

TOXTALK



PRESIDENT'S MESSAGE



I remember the first time I donned my lab coat as a new Forensic Toxicologist. It was exciting and terrifying at the same time. I would look to my colleagues that have been in the field for years, even decades, wanting to achieve the knowledge and experience they have. However, the journey to the back of the book is more enriching than just immediately flipping to the last page. I am so fortunate to have grown through the forensic toxicology field with a tremendous number of mentors, brilliant colleagues and friends. My family has always been my rock and endured the conversations over dinner involving stories of casework in less than appetizing details. I would not be where I am today in this field without the support of my family, my Wisconsin State Lab of Hygiene (WSLH) colleagues and dear SOFT friends. The Society of Forensic

"In the book of life, the answers aren't always in the back" - Charlie Brown (Charles M. Schulz)

Toxicologists has always been the cornerstone of so many decisions and learning touchpoints throughout my career and I am so grateful and humble to be able to work with you all in the coming year.

I think we all released a sigh of relief when we watched the clock strike midnight on January 1st, 2021. For the world, 2020 was a year like none other, and this was so true for SOFT. During the May SOFT Board meeting, the members of the Board began to realize that the organization could not safely gather together to

TOXTALK

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PRESIDENT'S MESSAGE CONTINUED

celebrate the field and each other. The 2020 Planning Committee was led by Denice Teem and Dani Mata and their passion and dedication to planning an incredible, historical meeting was not going to come to fruition. It was a difficult decision and the Board was heartbroken to have to make the choice, but it was necessary. Without missing a beat, Beth Olson and CC Watson immediately began planning our virtual event which came to be known as SOFTember. Much was learned in the planning and execution of SOFTember and it was a great success. Some aspects will continue as part of SOFT as a result of SOFTember. I truly feel we are an even stronger organization due to the challenges Covid-19 presented in organizing our annual meeting.

As President-Elect last year, I had the privilege of working with SOFT's committees. Beth and I worked with each committee to determine if a handbook was appropriate and, if so, requested those committees to create one. Having the opportunity to work with Beth on that project was extremely rewarding and allowed me to see just how much time and care the SOFT office puts into this organization. Needless to say, even while making the massive pivot to a virtual platform, Beth kept me on task and the handbooks were completed. It was exciting to not only watch Beth work but to really see the work of the committees. I want to thank all of the committee Chairs and Co-Chairs for completing the handbooks. This organization is strong in large part because of the essential work all of its committees perform.

In December, then President Suman Rana stood up the Toxicology Resource Committee and asked that I take on the role as Chair. Forensic toxicology laboratories have long described our lack of funding, personnel and workforce development which is necessary to

provide the best testing possible and have received little action or support through the years. The committee consists of Dan Anderson, Dani Mata, Karen Scott, Sara Short, Ruth Winecker and Lucas Zarwell. To be in the company of this committee is awe inspiring and so incredibly motivating. The committee had our first meeting in January and I found myself furiously trying to take notes and keep up with all of the tremendous ideas and thoughtful insights the members brought forward. We hit the ground running and we will be communicating with the SOFT membership soon of our path moving forward. I am also excited that this committee will be reporting our work to our CFSO representative, Dr. Tim Rohrig with specific needs. This will assist the CFSO in further advocating for forensic toxicology on the Hill.

As I have zigzagged my way through my career, I have found that there are so many opportunities and partnerships outside of the forensic toxicology field that we often overlook. I encourage you to look for opportunities to connect and engage partners that we may not always realize. Reaching out to your state's Highway Safety Office, your NHTSA Region Representative or your state's Public Health Department can often bring about collaborations that are beneficial to your laboratory and your partners. Many of these partners use our data in ways we do not fully understand and having a seat at their table can be educational and beneficial to all. National groups such as Responsibility.org are aware of the needs of the forensic toxicology laboratories and are working to provide support and advocacy. It always helps to have a few others in the back of the classroom with us, raising their hands and jumping up and down on our behalf.

The SOFT annual meeting is so important in many ways, one

of the greatest benefits is the ability to connect with our friends and colleagues and make new connections. Since we were not able to network and collaborate in-person last year, I thought I would bring some connections to you. In each issue of ToxTalk I would like to introduce you to my colleagues at the WSLH. Each person brings a unique set of skills to the table and I encourage you to reach out to them if you want to know more about what they do or just to simply introduce yourself!

I wish you all happiness and health in the coming year and I look forward to working for SOFT!



AMY MILES, B.S.
SOFT PRESIDENT

John Olberding (video)
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Ryan Pieters (video)
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Diane Kalscheur (video)
diane.kalscheur@slh.wisc.edu

WSLH Intros:

Name: Dan McManaway

WSLH employment: 20 years

Current role: Lab Manager and Supervisor

**Areas of expertise:
Instrument maintenance and troubleshooting for the Agilent GCMS and Perkin Elmer HSGC, oversight of day to day operations, QToF**

FROM THE EXECUTIVE DIRECTOR'S DESK

Submitted by: Beth Olson, SOFT Executive Director
beth@soft-tox.org



As we kick off 2021, SOFT is looking forward to a busy year! Most importantly, planning is in the works for an in-person SOFT Annual Meeting in Nashville in September. As we are planning for our Nashville meeting I'd like to reassure everyone that we'll be keeping a close eye on local, state and CDC guidelines. In order to have a successful meeting, we need to keep our attendees, exhibitors and staff safe, and we'll be following best practices at the time of the meeting. Look for our Covid Meeting Health and Safety web page when registration opens on May 3. This is where we'll keep everyone apprised of the precautions that we'll have in place for the meeting.

In addition, we'll be continuing many

of the online opportunities that were started in 2020. During SOFTember, we were able to hold open committee meetings for most of the SOFT committees. All SOFT members were able to attend to hear about the work our committees do, and to contribute and ask questions. We were surprised at the number of members who showed up for these meetings! At a couple of meetings, there were more than 100 SOFT members in attendance. What we learned from this was that this is a much-needed addition to what we offer as an organization and a great opportunity to build connections between members. We're going to continue the practice this year, with the committees scheduling at least one open meeting via Zoom during the first half of the year; and a second open meeting at SOFT in Nashville in September. Please keep an eye on your SOFT email for dates and times of these meetings so that you can participate.

We'll also be holding Continuing Education Committee workshops via webinar throughout the year. The two that were held last year were wildly

successful. Holding shorter sessions (2.5 hours) at a low cost met the needs of many participants, so we will continue this practice in 2021.

Our first SOFTopics of the year was held in February with nearly 100 participants. Several more SOFTopics sessions are in the works for 2021 and we plan to hold some in conjunction with webinars so participants will have the opportunity to discuss the webinar topics in small groups.

I look forward to seeing you all in Nashville in September. In the meantime, I hope to see many of you in our Zoom committee meetings, SOFTopics, continuing education workshops, and mentoring program sessions over the next few months!

BETH OLSON, MBA
SOFT EXECUTIVE DIRECTOR

SOFT Professional Mentoring Program

In each issue of ToxTalk, the SOFT Professional Mentoring Program Committee plans to bring you a glimpse of the activity going on within our program. Hear from committee members, program participants, and receive resources you can utilize in your own work life!

We wrapped up **Year 1** of the Professional Mentoring Program with a commencement celebration on December 10, 2020. Hear from the committee and Year 1 participants in this video.

Year 2 started off full steam ahead with 47 new pairs (75 total registrants), and seven pairs continuing from 2020, for a total of 54 mentor/mentee pairings for 2021.

We have planned a robust schedule of programs for the year, including meetings, trainings and webinars. If you have any questions, please feel free to reach out to any member of the committee.

**CLICK FOR
VIDEO!**

SOFT Professional Mentoring Committee

Michelle Peace, Chair

Jim Burris

Amanda Cadau

Marisol Castaneto

Marta Concheiro-Guisan

Lindsay Glicksberg

Helen Ha

Erin Karschner

Beth Olson

Kim Samano

Andre Sukta

Courtney Wardwell

SOFT TREASURER REPORT

Submitted by: Tate Yeatman M.S., F-ABFT
yeatmand@pbsso.org



In January, I was honored to take on the role of Treasurer of SOFT after serving on the Finance Committee last year and the past three years on the Board of Directors. I'd like to thank Robert Sears for serving as SOFT's Treasurer for the past two years. During my term, I look forward to continuing the work of the Finance Committee in overseeing SOFT's financial practices and making strategic recommendations to the Board to further strengthen SOFT's financial position.

The Finance Committee is made up of myself, Robert Sears, Suman Rana, Joe Saady, Bill Johnson, Russell (Rusty) Lewis, and Ayana Chan-Hosokawa. This committee helps provide financial oversight for the organization and provides guidance and recommendations to the Board on financial matters. As we begin 2021, SOFT remains in a strong financial position. As of January 28, 2021, SOFT's bank account balances totaled \$1,302,322. SOFT retained the services of an external audit firm to conduct an audit for 2020, the results of which were reviewed by SOFT's accountant, Board of Directors and Finance Committee. No material weaknesses or deficiencies were found by the audit firm.

Revenue

Annual Meeting

The budget for 2020 was approved by the Board with the expectation that SOFT would hold the annual meeting in San Diego. By March, we found ourselves in the early stages of the COVID-19 pandemic which would alter those plans drastically. In Mid-May, the SOFT BOD made the difficult decision to cancel the annual meeting. Based on

the timing of the meeting cancellation, some of our vendors had already paid for their sponsorship in the amount of \$13,375. These vendors requested that SOFT hold the funds and apply to the next in-person annual meeting.

SOFTember

SOFTember was developed which provided virtual training throughout the month of September. This event was a resounding success generating revenue lost due to the cancellation of the annual meeting.

Net Revenue

Our previously approved budget for 2020 projected a loss of \$79,956. Through the diligent work of the SOFTember planning committee, Beth, CC, the SOFT Board, and the entire SOFT family, SOFT finished 2020 with a positive cash flow.

Expense

Legal and Professional Services

The expenses in this category include the cost of the external audit that is performed every 2 years. Other expenses are associated with accountant fees, attorney's fees, payroll management costs, and IT support.

Administrative Expenses

This category includes the costs for the JAT subscriptions for SOFT members (approximately \$48,000 per year). Other expenses in this category include computer maintenance, software subscriptions, and professional development of staff.

Budget

The Finance Committee has prepared the budget for 2021 and forwarded to the Board for review and approval. The budget will take into consideration the current state of the COVID-19 pandemic and its potential impact on attendance at the annual meeting in Nashville.

For your convenience, a copy of SOFT's 2020 budget vs actuals is below. If you have any questions, please don't

hesitate to contact me. Thank you again for the opportunity to serve SOFT.

TATE YEATMAN M.S., F-ABFT
SOFT TREASURER

FINANCE COMMITTEE

Tate Yeatman, Chair

Robert Sears, Past Treasurer

Beth Olson

Suman Rana

Bill Johnson

Joe Saddy

Aya Chan-Hosokawa

Rusty Lewis

SOFT TREASURER REPORT

Submitted by: Tate Yeatman M.S., F-ABFT
yeatmand@pbso.org

Budget vs Actuals: FY 2020 Budget		
REVENUE		
	Actual	Budget
Membership	\$143,859	\$144,000
Annual Meeting	\$13,375	\$1,331,166
SOFTember	\$434,175	\$0
Merchandise	\$200	\$0
Advertising	\$500	\$0
Continuing Education	\$9,345	\$10,000
Contributions	\$3,372	\$3,000
Interest	\$138	\$750
Total Revenue	\$604,764	\$1,489,116
EXPENDITURES		
Payroll	\$149,384	\$146,157
Occupancy	\$2,109	\$4,000
Office	\$876	\$1,800
Administrative Expenses	\$69,001	\$72,500
Membership	\$7,500	\$5,000
Insurance	\$9,179	\$8,000
Appreciation Gifts	\$458	\$500
Legal and Professional Services	\$17,682	\$15,850
Meals and Entertainment	\$67	\$500
Awards	\$8,000	\$10,000
Annual Meeting *Refunded for more than the initial payment	(\$6)	\$1,242,765
SOFTember	\$45,447	\$0
Merchandise	\$0	\$0
Board and Committees	\$8,433	\$57,000
Bank Charges	\$6,169	\$5,000
Total Expense	\$324,299	\$1,569,072
Net Income/(Loss)	\$280,465	(\$79,956)

ANNUAL MEETING UPDATE - NASHVILLE, TN

After a very long and socially distant 2020, we are very excited to welcome you to Music City for the 2021 Annual SOFT meeting! While we are closely monitoring the COVID situation, we continue to plan for an in-person meeting. The 2021 meeting will be held September 26 – October 1 at the Gaylord Opryland resort complex (room rate \$209/night); a short trip from the airport and downtown Nashville. The resort is famous for its indoor attractions which include 9 acres of gardens, a 44-foot tall waterfall, and an indoor river. The resort also has on-site shopping and a variety of themed restaurants. Oh, and did we mention? The Grand Ole Opry, a world-famous country music institution, is just steps away.

The Scientific Program Chairs, the JAT Special Issue Editor, and the Workshop Chairs have sent out calls for abstracts and workshop proposals. Deadlines are fast approaching

and we need your submissions in all areas of forensic toxicology to help make the meeting a success! Are you interested in assisting with the scientific content as an abstract reviewer? Contact the Scientific Program Chairs. Would you like to volunteer at the meeting? Contact the Volunteer Coordinators.

We recommend that you make time to explore and see all that Nashville has to offer. We've put together a list [HERE](#) of our favorite places to eat, drink, and sightsee. See the sights of downtown Nashville, catch a live show, and enjoy the food scene while you're in town. We hope our off-site event will give you a taste of all that Nashville has to offer. We are very excited to host y'all in September!

See you soon!

Erin and Jen

IMPORTANT DATES AND DEADLINES

- JAT Special Issue Titles and Abstract Due, March 5, 2021
- JAT Special Issue Papers Due, March 19, 2021
- Workshop Submission Deadline, April 2, 2021
- ERA/YSMA Deadline, April 2, 2021
- ERA/YSMA Winners Announced, May 1, 2021
- Registration and Hotel Room Block Open, May 3, 2021
- Abstract Submission Deadline, May 10, 2021
- Registration Deadline to Avoid Late Fee, August 2, 2021
- Registration Deadline to Avoid On-Site Registration Fee, Sept. 1, 2021



2021 HOSTS



JEN COLBY



ERIN KARSCHNER

SCIENTIFIC PROGRAM COORDINATORS

KEN FERSLEW
REBECCA HARTMAN

WORKSHOP PROGRAM COORDINATORS

JARRAD WAGNER
CURT HARPER

EXHIBITOR LIAISON
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FOOD AND BEVERAGE
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ROXANE RITTER
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BETH OLSON
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CC WATSON

SEPTEMBER 26–OCTOBER 1, 2021
GAYLORD OPRYLAND



2021 PLANNING COMMITTEE MEMBERS

ANNUAL MEETING UPDATE - NASHVILLE, TN

Workshop Proposals for SOFT 2021 are due by April 2, 2021. Please contact the Workshop Program Coordinators, Curt Harper and Jarrod Wagner, in advance if you have a workshop idea you're planning to submit, even if you represent a SOFT committee. This will help avoid duplication of topics and aids in soliciting ideas if an area of inter-

est has not yet been met. If you have an idea but you are not quite sure whether you should submit, reach out so we can discuss it and get the best workshops in place in Nashville.

Workshop Program Coordinators

Jarrod Wagner and Curt Harper

2021 HOSTS



JEN COLBY



ERIN KARSCHNER

SCIENTIFIC PROGRAM COORDINATORS

KEN FERSLEW
REBECCA HARTMAN

WORKSHOP PROGRAM COORDINATORS

JARRAD WAGNER
CURT HARPER

EXHIBITOR LIAISON
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YFT CHAIR
VANESSA MENESES

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DAWN SWINEY
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MOBILE APPLICATION
RUSTY LEWIS
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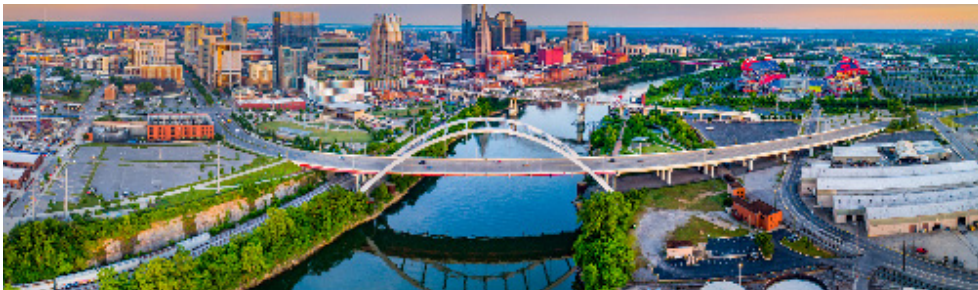
SEPTEMBER 26-OCTOBER 1, 2021
GAYLORD OPRYLAND



2021 PLANNING COMMITTEE MEMBERS

WORKSHOP PROPOSAL FORM

EMAIL PROGRAM COORDINATORS



SEE YOU IN NASHVILLE!

THE DFC COMMITTEE NEEDS YOUR HELP!

Happy New Year, Toxicology Community. The DFC committee needs your help! We are reaching out to request participation in the 2021 Drug Facilitated Crimes Survey from laboratories performing this type of casework. This questionnaire is short and should only take 10 minutes to complete. Your responses will guide our focus for the upcoming year on how the committee can contribute to the scientific community. Thank you for taking the time to complete.

**Click here for
Suvery Link**



**Please see the link and QR code to
complete the survey.**

**DON'T
MISS AN
ISSUE
OF JAT!**

Recently move? Change jobs? New last name?

Be sure to send your updated contact information to the SOFT office so your account with JAT can be updated.

Send your updates to cc@soft-tox.org

YOUNG FORENSIC TOXICOLOGISTS (YFT)

A Look at Suicides by Sodium Nitrite/Nitrate Ingestion: A possible new trend? A Case Report from the Young Forensic Toxicologists

Elisa N. Shoff, Erin Strickland, Aya Chan-Hosokawa

Introduction

Sodium nitrite (NaNO_2) and sodium nitrate (NaNO_3) are common inorganic substances found in food preservatives, fertilizer, and other industrial products (1). While safely consumed at low concentrations, at higher doses (exceeding 2 grams), sodium nitrite will oxidize the ferrous iron in hemoglobin, converting it to an inactive form, methemoglobin (MetHb). An increase in MetHb saturation hinders oxygen transport and subsequently causes asphyxiation and methemoglobinemia (2). When large amounts of sodium nitrate are ingested, the nitrate is first reduced to nitrite before toxicity can occur. Common pathological findings in decedents with fatal nitrite ingestion include cyanosis or the blue/grey discoloration of the skin, chocolate-brown discolored blood, and pulmonary edema (3). Sodium nitrite/nitrate is not a routinely tested substance in forensic toxicology, and published cases involving fatal ingestion of sodium nitrite/nitrate were at a minimum. However, since 2019, a recent increase in prevalence of these cases has occurred in various parts of the United States, primarily in teenagers and young adults, with the assistance of pro-suicide online forums and the ease of accessibility to purchase sodium nitrite online. The following cases were received and analyzed in 2019 and 2020, and provided by YFT committee members Elisa Shoff (Case 1 and 2) – Toxicologist II, Miami-Dade Medical Examiner Department (MDME) in Miami, FL, and Erin Strickland (Case 3 and 4) – Toxicologist 3/Supervisor, Harris County Institute of Forensic Sciences (HCIFS) in TX, as well as, fellow young forensic toxicologist, Aya Chan-Ho-

sokawa (Case 5 and 6) – Toxicology Technical Team Leader, NMS Labs. For all cases, %MetHb saturation and nitrite/nitrate levels, as applicable, were determined by spectrophotometry. A summary of all cases provided for this report can be found in Table 1.

Case Samples

Case 1: A 49 yr old white female was seen in the early morning via security footage smoking a cigarette and drinking coffee outside her apartment. Later that day, her partner tried contacting her numerous times with negative results. Her partner then proceeded to contact her landlord to conduct a welfare check, and upon entering the apartment, she was found unresponsive in bed. Fire rescue was called and pronounced her on the scene. The decedent had a history of bipolar disorder, depression, and previous suicide attempts, the most recent being three weeks prior by hanging. Upon investigating the scene, a large bottle of sodium nitrite was found on the decedent's bedroom side table. Additional substances found were bags of Kratom, and various prescription medications that the decedent was prescribed. Routine toxicology screening and confirmation was performed using gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-ion trap mass spectrometry (LC-Ion Trap MS) on both heart blood and iliac vein blood sources. Gabapentin, 9-hydroxyrisperidone, mitragynine, 7-hydroxy mitragynine, duloxetine, and ondansetron were detected qualitatively. Due to the presence of the bottle of sodium nitrite on the scene, an in-house quantitative analysis for



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YOUNG FORENSIC TOXICOLOGISTS (YFT)

carboxyhemoglobin, oxyhemoglobin and MetHb was performed. MetHb saturation was reported at 19% and the pathologist ruled the cause of death as “Acute Sodium Nitrite Toxicity”, and the manner of death “Suicide”.

Case 2: An 18 yr old white male was last seen by his mother around 10pm when she said goodnight to him. A short while later, she heard a noise and went into his room to find him unresponsive in bed and that he had vomited. Fire rescue responded to the scene and pronounced him dead. Police investigation discovered a suicide note and revealed the decedent had searched several methods of suicide, including sodium nitrate, and cyanide. The decedent had no known medical or mental health conditions and had never expressed any suicidal ideations in the past. All routine toxicology was negative, including a screen for cyanide in both blood and gastric. An in-house quantitative analysis for carboxyhemoglobin, oxyhemoglobin and MetHb was performed. MetHb saturation was reported at 64% and the pathologist ruled the cause of death as “Acute Sodium Nitrate Toxicity”, and the manner of death “Suicide”.

Case 3: A 17 yr old male was found unresponsive on his bedroom floor with a suicide note next to him stating he ingested sodium nitrite (NaNO_2). A bottle of sodium nitrite was found on scene. Medical intervention was attempted. His abdomen appeared slightly distended and it was observed at autopsy that the body looked blue/gray and his blood turned brown. In-house testing included a volatiles analysis (ethanol, methanol, isopropanol, and acetone), a basic drug screen by GC-MS, and a 10-panel ELISA (Enzyme Linked Immunosorbent Assay) screen. MetHb analysis was performed by NMS Labs. In-house test results did not yield any significant toxicological findings.

The MetHb saturation was reported as 44% and the cause of death was classified as “acute toxicity of sodium nitrite” and the manner of death was ruled as “suicide”.

Case 4: A 20 yr old female with a past medical history of depression was found with vomitus and in convulsions at her residence and pronounced <1 hr after hospital arrival. The decedent was reported to have consumed an “entire” bottle of sodium nitrate. This was suspected from social media posts. Bottles of sodium nitrate and sodium nitrite were recovered from the scene. At the hospital, her blood was reported to be black in color. In-house testing included volatiles analysis, vitreous chemistries, basic drug screen by GC-MS, and a 10-panel ELISA screen. MetHb analysis was performed by NMS Labs. In-house results did not yield any toxicological significant findings. The MetHb saturation was reported as 31% and the cause of death was classified as “acute sodium nitrate toxicity” and the manner of death was ruled as “suicide”.

Case 5: Decedent was a 17 yr old male. A moderator of a mental health/suicide knowledge website called emergency services after locating a post made by the decedent stating his intention and method he was going to use that evening. The internet provider address was tracked, and police were dispatched for a welfare check. Officers reached a family member and made entry into the home. The decedent was found deceased on the floor of his bedroom. A note of intent was located along with a bottle of sodium nitrate and an empty water glass. Apparent suicide by ingestion of sodium nitrate was suspected. Screening was performed using

immunoassay, and liquid chromatography-time of flight mass spectrometry (LC-TOF) on peripheral blood. Metoclopramide and cannabinoids were detected qualitatively. MetHb saturation was reported at 34% and nitrates/nitrites were quantified at 4400 mcM.

Case 6: Decedent was a 20 yr old male who had voiced threats of suicide by ingesting sodium nitrite to a girlfriend on the phone. The decedent’s mother called in a missing/suicidal person and the decedent’s phone was pinged at the hotel. Police were dispatched and found the decedent unresponsive on the floor lying face down. Two bottles of sodium nitrite were found on a table in a hotel room in addition to a bottle of antacid, a scale, measuring spoons, and a cup with residual white powder and water. In the trash can was found a shipping bag with a label that later matched to an Amazon shopping page that sells chemicals including the same brand sodium nitrite that was located at the scene. The note found had random thoughts but did not mention suicide. MetHb saturation was reported at 47% and nitrates/nitrites were quantified at 5500 mcM. Additional toxicological findings included the detection of metoclopramide qualitatively by LC-TOF.

Discussion

Since 2019, three cases from MDME, eight cases from HCIFS, and over 100 cases from NMS have been reported for fatal sodium nitrite/nitrate ingestion. While nitrite/nitrate exposure is typically accidental in nature, the rise of “suicide kits” available on the internet (2) has led to this possible new increase/trend observed in the past two years. The fourteen cases presented here is in comparison to

five previously published reports of suicide due to nitrite/nitrate toxicity from 1975-2020 (2-6). Investigative information, such as scene photos and evidence, is imperative in the determination of cases like this as specialty testing is needed, especially considering there is often minimal or no other toxicological relevant findings in these cases. In conjunction with crime scene investigation, MetHb saturation is utilized as an indication of sodium nitrite/nitrate toxicity. However, there is limited data supporting a correlation of MetHb saturation to measured sodium nitrite/nitrate levels. Falsely elevated or decreased levels of MetHb can occur in postmortem specimens, emphasizing the importance of testing soon after blood collection, minimizing the postmortem interval, and proper storage of specimens (7). Advertised as a “peaceful” method of suicide, sodium nitrite/nitrate toxicity is an attractive, easy alternative that appears to be on the rise and underscores that easily accessible toxins should not be overlooked in death investigations.

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YOUNG FORENSIC TOXICOLOGISTS (YFT)

Table 1. Case Result Summary

Case Number	State	Gender	Age	Collection Site	% MethHb Saturation	Cause of Death	Estimated PMI (hrs.)	Total Nitrite/Nitrates	Other Toxicology Findings
1	FL	Female	49	Heart	19	Acute NaNO ₂ Toxicity	N/A	N/A	Gabapentin 9-Hydroxyrisperidone Mitragnine 7-Hydroxy Mitragnine Ondansetron
2	FL	Male	18	Aorta	64	Acute NaNO ₂ Toxicity	N/A	N/A	None
3	TX	Male	17	Iliac	*44	Acute NaNO ₂ Toxicity	29-46	N/A	None
4	TX	Female	20	Femoral	*31	Acute NaNO ₂ Toxicity	19	N/A	None
5	CA	Male	17	Peripheral	*34	Apparent suicide by ingestion of NaNO ₂	N/A	4400	*Metoclopramide *Cannabinoids
6	FL	Male	20	Femoral	*47	N/A	N/A	5500	*Metoclopramide
7	FL	Female	17	Peripheral	79	Acute NaNO ₂ Intoxication	N/A	N/A	None
8	TX	Male	21	Femoral	*47	Acute NaNO ₂ Toxicity	16-34	N/A	None
9	TX	Male	29	Femoral	*35	Toxic Effects of NaNO ₂ & Etizolam	19-21	N/A	Etizolam
10	TX	Male	25	Femoral	*31	Methemoglobinemia Due to Acute NaNO ₂ Toxicity	17-33	N/A	None
11	TX	Female	17	Heart	*16	Toxic Effects of NaNO ₂	18-29	N/A	None
12	TX	Male	65	Heart	*77	Toxic Effects of NaNO ₂ & propranolol	50-66	N/A	*Sulfahemoglobin - 15% *Carboxyhemoglobin - 2% *Cimetidine - 2.5 µg/mL *Propranolol - 2100 ng/mL *Caffeine *Metoclopramide *Lorazepam - 14 ng/mL *Alprazolam - 48 ng/mL *Sertraline - 620 ng/mL *Desmethylsertraline - 1100 ng/mL *Mirtzapine - 310 ng/mL *Aripiprazole - 120 ng/mL
13	TX	Female	18	Femoral	*46	Toxic Effects of NaNO ₂	6-9	N/A	Diphenhydramine - 0.22 mg/L Methorphan <0.010 mg/L
14	IN	Male	14	Subclavian	*17	Suicide by NaNO ₂	N/A	2400	None

1. N/A - Data was not available or not applicable for the case. *NMS Labs test result

Designer Benzodiazepine Detected in a Sexual Assault Victim

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Introduction:

Benzodiazepines are a commonly prescribed class of drugs that are used in the treatment of anxiety disorders, depression, and insomnia. The illicit market has seen an emergence of novel or “designer” benzodiazepines that are readily available to order online and are not controlled at a federal level (1).

Flubromazolam is a potent triazolo-benzodiazepine that has no accepted clinical use and is currently not classified as a controlled substance at the federal level (1). Effects produced by flubromazolam can be observed after doses of less than 1 mg. Huppertz et al. (2) estimated a terminal half-life of 10-20 hours for Flubromazolam. Carpenter et al (3) evaluated clinical effects after exposure to flubromazolam, which included drowsiness/lethargy, slurred speech, confusion, ataxia, and hypotension.

Lorazepam (Ativan®) is a 3-hydroxy-benzodiazepine with a half-life of 9-16 hours (4). Like other benzodiazepines, the effects produced by both flubromazolam and lorazepam are those of central nervous system depression including sedation, dizziness, confusion, memory impairment, and decreased coordination. Due to their effects, the detection of benzodiazepines in drug-facilitated sexual assault [DFSA] cases has become more frequent.

Case History:

An eighteen-year-old female and her roommate invited their neighbor’s brother over to their apartment to hang out with them. The victim stated that the three of them were sitting around and talking for a few hours when she fell asleep around midnight. Her roommate later told her that both he and the other man moved her to the bedroom as she was in a state of sleep.

The roommate had left the apartment,

and while the roommate was gone, the victim awoke to the male assaulting her. She reports that she was confused and at the time felt like she was in a state of trying to wake up and was not certain if she was in a dream or not. The roommate asked the victim if she felt like she was assaulted and the victim replied yes. At this time, the roommate called and reported the assault to law enforcement. The victim stated that her last memory was around midnight until the next morning when law enforcement arrived somewhere between 0700 and 0900 hours.

The morning after the assault the victim was examined at a local hospital by a sexual assault nurse examiner [SANE]. The victim admitted to using marijuana approximately 12 hours before the assault occurred and stated that she has not taken any over the counter or prescription medications for as long as she could remember. In addition, the victim reported that she had not consumed any ethanol. The victim reported the following symptoms of drowsiness, confusion, memory loss, dizziness, and loss of consciousness over a period of approximately 7-9 hours.

During the exam, the victim repeatedly stated that she was “so tired” and dozed off periodically.

Blood and urine specimens were collected by the SANE at 1324 and 1504 hours, respectively.

Toxicological Analyses & Results:

Based on case history and period between the assault and collection of samples, volatile analysis was not performed. The urine specimen was screened using an eight panel [amphetamines, barbiturates, benzodiazepines, benzoylcegonine, cannabinoids, opiates, methadone, and phencyclidine] immunoassay by EMIT II Plus

(Siemens Healthcare Diagnostics, Inc., Newark, DE, USA) and a five panel [carisoprodol/meprobamate, fentanyl, oxycodone, tramadol, and zolpidem] immunoassay by ELISA (Immunoanalysis Corporation, Pomona, CA, USA). All positive immunoassay results were followed by a gas chromatographic-mass spectroscopic (GC/MS) or liquid chromatographic-tandem mass spectroscopic (LC-MS/MS) analysis for confirmation of analytes in the urine specimen. Based on the time frame of the assault/specimen collection and urine confirmation results, additional analyses were performed on the blood specimens by GC/MS or LC-MS/MS. The toxicological findings are presented in Table 1.

Discussion:

Flubromazolam is a novel benzodiazepine, which has recently been detected in drug driving cases in our jurisdiction (1). This case is the first DFSA case in our jurisdiction where flubromazolam has been detected.

The victim stated she had not taken any prescription or over the counter medications and failed to report her methamphetamine use, but she did report recent use of marijuana. This failure to report the usage of an illegal drug is consistent with the findings of Negrusz, et. al. (5) and could be due to the victim feeling that their recreational drug use would negatively affect the course of the sexual assault prosecution. The study determined that more DFSA cases occur due to an assailant taking advantage of someone after their own drug use rather than a perpetrator surreptitiously administering a drug.

The victim did report loss of consciousness, drowsiness, confusion, memory loss and stated during the examination she was tired, with the nurse examiner noting that she “dozed off” during the examination. These reported symptoms and ob-

Designer Benzodiazepine detected in a Sexual Assault Victim

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servations are consistent with the use of benzodiazepines and possibly the downside effect of methamphetamine intoxication.

Benzodiazepines have become commonly associated with DFSA due to their incapacitating effects such as sedation, dizziness, disorientation, lack of coordination, and memory impairment. The combination of two different benzodiazepines [flubromazolam and lorazepam] would enhance these effects.

Given the short half-lives of both Lorazepam and Flubromazolam and the time of ingestion to the collection of the blood specimens, a negative result is not unexpected. Although the victim admitted to smoking marijuana twelve hours before the assault, the urine immunoassay was negative for cannabinoids.

Fiorentin and Logan reviewed 1000 DFSA cases and found 784 cases were positive for one or more intoxicating

substances, and 210 of those cases were positive for benzodiazepines (6). Given the ready availability of these novel non-controlled benzodiazepines and their increased usage, it is important that the laboratory have methods in place to detect these emerging drugs.

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Table 1. Toxicology Results

Drug	Blood	Urine
Methamphetamine	0.13 mg/L	Positive
Amphetamine	Positive < 0.10 mg/L	Positive
Flubromazolam	Negative ¹	Positive
Lorazepam	Negative ²	Positive

¹ LOD: 4.0 ng/ml

² LOD: 10 ng/ml

ESTIMATING NORMAL RITALINIC ACID CONCENTRATIONS IN URINE

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Introduction:

Methylphenidate has been used to treat symptoms of Attention Deficit Hyperactivity Disorder (ADHD) for over 50 years (1). Additionally, it has been used to treat childhood bipolar disorder (2). Various reports suggest that a diagnosis of ADHD and subsequent treatment with stimulant drugs such as methylphenidate have increased to as much as 15% of the population (3). In that time, the nature of the formulation has evolved from immediate release to include transdermal patches and extended release oral tablets. Inasmuch as methylphenidate is a stimulant, it has been and continues to be abused (4,5).

Urine drug testing (UDT) is often employed to help assess patient adherence to chronic drug prescriptions (6). Since 80% of the oral dose of methylphenidate is excreted in urine as ritalinic acid (RA), the primary metabolite of methylphenidate, the resulting concentrations can be relatively high making identification of diversion and other abuse pathways difficult in the absence of historical metabolite levels from a normal patient population (7).

The work reported herein was directed at defining "normal" for urine levels of ritalinic acid. Over 28,000 test results were examined to reach this goal including 3 different groups of patient samples: positive with a prescription, negative with a prescription, and positive without a prescription. However, in addition to trying to define "normal", the data revealed several interesting conclusions involving age dependent concentrations of RA. Further, sample validity test results were evaluated for patients below age 18 down to age 4 and compare with normal adult levels of creatinine, pH, and specific gravity as well as RA concentrations.

Materials and Methods:

Ritalinic acid analysis is part of a larger test panel. Details of the full method and validation can be found in an earlier report by Enders et al. (8). Ritalinic acid and the corresponding internal standard, ritalinic acid-D10, were purchased from Cerilliant Corporation (Round Rock, TX) in 1 mg/mL stock solutions. An enzyme solution was prepared by diluting IMCSzyme® β -glucuronidase solution (IMCS, Irmo, SC) to 10,000 units/mL in 0.02 M sodium phosphate buffer, at pH 7.5. Normal, drug-free urine was purchased from UTAK (Valencia, CA). Samples (30 μ L) were diluted six times with 120 μ L of enzyme solution and 30 μ L of 1,000 ng/mL ritalinic acid-D10 internal standard. After dilution, samples were incubated at 60°C for 60 minutes for hydrolysis and then extracted using a solid-phase extraction method. Ultimately, samples were diluted ten times in 300 μ L of 10% methanol:90% water prior to injection and LC-MS/MS analysis. A morphine-3 β -D-glucuronide (Cerilliant, Round Rock, TX) standard was used as a hydrolysis control for the method. While conjugation of RA has not been reported, other analytes in this method (e.g., benzodiazepines, opiates, etc.) required hydrolysis for testing. Thus, RA was "hydrolysed" as part of the overall work flow in this test pathway.

LC-MS/MS Method

The method used a Thermo Ultra LC-MS/MS system utilizing mobile phases A (5mM ammonium formate with 0.1 % formic acid [aqueous]) and B (5mM ammonium formate in 75:25 methanol:acetonitrile with 0.1% formic acid) to provide a gradient shown in Table 1. A flow rate of 0.8 ml/min was used throughout and yielded a total cycle time of roughly 6.5 minutes. A Phenomenex (Torrance, CA) Kinetex 2.6

μ m Phenyl-Hexyl 100Å, 50 x 4.6mm (00B-4495-E). The injection volume was 15 μ L and column temperature was 30°C. The RA transitions and MS details for the Thermo Ultra can be found in Table 2. Ritalinic acid produced a quadratic response from 100 ng/mL to 100,000 ng/mL with 1/X² data weighting. A reporting cut-off of 500 ng/mL was used.

Data Analysis:

In an attempt to identify an adherent population of patients, the test results for ritalinic acid were curated as follows:

1. Only patients who were prescribed methylphenidate (e.g., Ritalin®, Concerta®, etc.) and tested positive for RA were included.
2. Patients testing positive for any illicit drugs were excluded.
3. Patients who did not test consistent with any other prescription(s) were excluded.
4. Patients who failed sample validity testing (e.g., pH, creatinine, and specific gravity) were excluded.
5. Patient samples without a UDT quantitative result (i.e., >ULOL) were not included.

This filtering process took the original 11,383 data points for ritalinic acid patient samples that were positive with a prescription down to 10,738 data points post cleaning. The data in Figure 1 are shown as box and whisker plots of the data with 2.5% and 97.5% limits. This was an attempt to remove "outliers" from the clean data set and provide more robust ranges.

Results:

Figure 1 illustrates the data post filtering for RA as described above. The

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graph is displayed on a logarithmic scale so that the plots can be displayed in the same graph. However, that is strictly a function of the display and has no bearing on the actual data. Nothing in this display reflects a “normal” distribution as expected from previous data displays from UDT (9-13) which is why box and whiskers plots were chosen for these data. Notably, the median for patients under 18 years old is much higher (14,246 ng/mL) than the median for patients 18 and over (8,714 ng/mL) (Figure 1b, 1c). A nonparametric Mann-Whitney U test indicates there is a significant difference between the mean of the ritalinic acid concentration in patients younger than 18 years and that in patients 18 years and older ($p < 0.001$).

Table 3 shows a variety of data listed by patient age. Interestingly, the median RA concentrations for patients below 18 are significantly higher than those for patients over 21 years old. This does not seem to be a function of body weight, creatinine or daily dose (also shown in Table 3). While creatinine concentration does appear to increase with age until about age 18, it is neither a big change nor unexpected (14,15). Specific Gravity and pH are consistent across all ages.

Table 3 also reports median data for patients under 6 suggesting lower median levels of RA. Patient samples from 4-year-olds have a median value of 5,716 while 5-year-olds exhibit a value of 12,172 ng/mL. The median pH is 7.02 for 4-year-olds and 6.87 for 5-year-olds while “normal” is approximately 6.5. Additionally, creatinine is lower for this age group at approximately 90 mg/dL and seems to hold there through age 8 before jumping to nearly 148 mg/dL in 15-year-olds.

Discussion:

Box and whiskers plots of the RA data are shown in Figure 1. The data was curated as discussed in the methods section in an attempt to define “normal” ranges of RA from methylphenidate patients. While the overall range (all data) in Figure 1a is interesting, the box and whiskers plots representing patients less than 18 years old and patients 18 years old and over demonstrate the difference between these unique populations (Figure 1b and 1c). It is clear that these two populations have statistically significant different average RA concentrations with the younger patients exhibiting the highest concentrations. A close look at Table 3 does not indicate any correlation between these average/median values and body weight, creatinine, or daily dose. Indeed, the daily dose does not vary tremendously between younger patients and those over 18. Normalizing dose by weight as shown in table 3 reveals slowly increasing dose/lb with age even though the median concentration of RA in younger patients is almost two fold greater than that of the older patients. Lastly, no difference was found between male and female patients.

Abuse of methylphenidate is acknowledged especially on college campuses where it is used to enable all night studying and is used recreationally via intranasal administration (5). The patient samples examined in this work fall into 3 broad categories: those with a prescription and positive for RA (11,384, 39.8%), those with a prescription but negative for RA (10,421, 36.4%), and those without a prescription but positive for RA (6,792, 23.8%). Of the patients with a prescription, only 11,383 tested positive for the metabolite (52.2%). The other 10,421 (47.8%) samples with a prescription

did not test positive suggesting either nonadherence of the patient or, more dangerously, diversion of the prescribed methylphenidate. Another group of 6,792 patients were positive for RA without a prescription listed. However, the ages of this group match those of the others and it is difficult to assign this group to either abuse or diversion.

One aim for this paper was to aid physicians in determining patient adherence. To be successful, RA outliers should be readily identified from a comparison with Figure 1. Necessarily, 5% of the patient data used in making Figure 1 is outside the limits of this box and whiskers plot. The ability to quickly compare UDT results without further mathematical manipulation to results from a large test population should help physicians determine patient adherence from their UDT data. Looking at the population of positive without a prescription, the data ranges from 500 ng/mL (administrative cutoff) to over 900,000 ng/mL. However, the 25%, median, and 75% values for this group are within those of the “positive with a prescription” group suggesting they do not account for abuse or diversion. In addition, the age ranges are similar to those of “positive with prescription”. If it is assumed that values over 120,000 ng/mL suggest abuse of the drug, then 167 patient samples from the positive with prescription are over this limit and as high as 900,000 ng/mL. At the very least, these patients should be consulted about their use of methylphenidate.

While an ever increasing number of children and young adults continue to be dosed with methylphenidate as well as other stimulants, little has been written about testing concentrations of RA in urine and what is “normal” vs. what is diversion/abuse

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(3,16). The data presented herein provide an estimate of “normal” such that physicians can readily assess whether a patient is “normal” or may be abusing/diverting their prescription. Interestingly, the “normal” concentration of RA in urine is different for patients under 18 years old from those 18 years and older while dose levels seem to be consistent across all age groups. This is the same across all data regardless of sex or dosage format. Thus, analysts should consider that the “normal” range of RA in urine is different for children than for adult patients.

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Table 1. The LC Gradient Parameters for Ritalinic Acid Method.

Step	Start (min)	Flow rate (mL/min)	%A	%B
1	0	0.8	95	5
2	1.08	0.8	80	20
3	2.25	0.8	35	65
4	4.12	0.8	2	98
5	6.45	0.8	2	98
6	6.95	0.8	95	5

Table 2. MSMS Method Acquisition Parameters.

Analyte	Transition	Collision Energy (V)	Tube Lens Voltage (V)	Retention Time (min)	Time Window (min)
Ritalinic Acid	220.137 → 84.135	35	107	1.75	0.4
	220.137 → 56.199	36	107	1.75	0.4
Ritalinic Acid D10	230.194 → 93.194	21	75	1.75	0.4
	230.194 → 61.132	42	75	1.75	0.4

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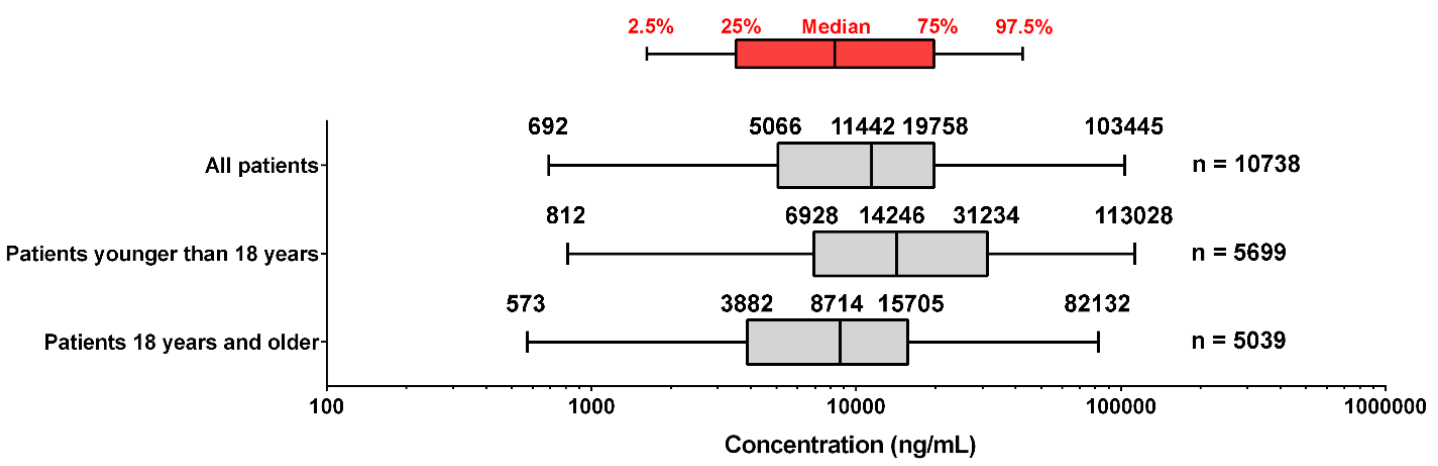
Table 3. Results by age.

Age (yrs)		Patient Specific Criteria (SVT and Demographics), Averages						RA Concentration (ng/mL)			Average Dosage	
Age Group	Avg. Age	N	Weight (lbs)	Ht (in)	pH	specific gravity	creatinine (mg/dL)	Mean	Std Dev	Median	Daily Dose (mg)	dose / Wt
4	4.74	13	50	44.33	7.02	1.0135	90.16	11762.1	18257	5766	12.2	0.24
5	5.6	153	54.81	45.52	6.87	1.0124	92.11	19750.7	24042	12122	13.1	0.24
6	6.56	322	64.71	47.49	6.88	1.0133	91.96	25183.3	30491	13996.5	20	0.31
7	7.51	438	60.35	50.04	6.82	1.0131	94.26	25637.5	33767	13725	16.4	0.27
8	8.51	536	67.3	51.54	6.73	1.0132	95.78	23934.0	27714	13685	21.4	0.32
9	9.52	614	74.61	53.61	6.75	1.0133	105.61	29054.6	34562	14886.5	24.2	0.32
10	10.51	651	85.78	54.94	6.6	1.0136	115.51	27175.6	30645	15654	26.2	0.31
11	11.48	643	96.1	57.28	6.56	1.0136	120.78	26671.1	31435	15205	28.9	0.30
12	12.5	827	112.6	59.8	6.51	1.0142	132.34	26045.0	30754	13590	28.5	0.25
13	13.47	444	125.06	62.09	6.58	1.0143	142.3	24042.8	27017	14440.5	29	0.23
14	14.46	429	140.69	64.05	6.53	1.0144	148.96	27203.6	36637	14246	32.86	0.23
15	15.51	360	154.76	65.96	6.6	1.0141	146.91	31844.1	86842	13207.5	35.15	0.23
16	16.48	282	164.72	66.92	6.5	1.0137	156.98	20523.5	24301	13353.5	31.95	0.19
17	17.46	240	164.71	66.53	6.69	1.013	161.32	22968.8	24680	15000.5	34.27	0.21
18	18.39	177	167.64	67.26	6.7	1.0131	148.46	16093.8	15700	11988	37.41	0.22
19	19.49	117	181.94	67.59	6.64	1.0137	156.67	19658.6	22502	11743	33.26	0.18
20	20.48	107	171.82	67.42	6.67	1.0128	142.82	13086.2	22802	7737	30.46	0.18
21	21.51	97	169.94	66.48	6.74	1.0125	138.96	21288.1	25409	11994	37.84	0.22
22	22.48	86	176.57	67.57	6.6	1.0127	134.95	11700.7	12127	8077	33.46	0.19
23	23.54	108	183.53	67.53	6.51	1.0126	138.42	16635.1	28106	7293.5	31.03	0.17
24	24.53	96	178.55	67.08	6.61	1.0119	129.55	14766.7	21196	8108.5	36.5	0.20
25	25.54	92	185.22	67.72	6.59	1.0118	119.13	10874.5	11607	8586.5	33.3	0.18
26	26.5	97	184.91	66.92	6.65	1.0118	115.89	13319.3	19532	8839	28.9	0.16
27	27.53	186	180.44	65.68	6.55	1.0125	115.56	13239.1	22155	6425.5	30.89	0.17
28	28.51	157	176.36	65.89	6.54	1.0112	120.55	16638.4	25897	9007	38.87	0.22
29	29.52	123	184.59	66.62	6.55	1.0128	124.75	15311.4	21773	8867	39.85	0.22
30	30.51	139	184.69	67.07	6.5	1.012	124.44	16368.6	24582	9124	37.4	0.20
31	31.55	154	184.27	67.01	6.35	1.0136	132.5	14242.4	20374	8395	39.71	0.22
32	32.5	152	185.27	65.68	6.51	1.0123	118.75	12783.9	16772	8552	36.26	0.20
33	33.47	103	184.08	65.8	6.42	1.0106	120.05	11611.8	15253	7528	36.99	0.20
34	34.53	148	172.99	65.95	6.57	1.0103	106.95	12536.0	18451	8712	37.68	0.22

ESTIMATING NORMAL RITALINIC ACID CONCENTRATIONS IN URINE

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Figure 1. Box and Whiskers plots for a) all patients, b) patients under 18 years, and c) patients 18 years and older.



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HUNTINGTON CONVENTION CENTER, CLEVELAND, OH
OCTOBER 30–NOVEMBER 4, 2022
DOUG ROHDE AND MICHELE MERVES CROSBY

2023

GAYLORD ROCKIES, DENVER, CO
OCTOBER 29–NOVEMBER 3, 2023
DAN ANDERSON AND JARRAD WAGNER

2024

UNION STATION, ST. LOUIS, MO
OCT 27–NOV 2
TBD

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