

Fatal 2,4-Dinitrophenol (DNP) Ingestion & Use of a Novel Analytical Methodology Testing Post-Mortem Blood Concentrations

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Abstract

Introduction: 2,4-Dinitrophenol (DNP) is readily available online as an unapproved weight loss supplement. Severe systemic toxicity and death have been reported following DNP use. Currently, routine laboratory testing of DNP concentrations is limited and seldom employed. We report a case of a fatal DNP ingestion and highlight the use of novel quantitative analytical procedures designed for post-mortem blood.

Methods: ToxB^ox[®] (PinPoint Testing, LLC), a drug elution kit designed to measure DNP in post-mortem blood specimens, was used. Samples were extracted using supported liquid extraction techniques and analyzed using liquid chromatography tandem mass spectrometry (LC-MS/MS).

Results: The patient's post-mortem femoral blood concentration of DNP was 72 µg/mL. The limit of detection was 0.001µg/mL.

Discussion: Cases of DNP toxicity are being increasingly reported, exacerbated by DNP's narrow therapeutic index, long half-life, and high lipid solubility. Our case involves an acute fatal ingestion with elevated DNP concentrations confirmed on post-mortem femoral blood. The patient's post-mortem blood concentration exceeds concentrations measured in the majority of other published cases of DNP fatalities.

Conclusion: Despite increasing DNP use and toxicity, readily available testing for DNP is limited. Our research team successfully used a novel analytical method to quantify post-mortem blood DNP concentrations. Forensic laboratories and clinicians may consider this test for future cases involving DNP.

Keywords: Forensic Science; Forensic Toxicology; 2,4-dinitrophenol; DNP; Post-mortem

List of abbreviations: DNP: 2,4-dinitrophenol; LC-MS/MS: Liquid Chromatography Tandem mass spectrometry; GC-MS: Gas Chromatography Mass Spectrometry

Introduction

2,4-Dinitrophenol (DNP) represented one of the earliest pharmaceutical approaches to treat obesity in the 1930s. It was also utilized by World War II soldiers to preserve body heat [1]. DNP is an odorless, yellow crystalline chemical with a narrow therapeutic index; it is well-absorbed orally, dermally, and via inhalation [2]. Although DNP remains a valuable industrial chemical used in the manufacture of dyes, explosives, and herbicides, it brings with it a checkered past and was removed as a dietary supplement in the late 1930s due to causing significant systemic toxicity [3]. Despite its dangers and narrow therapeutic index, disingenuous efforts have been made to market DNP on the Internet as an effective weight loss agent; this has resulted in a resurgence of use as well as increasing reports of toxicity and death [1].

DNP acts as a metabolic stimulant via uncoupling of oxidative phosphorylation [1]. Consequently, increased oxygen consumption and a subsequent hypermetabolic state develop, resulting in increased body temperature, heart rate, and respiratory rate [2]. This leads to the depletion of adenosine triphosphate and development of lactic acidosis [3].

Clinical manifestations of DNP toxicity include fever, headache, nausea, vomiting, diaphoresis, tachycardia, and tachypnea; yellowing of skin, sclera, and body fluids; and metabolic acidosis. More profound poisoning includes life-threatening hyperthermia, respiratory failure, cardiovascular collapse, and death. Treatment is largely supportive as no established approach or antidote currently exists [3].

Analytical testing for DNP is not commonly available and there is a paucity of data correlating serum concentrations with toxicity and death. The objective of this study is to report a case of fatal DNP ingestion and the use of a novel analytical method capable of confirming post-mortem blood concentrations.

Case Report

A 27-year-old male, with a past medical history of post-traumatic stress disorder, major depressive disorder, and attention deficit hyperactivity disorder, was found unresponsive and pulseless with an unknown down time following ingestion of 4 to 8 grams of DNP. An empty bottle of DNP capsules was found at the scene. Upon Emergency Department (ED) arrival, the patient remained pulseless with a core temperature of 100.8°F. Standard advanced cardiac life support resuscitative efforts were immediately commenced but were futile and the patient was pronounced dead 30 minutes post-ED arrival. Post-mortem femoral blood samples were referenced to a private ISO17025 and COLA/Clinical Laboratory Improvement Amendments (COLA/CLIA)-accredited laboratory (PinPoint Testing, LLC, Little Rock, Arkansas) for DNP testing.

Methodology

A custom ToxB^ox[®], a proprietary drug elution kit available from PinPoint Testing, LLC (Little Rock, Arkansas), was specifically designed to measure DNP in post-mortem blood specimens. Standards, second source quality control samples (QCs), and the isotopically-labeled internal standard were manufactured in a 96-wellplate format to deliver precise concentrations. Prior to analysis, drug residue in each well was reconstituted in 250 µL of whole blood to build six levels of analytical standards (0.5 µg/mL to 10 µg/mL) and three second source QCs spanning the linear working range (2.5 µg/mL, 5 µg/mL, and 10 µg/mL). The isotopically-labeled internal standard was also pre-manufactured in each standard and QC well in addition to blank wells for unknown specimen analysis. The final internal standard concentration in 250 µL blood samples was 0.05 µg/mL. As previously described by Patton *et al.* and McCain *et al.*, all blood calibration standards, QC material, and unknown samples were processed and analyzed identically [4,5]. Final sample extracts were immediately assayed or stored at 4 °C until analysis.

The analyte and internal standard were analyzed chromatographically using 5 µL injections on a 2.6 µm Phenomenex Kinetex Phenyl Hexyl (50 x 4.6 mm) LC column heated to 35 °C. Analytes were resolved at 0.5 mL/min using mobile phase A (10 mM ammonium formate in ultrapure 18.2 MΩ•cm water) and mobile phase B (0.1% formic acid in methanol). Analyte and internal standard were resolved using a gradient starting at 95% aqueous (Mobile Phase A) and ramping to 0% aqueous over 4 min, and holding constant for 1 min. The gradient returned to initial conditions over 0.1 minute and equilibrated for an additional 1.9 minutes. The total run time, including column equilibration period between injections, was 7.0 min. Specific mass spectrometer and analyte parameters are provided in Table 1 and 2. Two mass-to-charge ratio (*m/z*) transitions were monitored for each analyte. Ion ratios were matched to those of calibration standards to ensure interfering metabolites and other compounds were resolved. To ensure carryover was not present, matrix-matched samples containing no calibration standard material were injected, and blanks were injected following analysis of a known high-concentration sample (i.e., high level standards and QCs) and no carryover was detected. Retention time criterion for peak identification was a 0.2 minute retention time window relative to internal standard.

| Analyte | Retention Time (min) | Precursor Ion (<i>m/z</i>) | Product Ion (<i>m/z</i>) | Fragmentor (V) | Collision Energy (V) | Cell Accelerator Voltage (V) | Polarity |
|----------------------------------|----------------------|------------------------------|----------------------------|----------------|----------------------|------------------------------|----------|
| 2,4-Dinitrophenol | 4.35 | 183 | 123 | 112 | 17 | 4 | negative |
| | | | 109 | 112 | 29 | 4 | negative |
| 2,4-Dinitrophenol-d ₃ | 4.35 | 186 | 112 | 97 | 29 | 4 | negative |

Table 1: Specific Mass Spectrometer and Analyte Parameters

| Parameter | Setting |
|--------------------------|---|
| Mass spectrometer mode | Positive electrospray ionization, dynamic multiple reaction monitoring (dMRM) |
| Gas Temperature | 350 °C |
| Gas Flow | 12 L/min [Nitrogen] |
| Nebulizer Gas | 50 psi [Nitrogen] |
| Capillary Voltage | 3500 V |
| Cell Accelerator Voltage | 4 V |

Table 2: Instrument configuration for Agilent 6420

Results

Analysis of post-mortem femoral blood confirmed DNP ingestion. The patient's post-mortem femoral blood DNP concentration was 72 µg/mL (Figure 1). The accuracy (% Relative Error < 2%) and inter- and intra-run precision (%CV < 4%) of the ToxBox® DNP method resulted in low levels of quantification (~ 0.5 µg/mL) and detection (0.001 µg/mL). Accuracy and precision measurements of three QC samples spanning the linear range of the assay are presented in Table 3. The limit of detection was estimated by analyzing spiked blank blood. Blood spiked at 0.001 µg/mL provided signal-to-noise >10. The limit of quantification was defined as the lowest calibrator (0.5 µg/mL) used to assess accuracy and precision.

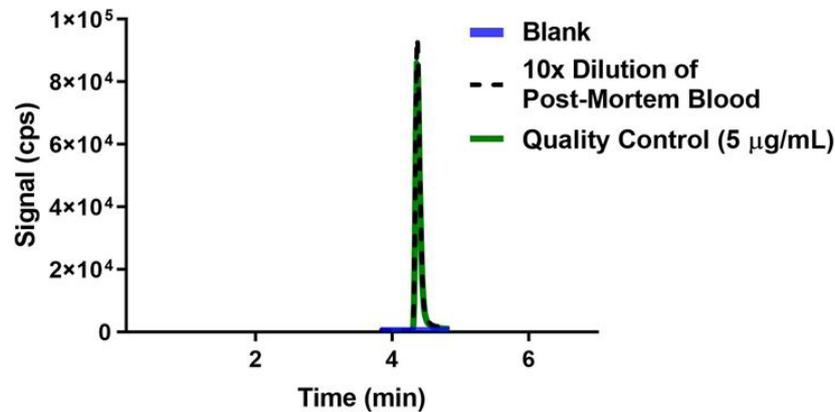


Figure 1: A representative LC-MS/MS chromatograph produced from a blank blood specimen (blue tracing), a quality control blood specimen fortified with DNP (5 µg/mL) (green tracing) and a 10-fold dilution of the case sample (dashed black line) (7 µg/mL). After applying appropriate correction factors, the final reported concentration of DNP was 72 µg/mL. Chromatography of all calibrators and quality control samples were similar throughout the analytical run.

| | QC 1 2.5 µg/mL | | | QC 2 5.0 µg/mL | | | QC 3 10 µg/mL | | | |
|--------------------------|-------------------|------------------|------------------|-------------------|------------------|------------------|------------------|------------------|------------------|------------|
| | Conc.±SD | %CV ^a | %RE ^b | Conc.±SD | %CV ^a | %RE ^b | Conc.±SD | %CV ^a | %RE ^b | Average r2 |
| 2,4-Dinitrophenol | 2.45 ± 0.04 | 1.7 | 2.1 | 4.97 ± 0.11 | 2.2 | 0.7 | 10.09 ± 0.38 | 3.7 | 1.0 | 0.9990 |

*Data obtained using an Agilent 6420.

^a %CV, Coefficient of Variation, Calculated as $([\text{standard deviation}/\text{mean}] * 100)$ at each concentration; ^b %RE, Absolute Relative Error, Calculated as $([(\text{expected concentration} - \text{calculated concentration}) / \text{expected concentration}] * 100)$ at each concentration

Table 3: Summary of Accuracy and Precision Measurements in Spiked Blood Samples (QC 1 – QC 3)

Discussion

Toxicity from DNP was appreciated as early as 1919 in French munitions workers who experienced chronic weight loss despite adequate caloric intake following exposure [6]. Initial studies in human subjects demonstrated that the increased metabolic energy was derived largely from body fat stores [6]. DNP was subsequently introduced as a weight loss supplement beginning in the 1930s. However, dangerous adverse effects and death were being increasingly reported and the compound was subsequently banned under the Food, Drug, and Cosmetic Act (FDCA) in 1938 [7].

There has been resurgence in the use of DNP due to its ease of access, purchase, and even manufacturing instructions disseminated via Internet discussion blogs and forums [1]. Typical audiences include bodybuilders and fitness enthusiasts due to DNP's alleged partitioning effects of fat catabolism with muscle sparing [1]. Case reports of DNP toxicity related to weight loss or suicidal ingestion are on the rise [8].

The toxicity of DNP is exacerbated by its narrow therapeutic index, high lipid solubility, and long half-life reported by some investigators to be 5 to 14 days in humans [7]. A definitively established half-life of DNP in humans, however, has yet to be elucidated. The average time to presentation following acute ingestion is between 7 to 8 hours, with the average time of death occurring at approximately 14 hours [1]. Our patient's post-mortem femoral blood DNP concentration was 72 µg/mL. This post-mortem blood concentration exceeds those reported in most previous cases, with the majority ranging from 12 to 48 µg/mL, however several of these cases do not specify whether blood specimens were centrally or peripherally sourced [9]. Additionally, a notable case reported a 17-year-old with a suicidal DNP ingestion with a post-mortem DNP serum concentration of 315 µg/mL, however the source of post-mortem blood was again not specified [7].

There are no currently available antidotal therapies and treatment is largely supportive. Extracorporeal removal methods, such as hemoperfusion, along with aggressive supportive care have been employed in few cases of successful patient management [10].

Readily available testing for DNP is limited. This case was an acute, fatal ingestion with elevated DNP concentrations confirmed with detection in post-mortem femoral blood. This highlights the need for heightened awareness from forensic laboratories regarding DNP abuse that may be a contributing factor in non-routine cases.

Our report has a few important limitations. We describe only a single case. Furthermore, capsules found at the scene were unable to be tested for potency. Only post-mortem blood was available for testing, therefore making it difficult to draw definitive conclusions correlating concentration and the development of toxicity. As a lipophilic drug, post-mortem DNP concentrations may be prone to significant post-mortem redistribution, potentially complicating interpretation of concentrations. Although limited, current literature suggests possible post-mortem release of DNP from depots in muscle and fat tissue to venous blood [11].

Conclusion

DNP has re-emerged after a prolonged absence from the market. Increasing access and use, combined with a narrow therapeutic index and currently limited available testing capabilities have rendered this agent a challenge for clinicians and forensic scientists. We report a case of a fatal DNP ingestion and describe a novel analytical procedure for measuring post-mortem blood concentrations.

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Conflict of Interest Disclosure

Joseph Jones and Jeffery Moran are employees and/or owners of PinPoint Testing, LLC

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References

1. Grundlingh J, Dargan PI, El-Zanfaly M, Wood DM (2011) 2,4-Dinitrophenol (DNP): a weight loss agent with significant acute toxicity and risk of death. *J Med Toxicol* 7: 205-12.
2. Miranda EJ, McIntyre IM, Parker DR, Gary RD, Logan BK (2006) Two deaths attributed to the use of 2,4-dinitrophenol. *J Anal Toxicol* 30: 219-22.
3. Hoffman, Robert S, Howland, Mary A, Lewin, et al. (2015) *Goldfrank's Toxicologic Emergencies* (10th Edn), McGraw-Hill Education, New York, United States.
4. Patton AL, Jones JO, Nord A, Eversole DW, Feazell EE, et al. (2018) Multi-laboratory validation of a Δ^9 -tetrahydrocannabinol LC-MS/MS test kit designed for quantifying THC and marijuana metabolites in blood. *Forensic Sci Criminol* 3: 2-8.
5. McCain KR, Jones JO, Chilbert KT, Patton AL, James LP, et al. (2018) Impaired driving associated with the synthetic cannabinoid 5F-ADB. *J Forensic Sci* 6: 1-4.
6. Leftwich RB, Floro JF, Neal RA, Wood AJJ (1982) Dinitrophenol poisoning: a diagnosis to consider in undiagnosed fever. *South Med J* 75: 182-4.
7. Hsiao A, Santucci K, Seo-Mayer P, Mariappan MR, Hodsdon ME, et al. (2005) Pediatric fatality following ingestion of dinitrophenol: postmortem identification of a "dietary supplement". *Clin Toxicol* 43: 281-5.
8. Kopec KT, Freiermuth C, Maynard S, Beuhler M (2018) Dinitrophenol (DNP) fatality associated with a falsely elevated salicylate level: a case report with verification of laboratory cross reactivity. *J Med Toxicol* 14: 323-6.
9. Baselt, Randall C (2017) *Disposition of Toxic Chemicals and Drugs in Man* (11th Edn). *J Anal Toxicol* 42: 139.
10. Lu Y, Jiang J, Huang W (2011) Clinical features and treatment in patients with acute 2,4-dinitrophenol poisoning. *J Zhejiang Univ Sci B* 12: 189-192.
11. Zack F, Blaas V, Goos C, Rentsch D, Büttner A (2016) Death within 44 days of 2,4-dinitrophenol intake. *Int J Legal Med* 130: 1237-41.

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