Journal of Forensic and Legal Medicine 79 (2021) 102148

Contents lists available at ScienceDirect

Journal of Forensic and Legal Medicine

journal homepage: http://www.elsevier.com/locate/yjflm

Case report

2,4-Dinitrophenol overdose - Everything old is new again

A. Griffiths [a,*], N. Milne [a,b,c], B. Ong [a,b], T. Watkins [a,d]

[a] Queensland Health Forensic and Scientific Services, Queensland, Australia
[b] The University of Queensland, Queensland, Australia
[c] Bond University, Queensland, Australia
[d] Princess Alexandra Hospital, Queensland, Australia

ARTICLE INFO

Keywords:
2,4-Dinitrophenol (DNP)
Hyperthermia
Overdose
Death
Post-mortem
Stability

ABSTRACT

A complete case example of a fatal 2,4-dinitrophenol (DNP) overdose involving a 23-year-old male is described. Included are details of not only the patient's presentation symptoms and treatment, but also the subsequent findings of the coronial investigation process including the autopsy, post-mortem computed tomography (PMCT) scanning and toxicological analysis and results. The patient presented with elevated temperature, heart rate and blood pressure. Multiple treatments were conducted to counteract these symptoms, however the patient died approximately 1.5 hours after hospital admission and some 4.5 hours after the DNP was initially consumed. Autopsy revealed the presence of cardiovascular disease that was contributory to death and post-mortem computed tomography showed evidence of decompositional intravascular gas in the neck, head, face, lower abdomen, heart and hepatic systems. Toxicological analysis was completed by protein precipitation with methanol and subsequent instrumental analysis by LC/MS/MS in negative ion mode. The antemortem blood specimen showed the presence of tadalafil, two anabolic steroids and a DNP concentration of 110 mg/kg which is consistent with other reported DNP fatalities. Despite the small amount of time between the antemortem specimen collection and death, the DNP concentration identified in the femoral blood post-mortem specimen was comparably low (5.5 mg/kg). DNP concentrations also reduced during an extended period of specimen storage prior to analysis indicating some instability in biological specimens even when refrigerated or frozen. DNP was found to be distributed primarily in the aqueous tissues (blood, vitreous, bile) rather than solid matrices (liver, kidney, muscle).

1. Introduction

2,4-Dinitrophenol (DNP) exists at room temperature as a crystalline substance with a distinctive bright yellow colouration and musty odour.[1] Since the early 1900s DNP has been used in the manufacture of a variety of different commercial products including; explosives, dyes, photography developers, wood preservatives and pesticides.[2] The earliest DNP reported deaths were due to occupational exposure related to these uses.[3–5]

In the 1930s DNP was found to cause significant weight loss when taken orally and was soon being marketed as a weight loss drug.[6,7] In the human body DNP acts by 'uncoupling' mitochondrial glycolytic oxidative phosphorylation. DNP disrupts the formation of adenosine triphosphate (ATP), the major final product in the Krebs cycle, by inhibiting both the uptake of inorganic phosphate into the mitochondria and through its action as a chemical ionophore which impairs the energy conversion gradient required for ATP production. The potential energy is instead dissipated as heat leading to hyperthermia.[5] The use of energy reserves from adipose tissues is stimulated in the body which results in an increased metabolic rate and overall weight loss.[8] DNP was associated with multiple adverse effects, including cataracts, severe hyperthermia, nausea, vomiting, sweating, dizziness and headaches and its use as a drug was subsequently banned in the United States in 1938.[2]

In the last two decades DNP has had a resurgence as an unregulated weight loss aid that can be easily sourced by the public over the internet. The use of DNP for weight reduction is particularly prevalent in the body-building community.[1] Reports of toxicity incidents involving DNP have increased substantially in recent years[9] and there have been multiple cases of DNP related fatalities reported[1,2,10–15]

In Australia, due to ongoing reports of toxicity, the substance was up-scheduled in 2017 to Schedule 10 of the Poisons Standard "substances of such danger to health as to warrant prohibition of sale, supply and use".

* Corresponding author. Forensic and Scientific Services, Health Support Queensland, 39 Kessels Road, Coopers Plains, Queensland, 4108, Australia.
E-mail address: andrew.griffiths@health.qld.gov.au (A. Griffiths).

https://doi.org/10.1016/j.jflm.2021.102148
Received 1 September 2020; Received in revised form 19 February 2021; Accepted 23 February 2021
Available online 3 March 2021
1752-928X/

Since this time there have been a further two known DNP fatalities in Australia,[16] the case presented here and another involving a male after the apparent consumption of DNP in what was mistakenly thought to be capsules containing MDMA (methylenedioxymethylamphetamine).[17] In this paper we describe a complete case example of a fatal DNP overdose involving a 23-year-old male. We include details of not only the patient's presentation symptoms and treatment, but also the subsequent findings of the coronial investigation process. The results of the autopsy and post-mortem computed tomography (PMCT) scanning are described with an emphasis on the toxicological analysis and results.

2. Clinical presentation

A 23-year-old Caucasian man called emergency medical services 2 h and 15 minutes after taking an overdose of DNP with suicidal intent. He self-reported having ingested 8g of DNP orally, and denied taking alcohol, prescribed medications and illicit drugs. His past medical history was notable for a suicide attempt several years previously, and use of anabolic steroids via intramuscular injection to aid body building.

Paramedics arrived 15 minutes after the emergency call and the patient was noted to be fully conscious, speaking in sentences, diffusely diaphoretic and hot to touch. Initial observations recorded a Glasgow coma score (GCS) of 15, temperature of 38.5 °C (101.3 °F), regular tachycardia at a rate of 185 beats/min, raised blood pressure of 158/109 mmHg, raised respiratory rate of 40 breaths/min and oxygen saturations of 97% on room air. An electrocardiogram (ECG) showed sinus tachycardia. Active cooling was commenced and he was transferred to a tertiary hospital, arriving 20 minutes later, almost 3 h after taking the overdose.

On initial examination in hospital he remained diaphoretic, hot to touch and had a temperature of 38.7 °C (101.7 °F). Other observations were similar and although his GCS remained 15, he was becoming agitated and restless. Initial therapy consisted of applying ice packs to the neck, axillae and groins, and intravenous administration of cold saline, diazepam, insulin, dextrose and calcium gluconate.

Despite these measures there was progressive agitation and his temperature rose to 39 °C (102.2 °F). He was intubated following rapid sequence induction with rocuronium, propofol and midazolam. He was ventilated, and then hand bagged due to rising pCO_2. A nasogastric tube was sited, and aspiration of the gastric contents revealed them to be yellow, in keeping with oral DNP ingestion. Activated charcoal was administered. An indwelling urinary catheter with a temperature probe was also placed.

He deteriorated with lowering oxygen saturations and blood pressure before having a pulseless electrical activity arrest 4 h and 12 minutes after reported DNP ingestion. He was described as being rigid around this time. Advanced life support measures were commenced, including cardiopulmonary resuscitation, bilateral finger thoracostomies to exclude a pneumothorax, and intravenous adrenaline, sodium gluconate and sodium bicarbonate. He became asystolic and was declared deceased a short time later, approximately 4.5 hours after the self-reported overdose. His final bladder temperature was recorded as 41.6 °C (106.9 °F).

Multiple drugs/medications commonly used in bodybuilding were present at his residence; dinitrophenol (DNP), clenbuterol, the anabolic steroids oxymetholone, trenbolone and testosterone, as well as, modafinil, raloxifene, and the erectile dysfunction drugs tadalafil and sildenafil.

3. Autopsy findings

The death was reported to the Coroner and a full internal autopsy was ordered. The body was admitted to the mortuary 4 h and 3 min after death and was stored in a refrigerator set at a constant temperature of 4 °C. A post-mortem CT scan (PMCT) was performed 8 h and 44 minutes after death and the body was returned to the same refrigerator until the autopsy. It showed a generalised increase in musculature consistent with his history of body building and anabolic steroid use. There was layering of high attenuation material in the stomach, duodenum and proximal jejunum, considered to be due to any residual DNP and/or the charcoal that was administered (See Fig. 1). No trauma was evident.

Of particular interest, despite the short time interval after death PMCT showed evidence of accelerated decomposition, attributed to antemortem hyperthermia.[18] Intravascular gas was evident in the neck and superficial soft tissue of the head and face. There was extensive hepatic gas, predominantly in the portal venous system, as well as some gas in the hepatic venous system (see Fig. 2). There were separate more loculated foci of gas in the posterior right lobe hepatic parenchyma. More inferiorly there was intravascular gas in the abdomen. There was also gas in the right atrium and ventricle of the heart. This distribution of gas was not unusual, however its presence was unexpected for the post-mortem interval, considering the body had remained refrigerated since approximately 4 h after death. In the Authors' experience, and as has been reported in human cases and animal models where there is a known post mortem interval,[19,20] only minimal to no appreciable decomposition gas is typically observed in this short time interval, with more substantial hepatic and portal venous gas only being observed after greater than 12–24 hours, or longer.

An autopsy was performed four days and 9 h after death. The body weighed 89 kg (196 lb) and was 177 cm (69.7 inches), equating to a body mass index of 28.4 kg/m^2. There was generalised increased musculature. There was only early externally evident decomposition, with green discolouration of the right lower quadrant of the abdomen.

On internal examination there was also increased muscularity and minimal subcutaneous and visceral adipose tissue. The stomach contained charcoal, with black and granular fluid. No yellow discolouration was evident in the oesophagus, stomach or duodenum. There was severe, eccentric atherosclerotic stenosis of the proximal left anterior descending coronary artery. The heart weighed 360 g and there was symmetrical left ventricular hypertrophy, with a wall thickness of 1.7 cm. Histologically there was mild myocyte hypertrophy and mild perivascular fibrosis. The testes were atrophic.

Multiple biological specimens were collected at autopsy for toxicological examination including; two femoral vein blood specimens, vitreous humour, muscle, liver, kidney and bile. No urine was available in the bladder. An ante-mortem blood specimen collected during emergency treatment was also requested for toxicology analysis.

4. Toxicology

4.1. Materials and methods

Reference standards for DNP, nifedipine, ammonium formate and formic acid, were obtained from Sigma-Aldrich (Castle Hill, Australia).

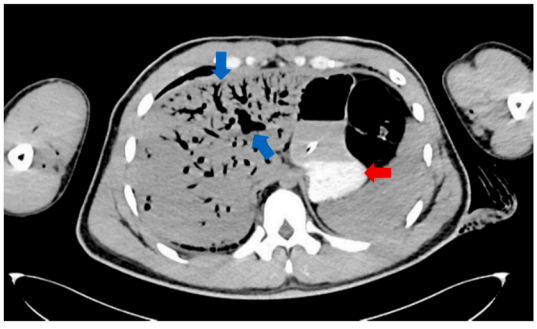

Fig. 1. High attenuation material in the stomach (red arrow), gas in the portal and hepatic venous system in the liver (blue arrows). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

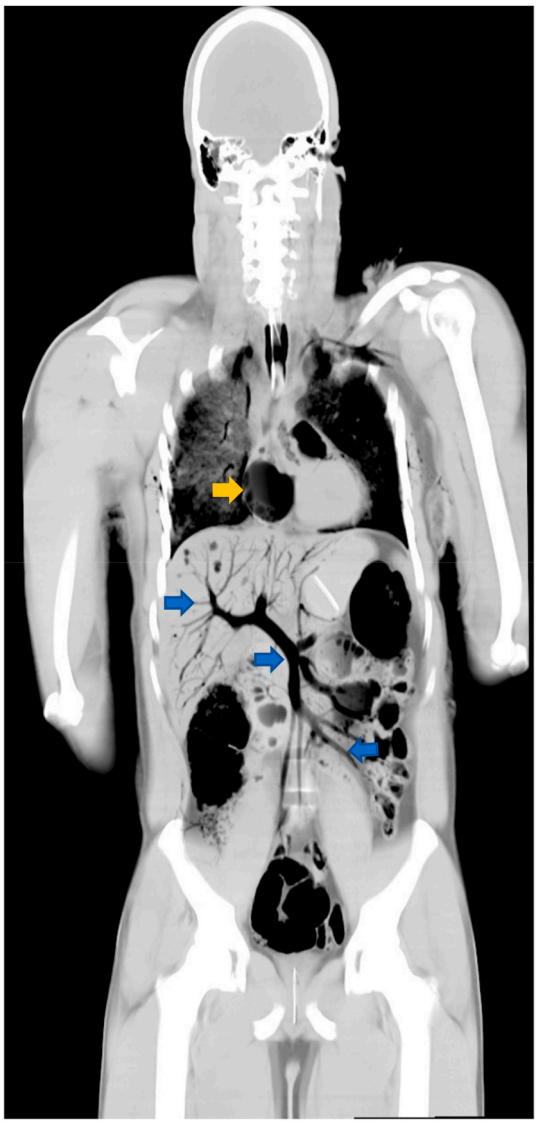

Fig. 2. Gas in mesenteric and portal venous system (blue arrows); gas in the right cardiac chambers (yellow arrow); note the paucity of PMCT decomposition changes elsewhere in the soft tissue and abdominal contents. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Nifedipine was chosen as internal standard as it gave a good negative ion response and its chemical structure had some structural similarities (nitrate moiety) to DNP. Supergradient HPLC grade acetonitrile was from RCI Labscan (Bangkok, Thailand). Ultra-pure water (18.2 MVcm at 25 C) was used for the mobile phases and produced in-house using an Advantage Milli-Q filtration system (Millipore, USA).

4.2. Sample preparation

Protein precipitation was used for sample preparation. The internal standard nifedipine (720ng) was added to 10mL polypropylene culture tubes. A 100 ng/μL DNP standard prepared in methanol was spiked into polypropylene tubes to produce at least seven calibration standards with a calibration range of between 0.4 mg/kg and 100 mg/kg. For all liquid specimens (blood, vitreous humour, bile) 0.25g was weighed into polypropylene tubes for sample preparation. For solid matrices (liver, kidney, muscle), a portion (0.25–0.5g) of specimen that had been macerated into a solid paste by blending in a stainless steel blender, was weighed and diluted 1 in 5 with deionised water then vigorously shaken prior to a 0.25 g aliquot being weighed and analysed as for other samples.

All samples were analysed in triplicate with one replicate spiked with 200 ng of DNP as a spiked matrix specimen. Pre-screened blank blood (0.25 g) was added to all standards and matrix blanks prior to protein precipitation to ensure matrix matching against samples. Deionised water (0.25 mL) was added to all tubes and protein precipitation facilitated by the addition of methanol (1.5 mL) whilst vortexing. Samples and standards were then centrifuged at 3000–4000 rpm for approximately 10 minutes. Following centrifugation, 50 μL of the supernatant was removed and diluted with 950 μL deionised water prior to analysis by negative ion liquid-chromatography/mass-spectrometry (LC/MS/MS).

4.3. Instrumental analysis

The instrument consisted of a Shimadzu 30A UHPLC coupled with a Shimadzu LCMS-8050 tandem MS detector. The separating column was a Waters Acquity UPLC BEH C18 1.7μm, 2.1 × 100 mm column held in a column oven at 40 °C. The mobile phase was Solvent A: 95/5 10mm Ammonium Formate/Acetonitrile with 0.1% Formic Acid, Solvent B: 5/95 Water/Acetonitrile with 0.1% Formic Acid. The gradient was 100% Solvent A initially, up to 100% Solvent B after 2 min, followed by a 2 min hold at 100% Solvent B. Mass Spectrometer parameters: interface temperature 300 °C, desolvation line (DL) temperature 250 °C, nebulizing gas flow 3.00 L/min, heating gas flow 10.00 L/min, heat block 400 °C, drying gas flow 10.00 L/min. The following MRMs were monitored in negative ion mode utilising electrospray ionisation: DNP, 183.05 > 153.00 (Collision energy (CE) 18, Q1 pre-bias (Q1) 13 V, Q3 pre-bias (Q3) 13 V), 183.05 > 137.00 (CE 22, Q1 13 V, Q3 11.5 V), 183.05 > 123.00 (CE 20, Q1 32.5 V, Q3 46 V), 183.05 > 108.90 (CE 26, Q1 13 V, Q3 32.5 V) and Nifedipine, 345.30 > 222.25 (CE 12, Q1 17.5 V, Q3 13 V), 345.30 > 121.95 (CE 12, Q1 17.5 V, Q3 25 V), 345.30 > 91.95 (CE 21, Q1 17.5 V, Q3 11.5 V). The dwell time for all MRMs was 10 milliseconds.

4.4. Validation

Partial validation of the method was conducted as per the laboratory's in-house protocols for one-off analyses. Recovery of DNP was measured in blood at two levels (0.5 mg/kg and 50 mg/kg, two replicates at each level) and averaged 69%. Ion suppression was measured at two levels (0.5 mg/kg and 150 mg/kg, two replicates at each level) by spiking protein precipitated blank blood matrix and comparing to a non-protein precipitated standard at the same concentrations. Only a slight enhancement (7%) of response was evident. At least seven calibration standards were prepared for each analysis. The regression model used was a quadratic fit, which gave r2 values of >0.998. Bias from the curve was assessed by comparing the measured and expected value of each calibrator over two separate runs. The measured result for all calibrators was within 15% of expected.

The completion of a spiked matrix replicate is standard practice for the laboratory when only partial validation has been completed for an analysis. The spike level (200 ng) was subtracted from the analysed concentration for this replicate to give a subtracted result that is directly comparable to the non-spiked replicates. The subtracted result was therefore included in the overall calculation of the mean analytical result. The spiked replicate is included as it gives a within-analysis indication of both analyte recovery and matrix effects for the specific sample matrix that is spiked. The limit of reporting (LOR) was set at the low std 0.4 mg/kg for aqueous matrices, while for solid matrices, as these samples were initially weighed and diluted 1 in 5 with water, the LOR was between 1.5 mg/kg and 2 mg/kg depending on the weight of sample (0.25–0.5g) taken prior to dilution. Not enough data was available to calculate a limit of detection (LOD), however, based on the response at the low calibrator and in specimens detected at less than the

low calibrator, the LOD was estimated to be approximately 0.1 mg/kg for liquid matrices and approximately 0.5 mg/kg for solid matrices.

4.5. Other testing

Routine toxicological screening was also conducted on the sample consisting of a variety of methods employing solid phase extraction (SPE), liquid-liquid extraction (LLE) and analysis by LC/MS/MS, liquid chromatography-quadrupole time of flight mass spectrometry (LC/QTOF), gas chromatography mass spectrometry (GC/MS) and high-performance liquid chromatography-diode array (HPLC/DAD). Alcohol was determined using direct dilution and analysis by packed column gas chromatography-flame ionisation detector (GC/FID). Methaemoglobin was determined by oximetry.

5. Results and discussion

Toxicology screening was initially performed on the antemortem blood specimen taken 8 min after hospital admission. Alcohol was negative, tadalafil was identified at 0.07 mg/kg and the synthetic anabolic steroids trenbolone and methandrostenolone were identified but not quantitated. Methaemoglobin was not markedly elevated in the ante-mortem specimen (1.6%). Whilst metheamoglobinemia is a recognised complication of DNP toxicity,[8] in general methaemoglobin levels appear to be either normal[8,12] or slightly increased[21] during patient treatment, though delayed onset metheamoglobinemia has also been reported.[17]

Initial testing for DNP was carried out using a non-certified reference standard already held by the laboratory. The results of this analysis gave an approximate DNP concentration of 110 mg/kg. Table 1 shows this initial result along with the re-analysis of the antemortem specimen and the analysis of all post-mortem matrices undertaken some eight months later against a newly purchased certified standard. Less than 20% variation from the mean was observed in all triplicate results for each sample matrix, including the spiked matrix replicates (with the DNP spike level subtracted). The non-certified standard was also compared to the certified standard at this time and shown to be of comparable purity.

Reported blood concentrations for fatal DNP overdoses vary widely in the literature, but the antemortem DNP concentration of 110 mg/kg in this case sits at the higher end of this range. This may reflect the acute nature of the exposure as other antemortem specimens taken after acute intentional exposure have also returned higher concentrations. For example, Kopec et al.[13] reported DNP concentrations of 120 mg/L in an antemortem blood taken 1 hour prior to death in a male who had died after acutely consuming 3g of DNP. Hsaoi et al.[10] reported an admission serum concentration of 315 mg/L in a female who had taken 12–15 pills in a suicide attempt. Miranda et al.[2] reported concentrations of 36.1 mg/L and 28 mg/L in hospital admission blood specimens relating to two cases of DNP toxicity which did not appear to relate to suicidal consumption.

Table 1
DNP concentrations in ante-mortem and post-mortem matrices.

Specimen Type	DNP Concentration (mg/kg)	Lab storage temp. (°C)
Ante-mortem blood	~110*	4
Ante-mortem blood	93^	4
Femoral vein blood#	5.5	4
Femoral vein blood (reference)#	14	−20
Vitreous	4.5	4
Liver	Not Detected <1.5	−20
Kidney	Not Detected <1.6	−20
Muscle	Detected <2	−20
Bile	8.5	4

*initial analysis -concentration against non-certified standard. ^reanalysis against certified standard after 8 months storage. #samples collected at the same time and stored at different temperatures.

In contrast, post-mortem blood concentrations are known to vary widely, with concentrations from <1mg/L to approx. 100 mg/L reported in the literature. Though not available in this case, post-mortem urine appears to be a valuable matrix in determining DNP consumption, with concentrations ranging between 14.3 and 220 mg/L in cases of either acute or chronic exposure (2,11,14–15). During patient treatment, bright yellow coloured urine,[14] gastric contents (as in this case), or sweat[1] are strong markers for DNP consumption, whereas, at autopsy, yellow discolouring of pleural cavity and fluids[2] and extremities has been observed.[15]

In the present case, much lower concentrations were identified in the post-mortem femoral vein specimens (5.5 mg/kg stored at 4 °C and 14.4 mg/kg stored at −20 °C) compared to the antemortem specimen (Table 1). There was only a 1.5-h time lapse between antemortem collection and death. However, during the intervening period multiple treatments were imparted on the patient and it is possible these treatments had a significant impact on the blood DNP concentration. Another possible reason for the lower concentrations identified in the post-mortem specimens is that a proportion of DNP was lost due to instability prior to blood collection at autopsy. PMCT scans showed evidence of accelerated decomposition some 8 h after death and it is therefore possible some DNP was lost during storage of the body prior to autopsy which was conducted 4 days 9 h after death. The different concentrations noted in the post-mortem blood specimens stored at 4 °C and −20 °C respectively further suggest that some DNP was also lost during the extended storage of the blood specimen at the higher storage temperature (4 °C). A recent study by De Campos et al.[14] indicated that DNP is unstable in blood and urine at room temperature but showed better stability at 4 °C and −20 °C, though slight decreases were observed after 14 days even at these lower temperatures. This loss of DNP during sample storage may therefore also potentially explain the approximately 15% lower concentration observed in the antemortem specimen (110 mg/kg to 93 mg/kg) after eight months storage at 4 °C. Even greater losses were evident in post-mortem blood specimens over the same period with an approximately 60% difference in DNP concentration observed in femoral vein blood specimens collected at the same time but stored at 4 °C and −20 °C (5.5 mg/kg and 14 mg/kg respectively).

Whilst DNP was identified in all liquid matrices (blood, vitreous, bile), the drug was either not detected or detected at low levels in all solid matrices (muscle, liver, kidney). It is possible that the analyte has not adequately moved into the solvent phase during protein precipitation for these matrices. However, this sample preparation has been employed successfully in solid matrices at our laboratory for the analysis of other small molecular weight analytes such as pregabalin.[22] It therefore appears more likely that the distribution of DNP within body tissues after acute intake is similar to that of other alcohols (eg ethanol) and is predominantly distributed in tissues that have a higher water content. Matrix concentrations could also potentially be influenced by post-mortem redistribution, though there is little information available to ascertain the significance of this phenomenon in relation to DNP. Only one other report of DNP concentrations in post-mortem solid matrices was identified in the literature.[15] The case related to a death following the chronic use of DNP over a period of 44 days. DNP was identified in higher concentrations in both venous blood and urine, compared to heart blood, cerebrospinal fluid, liver, kidney and lung. The authors surmised that the differences noted were due to post-mortem release from depots in the muscle and fat tissue to particularly venous blood and increased excretion to urine promoted by an infusion during clinical treatment.

The identification of two anabolic steroids in the admission specimen is consistent with other reported fatalities involving the apparent co-use of anabolic steroids with DNP (2,10,14). The drug is commonly marketed as a “fat-burner” to the bodybuilding community or as a weight loss drug to the general public. Whilst the adverse effects associated with DNP use appear to be well known in the community, the desire to lose

weight more quickly may cause some individuals to take larger doses which can result in either acute or chronic toxicity.[5] It would also appear that when taken acutely, the amount of DNP consumed has a distinct impact on the severity and onset of toxicity and the time taken for death to occur. The average time for death to occur in cases of acute toxicity has been estimated at 14 hours,[5] however the deceased in the present case died just 4.5 hours after acutely consuming 8g of the drug. A possible explanation for this is severe coronary atherosclerosis contributing to death, in the context of cardiovascular collapse associated with DNP toxicity. A similarly rapid time to death of just 5 hours was reported after a male had consumed 4.25g of the drug acutely[1] whereas, a survival time of 21 hours post-consumption was noted at the minimum reported lethal acute dose of 2.8g.[8]

6. Conclusion

The cause of death was certified as dinitrophenol toxicity. Coronary atherosclerosis was another significant condition which may have contributed to death in the context of cardiovascular collapse. The manner of death was suicide. Dinitrophenol is a highly toxic compound with a long history of causing adverse health events. While this case involved the intentional ingestion of a large dose of DNP there are multiple reports that it is being increasingly used as a weight loss supplement that is apparently easily obtainable over the internet. As such it is advisable for emergency departments to be alert for the potential symptoms of DNP toxicity and for laboratories to consider its inclusion into toxicological screening procedures.

Declaration of competing interest

The authors declare they have no conflicts of interest in relation to this study.

Acknowledgments

The authors thank Mr John Lock, Queensland Deputy State Coroner, for permission to publish details of this case and Mark Stephenson, Margaret Woolcock, Amanda Thompson and Karen Blakey, all of Forensic and Scientific Services (Queensland), for proof-reading the initial manuscript.

References

1. Holborow A, Purnell RM, Wong JF. Beware the yellow slimming pill: fatal 2,4-dinitrophenol overdose. *BMJ Case Rep*. 2016;2016, bcr2016214689. https://doi.org/10.1136/bcr-2016-214689.
2. Miranda EJ, McIntyre IM, Parker DR, Gary RD, Logan BK. Two deaths attributed to the use of 2,4-dinitrophenol. *J Anal Toxicol*. 2006;30:219–222. https://doi.org/10.1093/jat/30.3.219.
3. Warthin AS. A fatal case of toxic jaundice caused by dinitrophenol. *Bull Int Assoc Med Mus*. 1918;7:123–126.
4. Perkins RG. A study of munitions intoxications in France. *Publ Health Rep*. 1919;34:2335.
5. Grundlingh J, Dargan PI, El-Zanfaly M, Wood DM. 2,4-Dinitrophenol (DNP): a weight loss agent with significant acute toxicity and risk of death. *J Med Toxicol*. 2011;7:205–212. https://doi.org/10.1007/s13181-011-0162-6.
6. Cutting WC, Mehrtens HG, Tainter ML. Actions and uses of dinitrophenol. *J Am Med Assoc*. 1934;101:193–195.
7. Tainter ML, Stockton AB, Cutting WC. Use of dinitrophenol in obesity and related conditions. A progress report. *J Am Med Assoc*. 1933;101:1472–1475.
8. Bartlett J, Brunner M, Gough K. Deliberate poisoning with dinitrophenol (DNP): an unlicensed weight loss pill. *Emerg Med J*. 2010;27(2):159. https://doi.org/10.1136/emj.2008.069401.
9. Kamour A, George N, Gwynnette D, et al. Increasing frequency of severe clinical toxicity after use of 2,4-Dinitrophenol in the UK: a report from the National Poisons Information Service. *Emerg Med J*. 2015;32:383–386. https://doi.org/10.1136/emermed-2013-203335.
10. Hsiao AL, Santucci KA, Seo-Mayer P, et al. Pediatric fatality following ingestion of Dinitrophenol: postmortem identification of a Dietary Supplement. *Clin Toxicol*. 2005;43:281–285. https://doi.org/10.1081/clt-58946.
11. Politi L, Vignali C, Polettini A. LC-MS-MS analysis of 2, 4-dinitrophenol and its phase I and II metabolites in a case of fatal poisoning. *J Anal Toxicol*. 2007;31:55–61. https://doi.org/10.1093/jat/31.1.55.
12. Siegmueller C, Narasimhaiah R. Fatal 2,4-dinitrophenol poisoning. Coming to a hospital near you. *Emerg Med J*. 2010;27(8):639–640. https://doi.org/10.1136/emj.2009.072892.
13. Kopec KT, Freiermuth C, Maynard S, Beuhler M. Dinitrophenol (DNP) fatality associated with a falsely elevated salicylate level: a case report with verification of laboratory cross reactivity. *J Med Toxicol*. 2018;14:323–326. https://doi.org/10.1016/j.ajem.2019.03.035.
14. De Campos EG, Fogarty M, De Martinis BS, Logan BK. Analysis of 2,4-dinitrophenol in postmortem blood and urine by gas chromatography– mass spectrometry: method development and validation and report of three fatalities in the United States. *J For Sci*. 2020;65(1):183–188. https://doi.org/10.1111/1556-4029.14154.
15. Zack F, Blaas V, Goos C, Rentsch D, Buttner A. A Death within 44 days of 2,4-dinitrophenol intake. *Int J Leg Med*. 2016;130:1237–1241. https://doi.org/10.1007/s00414-016-1378-4.
16. Cairns R, Raubenheimer J, Brown JA, McArdle K, Buckley NA. 2,4-Dinitrophenol exposures and deaths in Australia after the 2017 up-scheduling. *MJA (Med J Aust)*. 2020;212(9):434. https://doi.org/10.5694/mja2.50528.
17. Duflou J. A case of fatal 2,4-dinitrophenol (2,4-DNP) intoxication with delayed onset methaemoglobinaemia. *Pathology*. 2019;51(5):548. https://doi.org/10.1016/j.pathol.2019.03.006.
18. Zhou C, Byard RW. Factors and processes causing accelerated decomposition in human cadavers – an overview. *J Forensic Leg Med*. 2011;18(1):6–9. https://doi:10.1016/j.jflm.2010.10.003.
19. Sapienzaa D, Bottarib A, Gualnieraa P, Asmundoa A, Perria F, Gaetab M. Post mortem CT of intrahepatic gas distribution in twenty-seven victims of a flood: patterns and timing. *Leg Med*. 2017;29:18–21. https://doi.org/10.1016/j.legalmed.2017.09.002.
20. Iwamotoa C, Ohuchidab K, Okumurac M, et al. Postmortem interval estimation using the animal model of postmortem gas volume changes. *Leg Med*. 2018;32:66–70. https://doi.org/10.1016/j.legalmed.2017.12.010.
21. Lu Y, Jiang J, Huang W. Clinical features and treatment in patients with acute 2,4-dinitrophenol poisoning. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*. 2011;12(3):189–192. https://doi.org/10.1631/jzus.B1000265.
22. Thompson A, Morey S, Griffiths A. Pregabalin and its involvement in coronial cases. *J Anal Toxicol*. 2019;44(1):29–35. https://doi.org/10.1093/jat/bkz041.