.40 ± 0.10	0.05 ± 0.03	35.40 ± 9.30	73.20 ± 19.80
	5 ppm U	9.91 ± 0.62	14.20 ± 0.98	14.3 ± 0.45	43.60 ± 2.50	8.44 ± 1.36	7.08 ± 1.19	0.49 ± 0.29	0.87 ± 0.30	2.60 ± 0.20	5.90 ± 0.40	1.40 ± 0.20	0.02 ± 0.10	29.40 ± 8.40	78.00 ± 25.20
	50ppm U	9.65 ± 0.81	13.90 ± 1.20	14.4 ± 0.20	42.80 ± 3.20	7.12 ± 2.07	5.68 ± 1.53	0.39 ± 0.35	1.04 ± 0.70	2.80 ± 0.70	5.80 ± 0.20	1.40 ± 0.20	0.05 ± 0.01	30.50 ± 6.60	87.0 ± 17.50

^aRBC = total red blood cell count, Hgb = hemoglobin, MCH = mean corpuscular hemoglobin, HCT = hematocrit, WBC = total white blood cell count, Lym. = lymphocytes, Mon. = monocytes, Neut. = neutrophils, BUN = blood urea nitrogen, TP = total protein, GLOB = globulin, CRE = creatinine, ALT = alanine aminotransferase, and ALP = alkaline phosphatase.

^bData expressed as mean ± SD of each group (control, 5 and 50 ppm uranium).

^cStatistical significance was determined using a one-way ANOVA with a Dunnett’s post-hoc test (p<0.05) compared to control values for each category.