REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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1. REPORT DATE *(DD-MM-YYYY)*
17-08-2018

2. REPORT TYPE
Journal Article

3. DATES COVERED *(From - To)*

4. TITLE AND SUBTITLE
The cardiopulmonary effects of sodium fluoroacetate (1080) in Sprague-Dawley rats
rats

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
McCranor, BJ, Young, TD, Tressler, JA, Jennings, L, Irwin, J, Ali, NA, Abilez, MK,
Stone, S, Racine, M, Devorak, JL, Sciuto, AM, Wong, B

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
US Army Medical Research Institute of
Chemical Defense
8350 Ricketts Point Road
Aberdeen Proving Ground, MD
21010-5400

8. PERFORMING ORGANIZATION REPORT NUMBER
USAMRICD-P18-016

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
National Institutes of Health
National Institute of Allergy & Infectious
Diseases
5601 Fishers Lane
Bethesda, MD 20892

10. SPONSOR/MONITOR'S ACRONYM(S)
NIAID

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES Published in Cogent Biology, 5, 1568669, 2019. The research described was supported by an interagency agreement (AOD18015-001-00000) between the NIH Office of the Director and the USAMRICD under the oversight of the Chemical Countermeasures Research Program within the Office of Biodefense Research at NIAID.

14. ABSTRACT
See reprint.

15. SUBJECT TERMS
Sodium fluoroacetate; 1080; metabolic poison; cardiopulmonary

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Bryan J. McCranor
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UNLIMITED	19	19b. TELEPHONE NUMBER *(include area code)* 410-436-9354

cogent biology

TOXICOLOGY | RESEARCH ARTICLE

The cardiopulmonary effects of sodium fluoroacetate (1080) in Sprague-Dawley rats

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Received: 23 October 2018
Accepted: 08 January 2019
First Published: 17 January 2019

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Abstract: Sodium fluoroacetate (1080) is a highly toxic metabolic poison that has the potential because of its lack of defined color, odor, and taste and its high water solubility to be intentionally or unintentionally ingested through food adulteration. Although the mechanism of action for 1080 has been known since the 1950s, no known antidote exists. In an effort to better understand the cardiopulmonary impacts of 1080, we utilized whole-body plethysmography and telemeterized Sprague-Dawley rats which allowed for the real-time measurement of respiratory and cardiac parameters following exposure using a non-invasive assisted-drinking method. Overall, the animals showed marked depression of respiratory parameters over the course of 24 h post-exposure and the development of hemorrhage in the lung tissue. Tidal volume was reduced by 30% in males and 60% in females at 24 h post-exposure, and respiratory frequency was significantly depressed as well. In telemeterized female rats, we observed severe cardiac abnormalities, highlighted by a 50% reduction in heart rate, 75% reduction in systolic blood pressure, and a 3.5-fold lengthening of the QRS interval over the course of 24 h. We also observed a reduction in core body temperature of nearly 15°C. Our study was able to describe the severe and pronounced effects of sodium fluoroacetate poisoning on cardio-pulmonary function, the results of which indicate that both tissue specific and systemic deficits contribute to the toxicological progression of 1080 intoxication

ABOUT THE AUTHOR

Bryan J. McCranor is a research biochemist at the US Army Medical Research Institute of Chemical Defense where he is a principal investigator with the Inhalation Toxicology Team. Overall, his research focuses on the development of novel prophylactic, therapeutic and medical countermeasure treatments for exposure to chemical threat agents and toxic industrial compounds. His work centers on the identification of toxicological characteristics of toxic chemicals, investigation of potential novel therapeutic targets, and advancement of medical countermeasures.

PUBLIC INTEREST STATEMENT

The U.S. Army Medical Research Institute of Chemical Defense (USAMRICD) is the United States' leading science and technology laboratory in the development of medical chemical countermeasures. At USAMRICD, the Inhalation Toxicology Team aims to support military and civilian populations through research in toxicology aimed at discovering and developing medical products and knowledge solutions. This group has used its expertise in the fields of toxic chemical toxicology and physiology to investigate the effects of sodium fluoroacetate (1080) on lung and heart function. Using real-time physiological measurements in a rat model, the team was able to visualize the significant impact of 1080 on both heart and lung function over the course of 24 h. In developing future treatments against 1080 intoxication, investigators will have to account for this severe weakening of heart and lung function.

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and will need to be accounted for when developing any potential countermeasure for 1080 poisoning.

Subjects: Toxicology; Pesticides; Biology

Keywords: Sodium fluoroacetate; 1080; metabolic poison; cardiopulmonary

1. Introduction

Sodium fluoroacetate (1080) is a colorless, odorless, tasteless, water-soluble metabolic poison that inhibits the citric acid cycle and reduces the rate of cellular metabolism (Goncharov, 2009; Parton, 2013). The United States tightly regulates the availability of the extremely toxic 1080 to sheep and goat farmers for managing coyote predation (Parton, 2013; Services, 2010), although other nations, including Australia, New Zealand, Mexico, Japan, South Korea, and Israel, use it as a rodenticide to control invasive and nuisance mammals (Eason, 2002; Proudfoot, Bradberry, & Vale, 2006; Sherley, 2007). Due to 1080's lack of a defining sensory characteristic and ease of solubility, it has the potential for harm through means of intentional or unintentional food adulteration. It is in that vein that in 2004 Rep. Peter DeFazio (D-Ore.) asked the Department of Homeland Security to ban its production due to terrorism fears (Holstege, Bechtel, Reilly, Wispelwey, & Dobmeier, 2007; Reeves, 2004). More recently, in March 2015, eco-terrorists in New Zealand threatened to poison infant and other dairy formula with 1080 due to their Department of Conservation's controversial use of the chemical for pest control (Brockett & Withers, 2015; Cooney, Varelis, & Bendall, 2016; Hume, 2015). 1080 has also been studied by nation states for potential use as a weapon, as evidenced by a CIA report that 1080 was found to be part of Saddam Hussein's Iraqi Intelligence Service covert laboratories, which developed chemical materials for assassination (CIA, 2007).

Fluoroacetate itself is not toxic, but must undergo a "lethal synthesis" (Peters, 1952) and be converted to fluorocitrate to elicit its effects. Since fluoroacetate is stereochemically similar to acetic acid, it is able to interact with the enzymes in the citric acid cycle where it is converted to fluoroacetyl-CoA and then enters the citric acid cycle (Savarie, 1984). Citrate synthase catalyzes the reaction of fluoroacetyl-CoA and oxaloacetate to form the highly toxic compound fluorocitrate (Peters & Buffa, 1949). Fluorocitrate, specifically the (-)-*erythro*-2-fluorocitrate (2R, 3R) isomer (Carrell et al., 1970), inhibits aconitase and arrests the citric acid cycle, leading to increases in citrate and the depletion of ATP (Buffa & Pasquali-Ronchetti, 1977). Although 1080 is highly toxic, only a small portion of fluoroacetate is actually synthesized to fluorocitrate, with rodent studies suggesting that only 1–2.5% of fluoroacetate is eventually converted to fluorocitrate (Gal, Drewes, & Taylor, 1961; Schaefer & Machleidt, 1971). 1080 may also have direct effects on the mitochondria, causing mitochondrial dysfunction through increased swelling and loss of the proton gradient (Buffa & Pasquali-Ronchetti, 1977). On the systemic level, seizures induced by 1080 poisoning are often attributed to the increase in citrate, which chelates Ca^{2+} in the central nervous system (Hornfeldt & Larson, 1990). The primary process for clearing fluoroacetate from the body is mediated by a glutathione-*S*-transferase and therefore dependent on glutathione concentration (Soiefer & Kostyniak, 1983). Complete clearance of 1080 takes no less than 48 h depending on the species (Goncharov, Jenkins, & Radilov, 2006; Tecle & Casida, 1989).

The median lethal dose (LD_{50}) of 1080 for humans is approximately 2–10 mg/kg (Egekeze & Oehem, 1979), and 1080 poisoning has a latent period of 0.5 to 6 h before clinical symptoms are observed (Egekeze & Oehem, 1979; Goncharov et al., 2006), due to the necessary conversion of fluoroacetate to fluorocitrate. Symptoms typically include nausea, vomiting, abdominal pains, salivation, irrational fear, weakness, tachypnea, cyanosis, sweating, and increased temperature (Brockmann, McDowell, & Leeds, 1959; Goncharov, 2009; Taitelman, Roy, & Hoffer, 1983). The main clinical presentation of 1080 poisoning is metabolic acidosis, a symptom also observed in

many disease states including heart failure, alcohol poisoning, several types of cancer, and malnutrition, among others (Seifter, 2012). Given the overlap of symptoms with many common diseases and no identified biomarker, 1080 poisoning is extremely difficult for medical professionals to correctly diagnose. Even if a correct diagnosis is made, no known antidote exists for 1080, and current treatment protocols consist only of general supportive measures to maintain airway, breathing, and circulation (Rippe & Irwin, 2008). Advances in therapeutics counteracting the effects of 1080 poisoning have been limited in their scope and success. Acetate donors (acetate, ethanol, glycerol) have been shown to be partially effective in mice, guinea pigs, and rabbits (Goncharov, 2009; Goncharov et al., 2006). *In vitro* studies investigating antioxidant compounds (e.g., glutathione, *n*-acetylcysteine, cysteamine) have also been encouraging (Mead, Moulden, & Twigg, 1985), but further evaluation is needed to assess their *in vivo* therapeutic effect.

In our study, we set out to assess the toxicological effects of 1080 in both male and female Sprague-Dawley rats, focusing on the cardiac and pulmonary impacts of the metabolic poison. We utilized a non-invasive assisted-drinking ingestion method (Rice, Rauscher, Langston, & Myers, 2018) to better model an accidental or intentional ingestion of 1080. We feel that when compared to more traditional models of ingestion (e.g., oral gavage), the assisted-drinking method is less stressful on the animal and better replicates human ingestion, since the poison is free to interact with all facets and tissues of the digestive system. With the use of whole-body plethysmograph (WBP) chambers and telemeterized animals, we were able to record real-time respiratory function and cardiac parameters following administration of 1080 over the course of 24 h, which allows for continuous measurement during the latency period and early time points of acute 1080 poisoning. It is our hope to aid in the development of an antidote and novel clinical treatment strategies for 1080 intoxication, with our investigation into the cardiopulmonary and general toxicological deficits caused by this metabolic poison.

2. Methods

2.1. Chemicals

Sodium fluoroacetate (CAT# N-13216-250MG) was purchased from Chem Service Inc. (West Chester, PA, USA) and dissolved in sterile water for injection (CAT# A1287301) (Thermo Fisher Scientific, Waltham, MA, USA). Formalin (CAT# 5705), hematoxylin (CAT# 72404), and eosin (CAT# 7111) were obtained from Thermo Fisher Scientific. 5% 5-sulfosalicylic acid (SSA) (CAT# S7422), glutathione (CAT# CS0260), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) (CAT# D8130), dimethyl sulfoxide (DMSO) (CAT# D2650), glutathione reductase (CAT# G3664), glutathione assay buffer (CAT# CS02060), and β-nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt hydrate (NADPH) (CAT# NADPH-RO) were obtained from MilliporeSigma (St. Louis, MO, USA).

2.2. Animals

Male (300–400 g) and female (150–250 g) Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA, USA) were housed individually under standard conditions with a 12 h light/dark cycle and standard rat chow and water available *ad libitum*. Telemeterized females were surgically implanted with HD-S21 probes ((Data Sciences International (DSI) St. Paul, MN, USA), placed in the ventral abdomen by DSI surgical staff and shipped after one week of recovery. All research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals. It adhered to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, published by the National Academy Press, 2011, and the Animal Welfare Act of 1966, as amended. The study protocol was approved by the Institutional Animal Care and Use Committee, United States Army Medical Research Institute of Chemical Defense (USAMRICD), Aberdeen Proving Ground, MD.

2.3. Rat median lethal dose

Both male and female Sprague-Dawley rats were utilized, and rats were administered sodium fluoroacetate via assisted drinking (Rice et al., 2018). Briefly, rats were held upright, and a 200 µl pipet tip filled with 75 µl of sodium fluoroacetate was placed in the side of the rat's mouth. The pipet tip was pointed to the back of the throat, and the solution was slowly injected into the mouth. The animal continued to be held upright and the pipet tip was held in place until the animal drank the solution. The median lethal dose (LD_{50}) was determined by the Dixon-Massey median lethal dose determination method (Dixon & Massey, 1983), wherein if an animal survived 24 h after exposure to a given dose, the dose for the next animal increased by a $\log_{10}$(dose) interval, and if the animal died within 24 h at a given dose, the dose for the next animal decreased by an equivalent $\log_{10}$(dose) interval. This dosing method continued one animal at a time until four reversals had been achieved or until the maximum number of animals per group was used. In total, 8 male rats and 11 female rats were needed to calculate the LD_{50} via assisted drinking, which was determined to be 2.08 mg/kg (95% CI: 1.73, 2.49) and 1.85 mg/kg (95% CI: 1.56, 2.19) for males and females respectively.

2.4. In vivo toxicology studies at 0.85 LD_{50}

For exposures, animals were individually placed into WBP chambers prior to administration of 1080 or water and permitted to acclimate for 10 min, after which baseline respiratory dynamics and cardiac parameters were recorded for 10 min. After baseline, animals were removed from the WBPs and administered either sodium fluoroacetate via assisted drinking (as mentioned in the previous section) at 0.85 LD_{50} (1.76 mg/kg for males and 1.52 mg/kg for females) or sterile water. Immediately after administration, animals were returned to WBPs, where they remained for 24 h. Upon completion of the 24-h monitoring period, animals were deeply anesthetized using an intramuscular injection of ketamine (90 mg/kg) in combination with xylazine (10 mg/kg) and euthanized via exsanguination. Bronchoalveolar lavage was performed on the left lobe using 3 ml of phosphate-buffered saline with an average recovery of 57 ± 11% (standard deviation). Blood was collected from the descending aorta. Tissues were collected, processed, and flash frozen using liquid nitrogen. Total number male and female cohorts were; 24 exposed male rats, 13 control male rats, 12 exposed female rats, and 5 control female rats.

2.5. Comparative pathology of tissues

For hematoxylin and eosin (H&E) staining, tissues were held in 10% neutral buffered formalin until fixation was complete. The tissues were then trimmed to a thickness of 3 mm or less and processed in a routine manner. The tissues were then embedded with paraffin and sectioned at 5 µm. H&E staining was completed using the automated Leica ST5020 stainer and coverslipped by the Leica ST5030. The finished product was then submitted to the pathologist for review.

2.6. Blood analysis

For the comprehensive metabolic panel (CMP), blood was collected from the descending aorta and placed in an untreated collection tube and analyzed by an automated hematology analyzer for the following: Albumin, the alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, calcium, creatine kinase, chloride, creatinine, enzymatic carbonate, gamma-glutamyl transferase, glucose, potassium, lactate dehydrogenase, sodium, total bilirubin, and total protein. Anion gap (AG) was calculated from the CMP results using the equation $AG = (Na^{+}+K^{+})—(Cl^{-}+eCO_2)$.

2.7. BALF analysis

Bronchoalveolar lavage fluid (BALF) was centrifuged at 4000 rpm for 10 min at 10°C. Supernatant was collected, aliquoted, and stored at −80°C. For analysis, samples were thawed, and protein content was determined using a Pierce 660 nm protein assay reagent kit (CAT# 22662) (Thermo-Fisher Scientific) according to the manufacturer's protocol using a 96 well plate and a Spectramax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA) utilizing SoftMax Pro software (v6.5, Molecular Devices) set to an absorbance point measurement at 660 nm. Heme content was

determined by calculating the absorbance of the BALF at 540 nm using a Spectramax M5 microplate reader, using a 96 well plate and an absorbance point measurement at 540 nm.

2.8. Inflammatory cytokine analysis

Blood from the descending aorta was collected into a serum separator tube, and serum was fractionated by centrifugation. Serum samples were then aliquoted and frozen at −80°C. On the day of the assay, samples were thawed, and the ProcartaPlex (CAT# EPX220-30122–901) (Thermo-Fisher Scientific) Luminex multiplex immunoassay procedure was performed in accordance with the manufacturer's instructions. Samples were assayed in duplicate and allowed an 18-h incubation, then added into the 96-well plate containing the antibody coupled beads. The plate was run on a Bio-Plex 200 system (Bio-Rad Laboratories, Hercules, CA, USA) and analyzed with Bio-Plex manager software (v6.1, Bio-Rad Laboratories). The ProcartaPlex immunoassay contained beads for the following proteins: Granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), interferon gamma (IFN-γ), interleukin (IL)-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12 p70, IL-13, IL-17, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory proteins-1α (MIP-1α), regulated on activation, normal T cell expressed and secreted (RANTES), monocyte-chemotactic protein 3 (MCP-3), macrophage inflammatory protein 2 (MIP2), interferon gamma-induced protein 10 (IP-10), Eotaxin, Growth-regluated oncogene alpha (GRO-α) and tumor necrosis factor α (TNF-α). Manufacturer's assay range is; IL-1α: 12.2–50000 pg/mL; G-CSF: 3.7–15000 pg/mL; IL-10: 9.8–40000 pg/mL; IL-17: 3.7–15000 pg/mL; IL-1β: 15.9–65000 pg/mL; IL-6: 4.9–20000 pg/mL; TNF-α: 4.9–20000 pg/mL; IL-4: 1.2–5000 pg/mL; GM-CSF: 9.8–40000 pg/mL; IFN-γ: 9.8–40000 pg/mL; IL-2: 4.9–20000 pg/mL; IL-5: 2.4–10000 pg/mL; IL-13: 4.9–20000 pg/mL; IL-12p70: 9.8–40000 pg/mL; Eotaxin: 1.9–7700 pg/mL; GR-α: 11.4–46500 pg/mL; IP-10: 4.9–20100 pg/mL; MCP-1: 18.03–75000 pg/mL; MCP-3: 3.5–14200 pg/mL; MIP—1 alpha: 2.3–9500 pg/mL; MIP—2: 0.6–2400 pg/mL; Rantes: 85.5–350000 pg/mL.

2.9. Cardiac total glutathione analysis

Frozen cardiac tissue was cryopulverized using a mortar and pestle; then 100 ± 10 mg of cryopulverized powder was added to 15 ml conical tubes, followed by 10 volumes (in µl) of 5% SSA. Conical tubes were inserted into the Omni Processing Rack attached to an Omni Prep Homogenizer and homogenized for 1 min at 10,000 rpm. Homogenized samples were then placed on ice for 10 min to allow settling and then centrifuged for 10 min at 4,700 rpm and 4°C. The lysate was then extracted. To acquire appropriate starting concentration for the assay, a 4-fold dilution of lysate was performed. Diluted samples were kept on ice until added to 96-well plates. 10 µl of prepared glutathione standard, diluted sample, and 5% SSA, all assayed in triplicate, were added into the designated standard, unknown and blank wells, respectively. 150 µL of working mixture, composed of 2.7% of 1.5 mg/mL DTNB in DMSO solution, 4.1×10^{-2}% glutathione reductase and 97.3% of 1X assay buffer, was added into all 96 wells of the plate and then incubated for 5 min at room temperature. 50 µL of 1.6 mg/ml NADPH was added into all 96 wells of the plate. The absorbance of the wells was then measured at 412 nm using a Spectramax M5 microplate reader, performing a kinetic read at 1-min intervals for 5 min.

2.10. Data and statistical analysis

Data are presented as the mean ± the standard deviation, unless otherwise noted. Where appropriate, the 1080-exposed group was compared to the control group using a Student's t-test with significance set to $p < 0.05$. Specialized software and customized routines were used to collect respiratory dynamics (FinePointe Software v2.3.1.16, DSI) and cardiac (Ponemah Software v5.2, DSI) data, and all raw data were exported and analyzed using custom-designed programs (Microsoft Visual Basic for Applications v7.0.1639; Microsoft Corporation; Redmond, WA, USA), spreadsheet software (Microsoft Excel v14.1.7166.5000 [32-bit]; Microsoft Corporation; Redmond, WA, USA), and statistical and graphing software (GraphPad Prism v5.04, v7.04; GraphPad Software, Inc.; La Jolla, CA, USA).

3. Results

3.1. General toxicity

We observed no significant difference between the LD_{50} for 1080 ingestion in male and female rats (Table 1) when utilizing the assisted-drinking method. The calculated LD_{50}s (male: 2.08 mg/kg 95% CI: 1.73,2.49, female: 1.85 mg/kg 95% CI: 1.56, 2.19) were in agreement with previous studies in which oral gavage was utilized (Gratz, 1973; Parton, 2013; Pelfrene, 2010). Following 1080 exposure, rats exhibited a latency period prior to major convulsions, in which we observed clinical signs including tongue fasciculation, frantic behavior, increased urination and defecation, facial tremors, tonus, clonus, and myoclonic jerk (Tables 2 and 3). Typical onset time of symptoms did not differ greatly between males and females, with the exception of muscle fasciculation, for which males had an earlier onset, and frantic behavior and motion arrest, which occurred later in males. Onset of convulsions typically appeared without any immediately preceding overt signs or symptoms and were substantial. When administered a 0.85 LD_{50} dose of 1080, the median times for onset of convulsions were 85 ± 21 min in males and 113 ± 5 min in females. Both male and female rats that survived to 24 h exhibited a significant decrease in body weight compared to control, with the exposed males' body weights decreasing 8.9 ± 1.4% ($p < 0.05$, Student's t-test, $n = 13–18$) and females' weights decreasing 6.7 ± 1.2% ($p < 0.05$, Student's t-test, $n = 4–5$). At 0.85 LD_{50} in male rats, we observed no changes in blood cell parameters (via complete blood count, not shown), but saw significant decreases in albumin, alkaline phosphatase, chloride, potassium, and total protein, and significant increases in aspartate transaminase, creatinine, and blood urea nitrogen (Table 4). The changes in the CMP could indicate impaired liver, heart, kidney, and lung function in animals dosed with 1080 (Gowda et al., 2009; Grossman, Yap, & Shafritz, 1977; Hosten, 1990; Teloh, 1978, Terzano et al., 2012; Vaduganathan, Pallais, Fenves, Butler, & Gheorghiade, 2016). Interestingly, we did not observe any significant change in AG (16.8 ± 3.6 mmol/L in controls vs. 15.1 ± 2.6 mmol/L in exposed), which is a typical indicator of metabolic acidosis (Zingg, Bhattacharya, & Maerz, 2018). We also observed no significant changes in circulating inflammatory cytokines, other than a slight increase in IL-17, at 24 h in the male rats exposed to a 0.85 LD_{50} of 1080 (Table 5).

3.2. Pulmonary effects

No gross tissue damage was observed in either male or female rats exposed to a 0.85 LD_{50} dose of 1080. H&E staining revealed hemorrhage in the lungs of 7 of the 8 rats exposed to 1080 (Figure 1), which occurred prior to euthanasia at 24 h, as indicated by the appearance of surrounding macrophages removing the associated protein and fluid. When performing bronchoalveolar lavage on the exposed animals, we observed pink-tinted fluid, a potential indicator of blood in the lungs. Although some of the BALF displayed an elevated absorbance at 540 nm (absorbance of hemoglobin), we did not observe a statistically significant increase in the average absorbance of exposed male rats (Figure 2). We did, however, see a statistically significant increase in BALF protein levels of exposed animals (Figure 2). Since we did not observe a corresponding statistically significant increase in circulating cytokines, the increase in BALF proteins can be interpreted as a local event rather than as a component of the systemic effects of 1080. This conclusion is supported by the comparative pathology analyses of the heart, gastrointestinal tract, liver, kidney, and brain tissue, all of which revealed no observable signs of damage to any of those tissues.

The use of whole-body plethysmography following exposure to 0.85 LD_{50} 1080 enabled us to quantify pronounced effects on respiratory function. Almost immediately after ingesting 1080, tidal volume (TV) decreased markedly and remained depressed over the course of the 24-h

Table 1. Calculated median lethal dose (LD_{50}) in male and female Sprague-Dawley rats

	LD_{50} (mg/kg)	95% confidence intervals
Male	2.08	1.73, 2.49
Female	1.85	1.56, 2.19

Table 2. Observable signs of intoxication in male rats

Signs	Average time to onset (minutes)	Standard deviation
Chewing	29.9	16.5
Oral tonus	30.2	9.7
Facial tremors	33.6	16.2
Muscle fasciculation	34.5	18.5
Scrotal tension	35.0	29.5
Hiccup	42.2	21.6
Excessive grooming	44.4	20.0
Whole-body shake	46.4	9.6
Excessive licking	47.9	32.9
Tongue fasciculation	52.9	37.0
Excessive defecation	53.2	20.4
Tonus	58.4	8.9
Frantic	62.4	27.0
Coprophagy	66.3	43.1
Myoclonic jerk	68.3	26.5
Piloerection	70.3	22.4
Head bob	71.6	28.6
Body tremors	74.0	28.3
Squinting	80.8	25.8
Opisthotonus	81.3	16.7
Convulsions	84.9	21.2
Pallor	88.3	25.5
Ataxic	88.8	84.5
Motion arrest	90.0	38.2
Retch	95.0	67.6
Lethargy	97.0	26.9
Salivation	103.9	38.7
Gasp	113.4	85.4
Clonus	122.5	74.2

Not every rat experienced all of the observed signs. n = 2–14.

experiment (Figure 3). This may indicate acute lung injury in the animals exposed to 1080 and would support the comparative pathology observed in those animals. The decrease was more severe in female rats, ~60% reduction, as compared to males, ~30% reduction, at the end of the study. We also observed a significant decrease in the respiratory frequency (f) in exposed animals, although the reduction occurs after the latency period is over and convulsions are observed. Unlike TV, we observed a sharp increase in f at around 30 min after exposure, in both males and females. Following the increase at 30 min there is a prolonged decrease throughout the 24-h observation period (Figure 4). At around 6 h post-exposure, in both the males and females, f for the exposed animals is lower than for the controls. The decrease in f in exposed animals of either sex eventually plateaued, with the males plateauing around 6 h post-exposure and the females at 10 h post-exposure. We observed an increase in f for control animals corresponding with their normal active period of the day (post-exposure hours 2–18), which was noticeably absent in all exposed animals. In total, the respiratory effects of 1080 were pronounced and lasted over the course of our 24-h observation, suggesting that pulmonary capacity is weakened and lung function is significantly impaired.

Table 3. Observable signs of intoxication in female rats

Signs	Average time to onset (min)	Standard deviation
Chewing	27.5	19.4
Excessive grooming	28.7	6.4
Tongue fasciculation	33.5	28.6
Hiccup	35.1	14.5
Frantic	36.5	23.3
Cyanosis	42.2	37.2
Myoclonic jerk	44.0	24.5
Ataxic	50.0	38.1
Facial tremors	51.4	23.9
Pallor	51.5	9.2
Body tremors	53.4	42.4
Motion arrest	54.0	48.1
Squinting	55.8	13.5
Tonus	59.0	72.1
Lethargy	63.6	37.6
Excessive defecation	63.7	40.5
Piloerection	65.3	28.2
Loss of righting	78.0	38.2
Opisthotonus	79.5	38.9
Muscle fasciculation	80.1	46.6
Gasp	80.5	26.2
Hunched posture	82.0	77.8
Retch	89.5	46.3
Clonus	90.4	36.3
Belly down	106.6	26.1
Head bob	107.9	15.0
Convulsions	112.5	4.7
Rearing	115.3	5.1

Not every rat experienced all of the observed signs. n = 2–12.

3.3. Cardiac effects

A cohort of female rats were implanted with telemetry devices that allowed for monitoring of heart rate, left ventricular pressure, and core body temperature over the course of the experiment. Once again, we administered a 0.85 LD_{50} dose of 1080 and observed the animals for 24 h while collecting real-time physiological data. We observed profound impacts of 1080 on cardiac function over the duration of our study. During the latency period (2-h period immediately after exposure and prior to the observation of whole-body convulsions), the heart rate (HR) of animals dosed with 1080 was normal as compared to controls (Figure 5(a)). Over the next 10 h HR steadily declined from approximately 400 beats per minute (BPM) to 150 BPM. For the remainder of the study, HR remained severely depressed (75% reduction from control) in exposed animals. The decrease in HR corresponded with an approximate 3.5-fold increase in average QRS interval over the same time period (Figure 5(b)). In humans, increased QRS interval has been implicated as an indicator of left ventricular dysfunction (Murkofsky et al., 1998), suggesting that impaired cardiac function follows whole-body convulsions in exposed animals. We also observed a steady decrease in systolic blood pressure (SYS) from 30 min post-exposure throughout the entire 24-h study (Figure 5(c)), which resulted in a nearly 50% reduction in blood pressure in exposed animals by the end of the study.

Table 4. Comprehensive metabolic panel in male Sprague-Dawley rats

	Control	0.85 LD_{50}
Albumin (g/dl)	3.2 ± 0.3	2.8 ± 0.2*
Alkaline phosphatase (U/L)	369.7 ± 82.6	223.4 ± 60.1*
Alanine aminotransferase (U/L)	66.4 ± 8.2	135.9 ± 204.3
Aspartate aminotransferase (U/L)	158.7 ± 56.3	378.1 ± 329.4*
Blood urea nitrogen (mg/dL)	16.4 ± 3.1	45.9 ± 29.6*
Calcium (mg/dL)	10.7 ± 0.5	10.7 ± 0.6
Creatine kinase (U/L)	945.7 ± 293.8	1202 ± 854.8
Chloride (mmol/L)	102.5 ± 1.1	99.4 ± 2.8*
Creatinine (mg/dL)	0.4 ± 0.1	0.6 ± 0.2*
Enzymatic carbonate (mmol/L)	27.5 ± 2.7	28.1 ± 2.1
Gamma-glutamyl transferase (U/L)	10.0 ± 0.0	10.0 ± 0.0
Glucose (mg/dL)	353.7 ± 138.1	318.8 ± 90.6
Potassium (mmol/L)	6.1 ± 1.1	4.7 ± 0.6*
Lactate dehydrogenase (U/L)	2291.8 ± 1316.2	2317.5 ± 1074.1
Sodium (mmol/L)	140.6 ± 2.4	138.8 ± 3.5
Total bilirubin (mg/dL)	0.5 ± 0.6	0.2 ± 0.1
Total protein (g/dL)	5.3 ± 0.5	4.7 ± 0.3*

n = 11–16, * $p < 0.05$, Student's t test, ± standard deviation, male rats.

Table 5. Cytokines and chemokines in male rats after exposure to 1080

Analyte	Control		0.85 LD_{50}	
	pg/ml	Standard deviation	pg/ml	Standard deviation
G-CSF	65.3	31.0	68.4	35.2
IFNy	29.8	13.0	30.6	13.9
IL-1β	11.2	2.3	16.9	10.4
IL-6	2.8	2.8	4.2	4.2
IL-10	53.1	31.9	62.9	48.2
GRO-α	354.5	166.3	434.8	225.2
MCP-1	5003.6	2559.0	4042.6	2133.6
MIP-1α	32.4	6.9	36.8	12.1
MCP-3	638.2	356.3	500.4	349.8
MIP-2	11.1	2.5	14.3	4.3
TNF-α	3.8	1.0	6.2	4.3
Eotaxin	1734.2	1253.2	1043.4	111.9
IL-2	10.7	3.1	25.5	25.0
IL-4	17.3	9.0	15.5	8.6
IL-5	38.6	23.3	30.0	18.5
IL-12 (p70)	11.5	18.9	16.9	18.2
IL-13	3.7	0.5	6.1	4.2
IL-17	4.1	4.2	11.7*	3.5
IP-10	188.4	110.0	157.6	105.3
RANTES	15,364.2	3628.2	10,714.0	8749.3

n = 8–11, *$p < 0.05$, Student's t test. All cytokines and chemokines not listed were below the limit of detection.

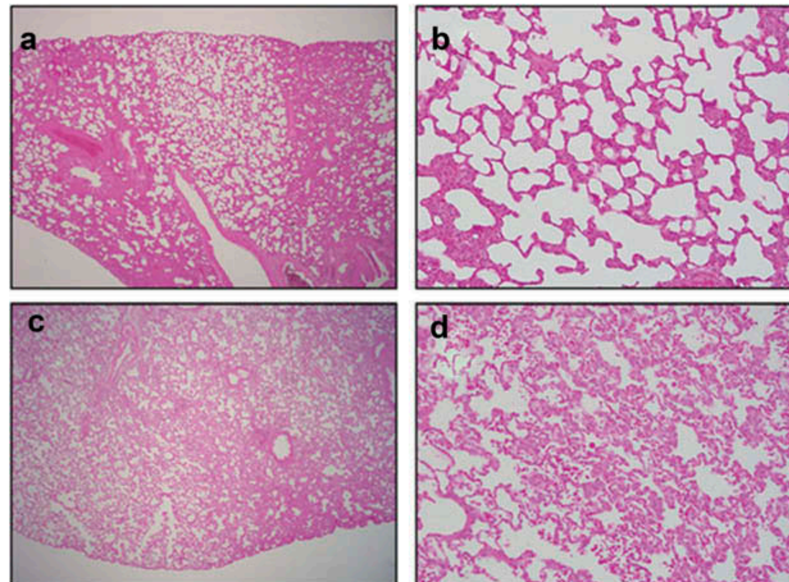

Figure 1. ***Lung histology of rats exposed to 0.85 LD_{50}.*** **Both male and female rats were exposed to a 0.85 LD_{50} dose of 1080. Rats were observed for 24 h in a whole-body plethsymograph chamber and euthanized at 24 h. Tissues were collected for histological analysis by H&E staining. Hemorrhage was observed in the lungs of 7 of the 8 exposed animals examined. There was no observed difference between the male and female tissues. (a) Control left lung lobe 40x, (b) Control left lung lobe 200x, (c) Exposed left lung lobe 40x, (d) Exposed left lung lobe 200x.**

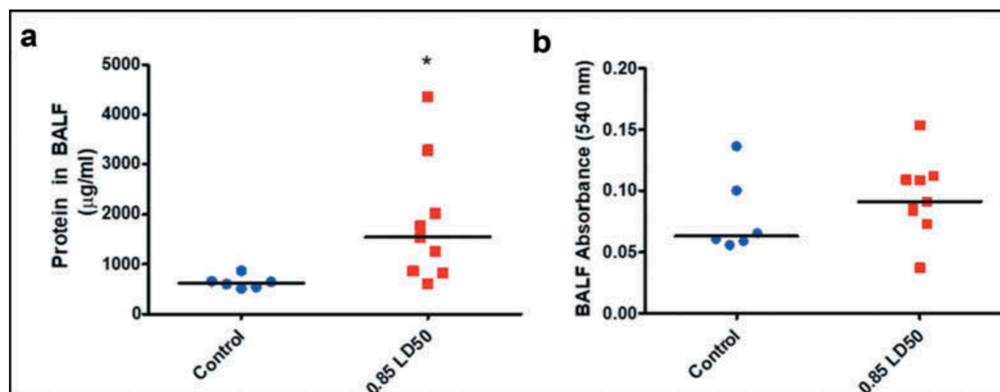

Figure 2. ***Analysis of BALF in exposed male rats.*** **Male rats were exposed to a dose of 0.85 LD_{50} 1080. Rats were observed for 24 h in a whole-body plethysmograph chamber and then euthanized. A bronchoalveolar lavage was performed on the left lung, and the fluid was collected for analysis. Protein content in the BALF was determined by a Pierce 660 nm assay. (a) Significant increase in BALF protein was observed in the exposed animals. (b) BALF absorbance at 540 nm, which corresponds with hemoglobin's absorbance peak, was investigated. Although we did observe red-tinged BALF in some of the exposed animals, we did not see a significant increase in all of the exposed. Bars = mean, n = 6–9, *$p < 0.05$, Student's t-test.**

We were able to assess total glutathione levels in the heart 24 h after exposure to 1080 (Figure 6). Glutathione is a endogenously synthesized antioxidant (Carocho & Ferreira, 2013) and is necessary for the enzymatic defluorination and clearance of fluoroacetate from the body (Soiefer & Kostyniak, 1983). We observed in male rats exposed to a 0.85 LD_{50} dose of 1080 an approximately 50% reduction in total glutathione levels in the heart at 24 h. Although overall cardiac function was significantly impaired, with left ventricular dysfunction in particular, as indicated by prolonged QRS interval and reduced cardiac glutathione in animals exposed to 1080, comparative pathology found

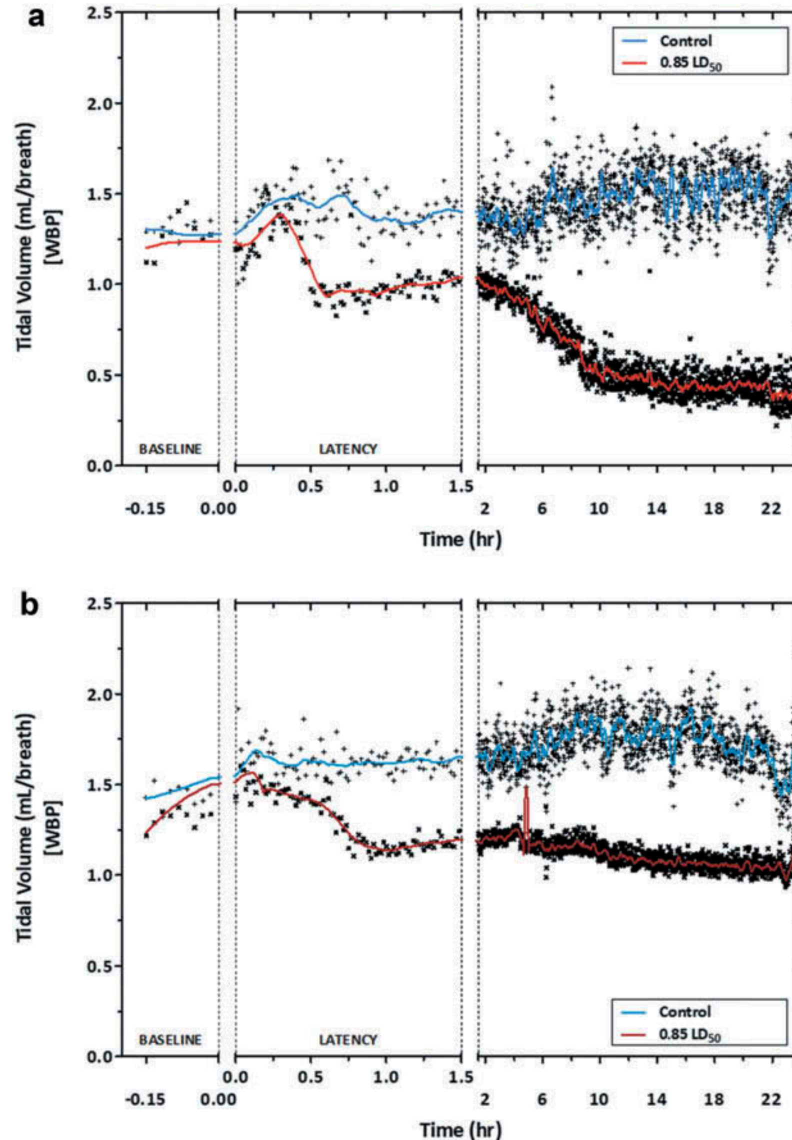

Figure 3. Tidal volume of rats after exposure to 1080. Both female (a) and male (b) rats were exposed to a 0.85 LD_{50} dose of 1080 and observed in a whole-body plethysmograph chamber for 24 h. We observed an overall decrease in the tidal volume, starting about 30 min after exposure. The decrease plateaued, even after major convulsions (~1.5 h). In females a second decrease occurred from 2 h to 10 h. In males we did not observe a further decrease in tidal volume after the initial decrease at 30 min. Blue = Smooth fit of Control, Red = Smooth fit of Exposed, Females *n* = 4–8, Males *n* = 7–19.

no observable tissue damage in the heart in any exposed animal at 24 h. This indicates that although cardiac function is clearly impacted, it may take more than 24 h for any gross tissue damage to occur in animals exposed to 1080.

We also observed a severe effect of 1080 on core body temperature (Figure 7), with a steady decrease in core body temperature in the 1080-exposed animals, starting around 30 min after exposure. Over the course of the 24-h study, the animals' core body temperatures decreased dramatically, to approximately 22°C, an approximate 15°C (or 39.5%) reduction as compared to controls. The data could suggest severe impairment of exposed animals' ability to thermoregulate, which combined with many of the observed signs and symptoms of intoxication could indicate impairment of the autonomic system. The decrease in core body temperature could also be an artifact from the weakened cardiac function, since hypothermia and cardiac failure have been linked in humans (Casscells et al., 2005; Payvar et al., 2013).

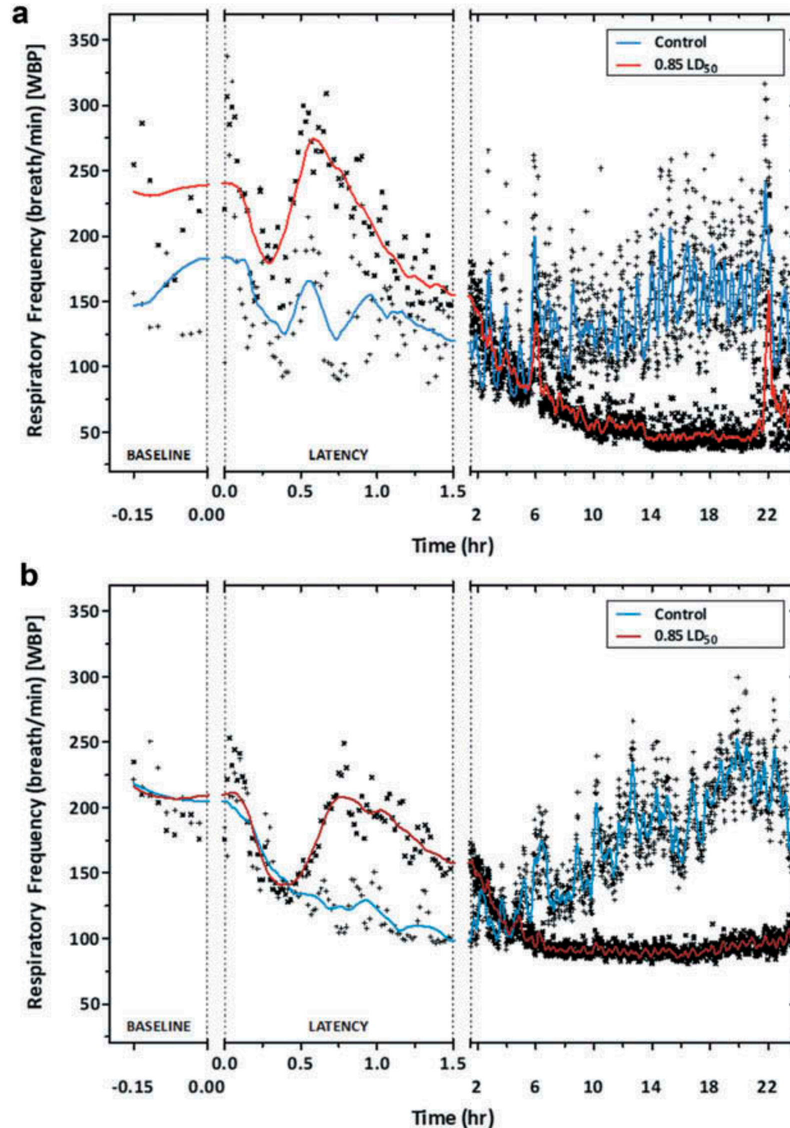

Figure 4. Respiratory frequency of rats exposed to 1080. Both female (a) and male (b) rats were exposed to a 0.85 LD_{50} dose of 1080 and observed in whole-body plethysmograph chambers for 24 h. We observed, in both female and males, a sharp increase in frequency around 30 min. The increase was short lived, and a steady decrease was observed in each sex. In the females the decrease in respiratory frequency plateaued at 10 h post-exposure, and in the males the plateau was at 6 h. The increase in control frequency, observed in post-exposure hours 2–18, corresponds with the animals' normal active periods. Blue = Smooth fit of Control, Red = Smooth fit of Exposed, Females *n* = 4–8, Males *n* = 7–19.

4. Discussion

Sodium fluoroacetate is a metabolic poison that is known to impair mitochondrial function through inhibition of the enzyme aconitase in the citric acid cycle (Goncharov et al., 2006; Peters, 1952; Savarie, 1984). This eventually leads to the clinical presentation of symptoms that typically include nausea, vomiting, abdominal pains, salivation, irrational fear, weakness, tachypnea, cyanosis, sweating, increased temperature (Brockmann et al., 1959; Goncharov, 2009; Taitelman et al., 1983), and death in humans when ingested (2–10 mg/kg (Egekeze & Oehem, 1979)). Although the mechanism of action for 1080 is known, research into therapeutics or diagnostics for intoxication has had limited success.

Our study aimed to quantify cardiopulmonary impairment in Sprague-Dawley rats exposed to 1080 through a non-invasive ingestion model (Rice et al., 2018) using real-time measurements of cardiac and respiratory parameters. We utilized both male and female cohorts of Sprague-Dawley rats in our study and found comparable susceptibility to 1080 intoxication, general agreement in presentation of signs and symptoms of intoxication, overall depression of respiratory parameters following exposure to 1080, and similar gross histological observations between the sexes. In the

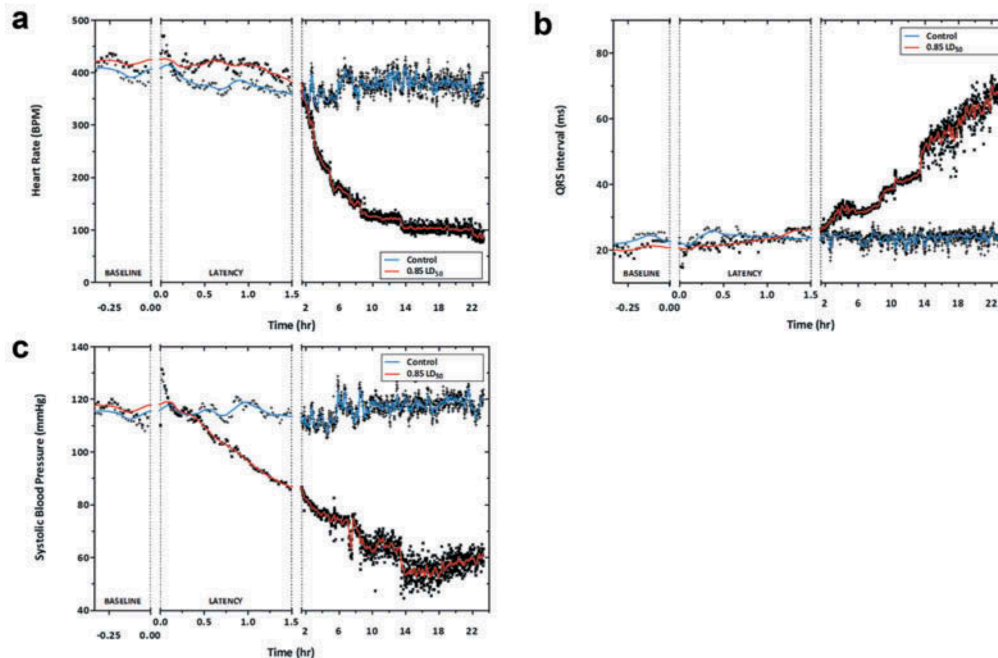

Figure 5. The effects of 1080 on cardiac parameters in female rats. Female rats were administered a 0.85 LD_{50} dose of 1080, and cardiac parameters were monitored using an implanted telemetry device. We observed pronounced impacts on cardiac function over the course of 24 h. (a) Heart rate remained normal in the controls during the latency period, but fell sharply over the course of 10 h following onset of whole-body convulsions. (b) QRS interval mirrored the heart rate and only increased following the onset of whole-body convulsions. (c) Systolic blood pressure started decreasing immediately after administration of 1080 and was reduced by 50% at the end of the study. Blue = Smooth fit of Control, Red = Smooth fit of Exposed, *n* = 4–8.

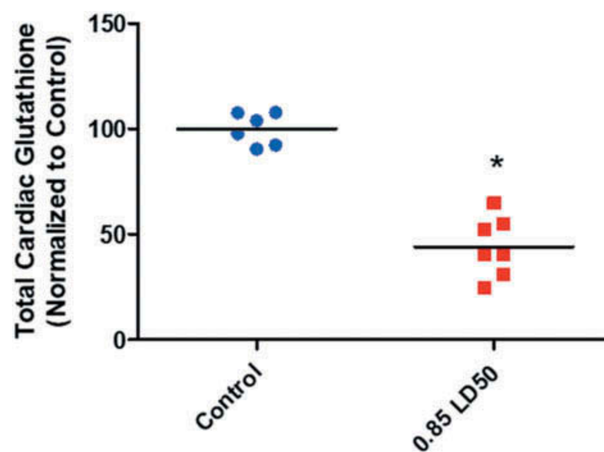

Figure 6. Cardiac glutathione levels following exposure to 1080 in male rats. Twenty-four hours after exposure to a 0.85 LD_{50} dose of 1080, male rats were euthanized, and heart tissue was collected. Total glutathione levels in the heart were assessed from homogenized tissue using a colorimetric assay. We observed a nearly 50% decrease in the total glutathione levels in animals that had been exposed to 1080. This may indicate both cardiac dysfunction and increased fluoroacetate defluorination in the heart tissue. Male rats, Bar = mean, *n* = 6–7, *$p < 0.05$ Student's *t* test.

progression of signs and symptoms of intoxication, the only slight difference we observed between males and females was that after exposure to 1080 female rats exhibited frantic behavior sooner and more frequently than male rats. In our model of exposure to a 0.85 LD_{50} dose of 1080, we observed a latency period of approximately 1.5 h (85 min in males, 113 min in females) before major symptoms of whole-body convulsions were observed. We saw no significant difference in the median lethal dose between male and female rats (2.08 mg/kg for males and 1.85 mg/kg for

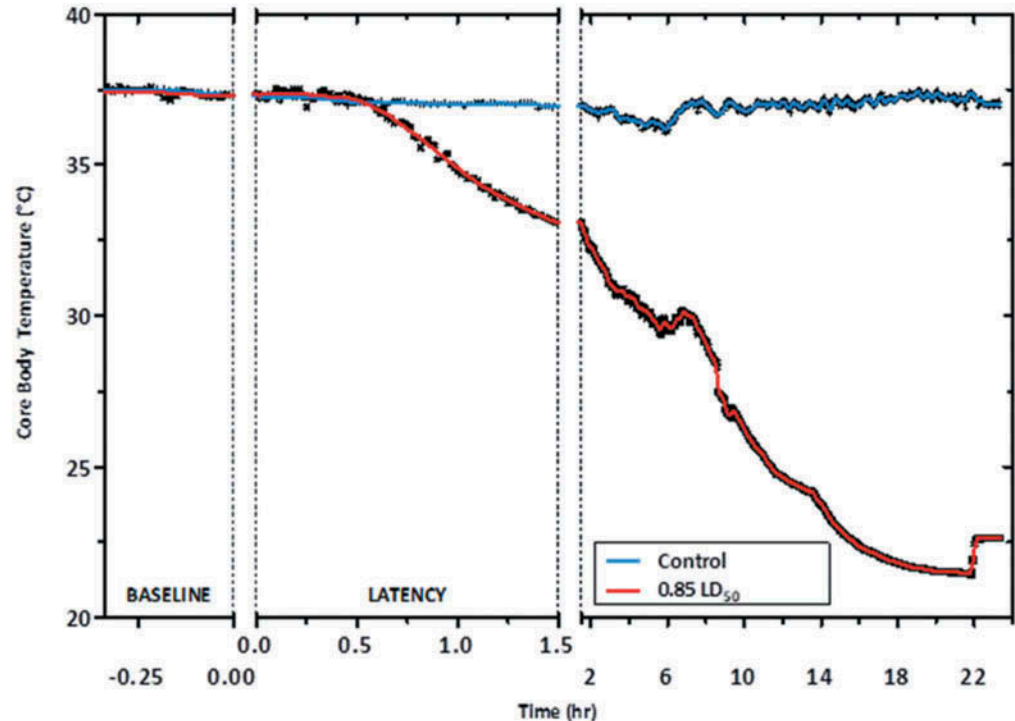

Figure 7. The effect of 1080 exposure on core body temperature in female. Female rats were exposed to a 0.85 LD_{50} dose of 1080, and core body temperature was measured using an implanted telemetry device. We observed a severe decrease in core temperature of approximately 15°C in the exposed animals. This indicates a significant inability to thermoregulate after 1080 exposure. Female rats, *n* = 4–8, Blue = Smooth fit of Control, Red = Smooth fit of Exposed.

females), which was in agreement with historical literature (Gratz, 1973; Parton, 2013; Pelfrene, 2010), thus validating the use of our assisted-drinking method. A comprehensive metabolic panel in male rats indicated impaired heart, liver, kidney, and lung function.

We observed severely impaired respiratory function in both male and female rats administered a 0.85 LD_{50} dose of 1080, as indicated by a depression in *f* and TV. The decrease in TV was more pronounced in the female rats (~60% reduction compared to ~30% reduction in males) and appears to be biphasic, with two periods of falling TV at 30 min and at 2–6 h post-exposure, while the males have a single period of falling TV at approximately 45 min post-exposure. Some of this difference, though, may be due to artifacts from the limited number of female rats in the study. The decrease in body weight experienced in both the exposed male and female rats over 24 h (8.94% decrease in males and 6.74% decrease in females) is only expected to contribute to a small portion of the reduction in TV (Stahl, 1967), with the toxic effects of 1080 playing the major factor. The depression of respiratory function correlates with lung hemorrhage observed in both male and female rats administered 1080, which is in agreement with other reports of animals and humans with lung insults following 1080 intoxication (Brockmann, McDowell, & Leeds, 1955; Goh, Hodgson, Fearnside, Heller, & Malikides, 2005, Gooneratne et al., 2008, Harrisson et al., 1952; Schultz, Coetzer, Kellerman, & Naudé, 1982).

We also report severe cardiac dysfunction in 1080–exposed, telemetered female rats. We observed profound depression of both HR (approximately 50%) and SYS (75% reduction) over the course of our 24-h study. While HR only decreases after the latency period, an interesting finding was that SYS decreases prior to the onset of convulsions and as early as 30 min after exposure. The depression of both HR and SYS is in agreement with previous studies which have seen similar trends in multiple species (Chenoweth & Gilman, 1947; Goncharov et al., 2006; Sherley, 2007). We also observed a 3.5-fold increase in the QRS interval following the onset of whole body convulsions, which may indicate left ventricular dysfunction (Murkofsky et al., 1998). Measurement of total glutathione levels in the cardiac tissue revealed that at 24 h after exposure, levels were reduced by 50% as compared to controls. In humans, reduced levels of both cardiac and systemic glutathione were found to be

related to the functional status of, and structural abnormalities in, both symptomatic and asymptomatic heart disease. In patients with left ventricular dysfunction glutathione levels were found to be decreased by over 50% compared to normal healthy adults (Damy et al., 2009). Since glutathione is the major endogenous antioxidant (Carocho & Ferreira, 2013), the reduced cardiac glutathione we observed most likely indicates an increased reactive oxygen species (ROS) generation (and subsequent scavenging) due to the mitochondrial impairment of 1080. Another factor in the decrease of cardiac glutathione is the potential increase in the defluorination process to rid the body of fluoroacetate (Soiefer & Kostyniak, 1983). Both the reduced cardiac glutathione levels and prolonged QRS interval in exposed animals suggest left ventricular dysfunction (Damy et al., 2009; Murkofsky et al., 1998), despite the lack of pathological findings.

The combination of substantial reductions in core body temperature in female rats exposed to 1080, which has been previously noted in rats and other species (Peters, 1957; Sherley, 2004; Sikulova & Novak, 1970) with some of their exhibited symptoms during the latency period (tongue fasciculation, frantic behavior, increased urination and defecation, facial tremors, tonus, clonus, and myoclonic jerk), also implicates disruption in the nervous system. Mitochondrial dysfunction can impact the autonomic system, as cardiovascular autonomic impairment has been indicated in patients with mitochondrial disorders (DiLeo et al., 2007). In the case of 1080 poisoning, overstimulation of the autonomic nervous system from increased metabolic acidosis has been implicated as the cause for many of the toxic symptoms in animals (Sherley, 2004). However, we did not observe any significant changes in the anion gap in the exposed animals, which would indicate systemic metabolic acidosis, although we were unable to directly measure blood pH in this study. Generally, our data are in agreement with this view that 1080 impacts the autonomic nervous system and leads to dysautonomia in exposed rats.

Our study was able to describe the severe and pronounced effects of sodium fluoroacetate poisoning on cardiopulmonary function. Taken together both male and female rats exposed to a potentially lethal dose of 1080 showed indications of general cardiac and pulmonary failure, left ventricle dysfunction, lung hemorrhage, and dysautonomia. Our results indicate that both tissue-specific and systemic deficits contribute to the toxicological progression of 1080 intoxication. Currently there is no approved antidote for 1080 poisoning, and clinical protocol suggests general supportive care. As we evaluate potential therapeutics or treatment strategies for 1080 intoxication, it would be wise to consider the profound cardiopulmonary deficits and develop treatment regimens to alleviate the impact on the heart and lungs.

Acknowledgements
We would like to thank Dr. Rebecca J. Lewandowski for her input and assistance in the development of our *in vivo* model. We would also like to thank MAJ William L. Wilkins and the Comparative Pathology team at USAMRICD for help with the tissue histology analysis.

Funding
The research described was supported by an interagency agreement (AOD18015-001-00000) between the NIH Office of the Director (OD) and the U.S. Army Medical Research Institute of Chemical Defense under the oversight of the Chemical Countermeasures Research Program (CCRP) within the Office of Biodefesne Research (OBRS) at the National Institute of Allergy and Infectious Diseases (NIAID/NIH).

Competing Interests
The authors declares no competing interests.

Disclaimers
The views expressed are solely those of the authors and do not necessarily represent the official views of the CCRP, NIAID, NIH, or HHS and do not reflect official policy of the Department of the Army, Department of Defense, or the U.S. Government. The experimental protocol was approved by the Animal Care and USE Committee at the United States Army Medical Research Institute of Chemical Defense, and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011), and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. N.A.A, M.K.A., and M.R. were supported in part by an appointment to the Research Participation Program for the U.S. Army Medical Research and Materiel Command administered by the Oak Ridge Institute for Science and Education through an agreement between the U.S. Department of Energy and U.S. Army Medical Research and Materiel Command.

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Citation information
Cite this article as: The cardiopulmonary effects of sodium fluoroacetate (1080) in Sprague-Dawley rats, Bryan J. McCranor, Talearia D. Young, Justin Tressler, Laura Jennings, James Irwin, Nazira A. Alli, Marilynda K. Abilez, Samuel Stone, Michelle Racine, Jennifer L. Devorak, Alfred M. Sciuto & Benjamin Wong, *Cogent Biology* (2019), 5: 1568669.

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