Case Report

Two Deaths Attributed to the Use of 2,4-Dinitrophenol

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Abstract

We report the cases of two individuals, one in Tacoma, WA, and the second in San Diego, CA, whose deaths were attributed to ingestion of 2,4-dinitrophenol (2,4-DNP). 2,4-DNP has historically been used as a herbicide and fungicide. By uncoupling mitochondrial oxidative phosphorylation, the drug causes a marked increase in fat metabolism that has led to its use to aid weight loss. Both cases reported here involved its use for this purpose. Features common to both cases included markedly elevated body temperature, rapid pulse and respiration, yellow coloring of the viscera at autopsy, history of use of weight loss or body building supplements, and presence of a yellow powder at the decedent's residence. Because of its acidic nature, the drug is not detected in the basic drug fraction of most analytical protocols, but it is recovered in the acid/neutral fraction of biological extracts and can be measured by high-performance liquid chromatography or gas chromatography–mass spectrometry. The concentration of 2,4-DNP in the admission blood samples of the two deaths reported here were 36.1 and 28 mg/L, respectively. Death in both cases was attributed to 2,4-DNP toxicity. Review of information available on the internet suggests that, although banned, 2,4-DNP is still illicitly promoted for weight loss.

Introduction

Dinitrophenols (DNPs) are a class of synthetic chemicals which do not occur in nature. Of the six dinitrophenol isomers that exist, 2,4-DNP (DNP; CAS # 51-28-5) is commercially the most important, and it has been used as an ingredient in agricultural pesticides, wood preservers, dyes, photographic developers, and as an explosive (1). All DNPs are highly toxic and well-absorbed through the skin, by inhalation, and orally. The mechanism for toxicity common to all DNPs is uncoupling of mitochondrial glycolytic oxidative phosphorylation, resulting in increased metabolism of lipids. This shift in metabolism led to the use of DNP as an ingredient in weight-loss pills in the early 1930s for the treatment of obesity. However, adverse effects including cataracts, renal failure, and deaths due to hyperthermia were attributed to the drug, and as a result, it was banned for weight-loss purposes in 1938 (2). DNP and its derivatives have remained in production, however, primarily for use as agricultural pesticides (3).

Recently, DNP has become the subject of renewed interest as a weight loss drug among bodybuilding enthusiasts. Although, the drug is illegal in the United States, it can be purchased on the internet under such names as Sulfo black, Nitro Klenup, or Caswell No. 392 from commercial web sites which sell and promote the use of anabolic steroids. Some of the websites promoting use of the DNP give no information about its dangers, though others include a disclaimer that it is not safe for human consumption. We also reviewed an internet journal of an individual who describes his experience while taking DNP. This individual was able to lose 20 pounds in 12 days by taking 400 mg/day of DNP at night, and his journal gives directions on how to use the drug successfully.

We describe toxicological findings from two deaths, one in Tacoma, WA and a second in San Diego, CA, following the ingestion of 2,4-DNP. Additionally, in the Tacoma case, 2,4-DNP was identified in capsules the decedent was taking for weight loss, while in the San Diego case, 2,4-DNP was identified in a yellow powder found at the scene.

Case Histories

Case 1

A 17-year-old female was admitted to a Tacoma, WA area hospital. For several weeks prior to this incident the patient had reported feeling fatigued and tired. The previous night, after returning from school, she developed nausea and muscle pain (myalgia). She vomited and became very thirsty and diaphoretic. Her breathing became rapid and shallow. She had no known allergies; however, she had recently started her period and had a tampon in place. There was no significant past medical history, specifically no cardiopulmonary disease or current abdominal conditions.

Her vital signs are listed in Table I. She was lethargic and had

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Table I. Clinical and Toxicological Data for Both Cases

	Case 1	Case 2
Clinical Data		
Gender/age	F/17	M/28
Pulse/respiratory rate	150/40	140/32
Blood pressure	135/44	180/20
Temperature	103°F	106°F
pO_2	214	
pCO_2	24	
pH	7.45	7.50
WBCs/platelets	14.9/adequate	15.8/54
SGOT/SGPT		624/713
Potassium	4.1	11.2
APTT		107
2,4-DNP Concentrations		
Admission blood (mg/L)	36.1	28
Admission serum (mg/L)	29.7	
Peripheral blood (mg/L)		31
Peripheral serum (mg/L)		29
Gastric contents (mg)		850
Urine (mg/L)		53
Vitreous (mg/L)		3.4
Bile/liver		present

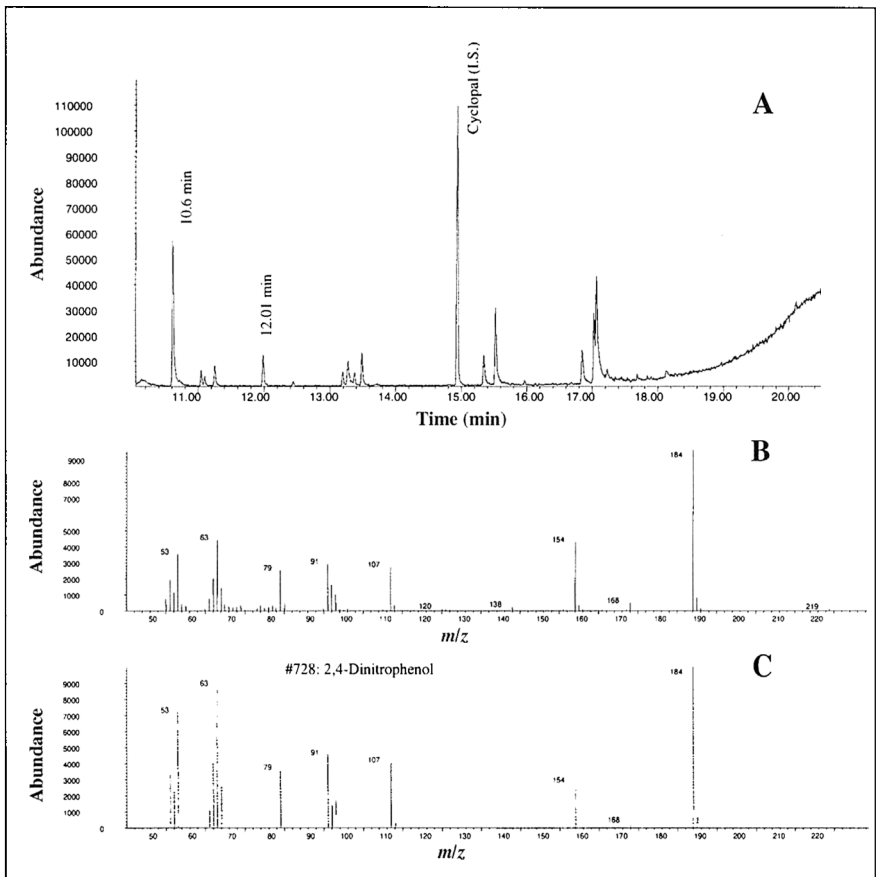

Figure 1. Total ion chromatogram of acidic/neutral extraction of blood specimen analyzed by GC–MS: cyclopal was used as the internal standard and ibuprofen is present at 12.01 min, and mass spectra of peak present at 10.6 min (A). 2,4-Dinitrophenol was identified by our mass spectral library (B) (Wiley) with a 99% match (C).

difficulty answering questions, often fading out as she responded to inquiries. She received a preliminary diagnosis of toxic shock syndrome and was given 2–3 L of normal saline and vancomycin and ceftriaxone for empiric antibiotic coverage.

She became progressively hypotensive, requiring pressor support with dopamine, and quickly developed respiratory failure, requiring intubation and mechanical ventilation. Shortly thereafter, she developed a cardiac arrhythmia that degenerated into asystole. Cardiopulmonary resuscitation was initiated but was unsuccessful, and she died approximately 3 h after her initial presentation.

The autopsy showed no acute inflammation of the endometrium or cervix, normally present in toxic shock syndrome. However, a yellowish serous fluid was noted to be present in each pleural cavity, the peritoneal cavity, and the pericardial cavity. The cause of death was undetermined pending toxicology.

Blood and serum samples collected concurrently on admission to hospital were submitted for a complete drug screen to the Washington State Toxicology Laboratory. Two capsules containing a yellow powder found in the decedent's bedroom, which she was reportedly taking for weight-loss purposes, were also submitted.

Blood specimens were screened for alcohol and other volatiles by headspace gas chromatography (GC) and found to be negative. Common drugs of abuse were screened by enzyme multiplied immunoassay technique, and none were detected. Analysis by GC with mass spectrometry (MS) and nitrogen-phosphorus detection for basic drugs involving extraction into butyl chloride with a back extraction were negative. Samples were tested for weakly acidic and neutral drugs using a liquid/solid partition extraction procedure followed by GC with flame-ionization detection (FID) and confirmation by GC–MS as described elsewhere (4,5). This revealed the presence of a small quantity of ibuprofen (< 2.5 mg/L) and a sharp, unidentified peak with a retention time of approximately 10.6 min by GC–FID. Analysis of the extract by GC–MS confirmed the presence of ibuprofen, and the unidentified peak at 10.6 min had mass fragments of *m/z* 184, 154, 107, 91, 79, 63, and 53. The spectrum had a 99% match by a mass spectral library (Wiley) for 2,4-DNP (Figure 1).

Capsule contents were analyzed by the same process using a 1 mg/mL methanol solution. Analysis by GC–MS showed a sharp peak that matched both the peak in the blood extract and the 2,4-DNP standard spectral library (Figure 2). A 2,4-DNP standard was purchased from Sigma® Chemical Company, and the identity of the peaks was confirmed. A standard curve was

prepared with a range of 0.1–1.0 mg/L, with excellent linearity (r^2 = 0.999). The DNP concentration in the blood collected at the time of admission to the hospital was 36.14 mg/L. The concentration in a serum sample collected at the same time was 29.68 mg/L. Gastric contents collected at autopsy did not contain DNP or any other drugs. The cause of death was attributed to 2,4-DNP intoxication, and it is believed to be the first death reported from this drug in a non-body builder.

Case 2

A 28-year-old male college student was found unconscious in a bathtub full of ice by his roommate, who took him to a local emergency department. On admission he was conscious, but confused and hyperthermic, with some muscle rigidity. He related he had an allergy to shellfish and had recently eaten some clam chowder, and he thought he may also have had an allergy to ephedrine, which he had taken. His roommate related that he had been taking some bodybuilding supplements from Mexico. The medications, syringes, and a yellow powder were later submitted to the Medical Examiner's office. The subject's vital signs on admission are listed in Table I. His condition deteriorated rapidly, and he died within 50 min of admission. At autopsy, he appeared jaundiced and had left ventricular hypertrophy of 1.6 cm. He had multiple organ congestion and severe pulmonary edema. Several of his ribs were fractured from CPR attempts. He had 150 mL of blood in his stomach and evidence of hemorrhagic gastritis. Also noted were severe cerebral edema with uncal herniation and other evidence of medical therapy. The liver had a soft, mottled purple and brown appearance, and microscopically, it showed severe sinusoidal congestion and subtle centrilobular hepatocyte necrosis.

Multiple samples were submitted for toxicological testing. Alcohol was tested by GC–FID and was negative. Drugs of abuse tested by enzyme-linked immunosorbent assay (ELISA) were also negative. A basic drug screen by GC–MS revealed the presence of diphenhydramine (0.13 mg/L) and lidocaine (< 0.05 mg/L). Specific analyses for ephedrine (antemortem blood) and steroids (postmortem urine) were negative. Vitreous electrolytes and glucose were within normal ranges. The medication bottles submitted contained thyroid hormones, ephedrine, and guaifenesin. An acidic/neutral drug screen by high-performance liquid chromatography–diode-array detection (HPLC–DAD) detected an unknown substance later confirmed to be 2,4-DNP. The yellow powder turned in by the roommate was analyzed by GC–MS and found to contain 2,4-DNP.

Subsequent analysis of the postmortem urine sample by Toxi-Lab® (Ansys) showed the presence of 2,4-DNP, which was confirmed by GC–MS in the Toxi-Lab extract. Blood and tissue homogenates were then extracted into *n*-butyl chloride after adding 500 µL of 1N HCl and analyzed by HPLC–DAD. The method used a Novapak phenyl HPLC column (2 × 150 mm, 4-µm particle size), with detection using diode-array detection (200–350 nm). The gradient program used a 0.031M phosphate buffer (pH 3.5), pumped at 0.6 mL/min, and modified with acetonitrile (15% acetonitrile (0–9 min, 28% acetonitrile 10–22 min, 15% acetonitrile 23–25 min). The results of the analysis are shown in Table I. The death was attributed to ingestion of 2,4-DNP.

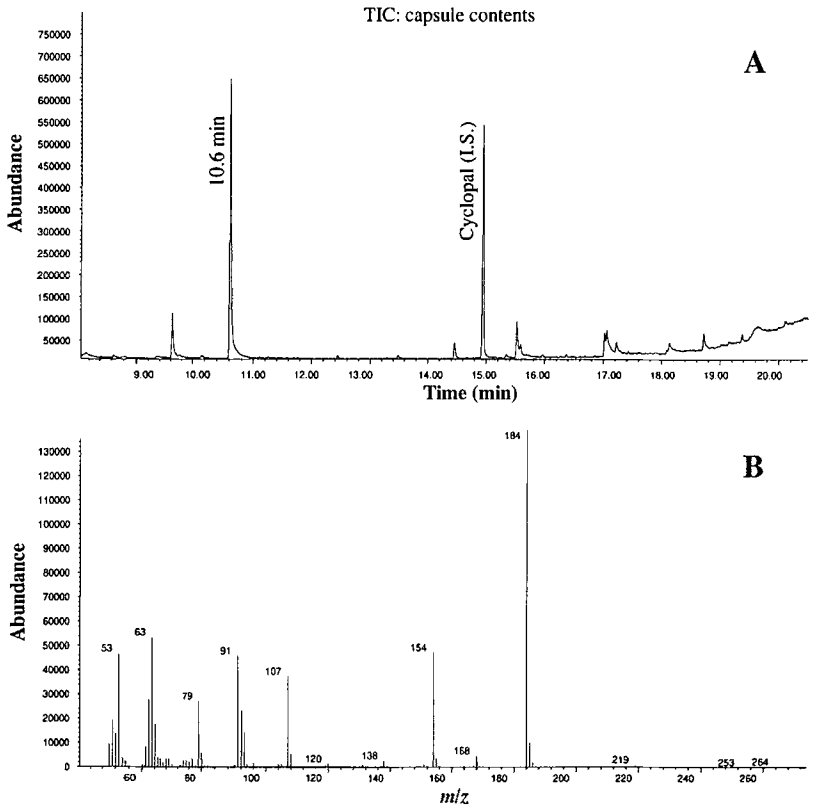

Figure 2. Total ion chromatogram of capsule contents following acidic/neutral extraction: mass spectra of peak at 10.6 min (A) and a 99% match for 2,4-DNP by a library match (Wiley) (B).

Discussion

2,4-DNP (synonyms: 1-hydroxy-2,4-dinitrobenzene; alpha-dinitrophenol; aldifen; and DNP) is a yellow crystalline solid with molecular weight of 184.11. It has a sweet, musty odor and it sublimes when carefully heated. The description of this compound fit well with the contents of the capsules and yellow powder submitted for investigations.

The acute effect of DNP in humans through oral exposure consists of hyperthermia, nausea, vomiting, sweating, dizziness, headaches, and loss of weight. In a poisoned person, the results are an almost immediate increase in oxygen consumption and elevated body temperature, respiration rate, and heart rate. In sub-acute poisoning due to repeated daily exposures, some individuals complain of lassitude, headache, malaise, and a disarming sense of energy. At high levels, this chemical may cause increased heart and breathing rates, and even death (6).

The fatal dose in adults is about 1–3 g by mouth, and 3 g has proven fatal, even in divided doses over a period of 5 days (7). According to the U.S. Department of Health and Human Services, deaths have occurred in people who ingested 3–46 mg of dinitrophenols per kg of body weight per day (3–46 mg/kg/day) for short periods or 1–4 mg/kg/day for long periods. Also, people who breathed air containing 40 mg dinitrophenols per cubic meter (40 mg/m^3) for long periods have died (6).

Most cases reporting DNP poisoning in the past have been in individuals handling pesticides containing one of the dinitrophenols or where consumption of the pesticide was accidental (8–11). A farmer who demonstrated DNP poisoning symptoms after crop spraying with a herbicide containing derivatives of DNP was hospitalized and treated for DNP poisoning. By the third day of admission, DNP concentrations were measured in urine at 52.7 mg/L. Urine concentrations on the 4th, 8th, and 9th days were 37.5, 49.3, and 34.4 mg/L, respectively. DNP levels in blood measured were measured at 11.5, 9.2, and 8.9 mg/L, during the 4th, 8th, and 9th days, respectively. Blood concentrations when signs of toxicity appeared range from 40 to 50 mg/L (12).

Reports of DNP poisoning related to weight loss appear to be becoming more common. McFee et al. (13) reported the death of a 22-year-old male 16 h after his last DNP dose, estimated at 600 mg/day over four days for weight loss. He presented with diaphoresis, and fever (102°F), but he was lucid and cooperative, gradually becoming agitated and delirious, bradycardic and asystolic, and he died within about an hour of admission. No autopsy information or toxicology data were available. The man's death led to the arrest of the individual selling DNP over the internet in Bloomington, IN. Hsaio et al. (14) reported the death of a 17-year-old female who had ingested a number of "diet pills" in a suicide attempt. She presented 4 h after ingestion, with a temperature of 98.8°F, a history of yellow emesis, heart rate 150 bpm, and respiration 42 breaths/min. She became increasingly agitated and combative, febrile to over 104°F, and died approximately 6 h after admission and 10 h after ingestion. A serum DNP concentration was determined by spectrophotometry as 315 mg/L, but the time that the sample was taken was not reported.

DNP poisoning has been reported frequently among body builders also. Suozzi et al. (15) reported the death of a 24-year-old male who collapsed at a health club with similar symptoms to those described elsewhere, fever of 104°F rising to 105.5°F. He had DNP capsules and literature on weight loss in his possession. A report from Norway (16) reported two active male body builders complaining of lassitude and malaise, who admitted to taking dinitrophenol (approximately 5 mg/kg bodyweight/day) to burn fat before a body-building competition. In this case, the individuals using DNP survived because they alerted the medical staff of their drug use.

As noted earlier, the diagnosis of DNP poisoning can go undiagnosed if individuals do not report its use. In Case 1, the young woman did not admit to DNP or any other drug use. Typical hospital drug screens would not disclose the presence of this toxic agent. Also of note is the fact that the drug, being acidic, will not be present in a basic drug fraction, but will require extraction at acidic pH into an organic solvent prior to chromatographic analysis.

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