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CASE REPORT

# Death of an apprentice bodybuilder following 2,4-dinitrophenol and clenbuterol intake

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## Abstract

We present the case of a 17-year-old man, who died after 2,4-dinitrophenol (DNP) and clenbuterol consumption, which he likely took for physical enhancement. Forensic post-mortem examination revealed a yellowish skin colour and nonspecific signs of asphyxia. Analytical confirmation of the intoxication was obtained in blood and urine, with high levels of DNP and clenbuterol. Both of these substances are used by bodybuilders as DNP enhance lipolysis and clenbuterol has anabolic properties, but their toxicity is underestimated. DNP uncouples oxidative phosphorylation, leading to thermogenesis and even relatively small doses can cause fatal hyperthermia. Clenbuterol is a β2 agonist that causes electrolyte disturbances (hypokalemia and hyperglycemia mostly) and death have been described through coronary vasospasm. Given the circumstances in which the body was found and toxicological results, we believe the cause of death to be fatal hyperthermia from DNP intake. These substances are illegal in many countries, but easily bought online. Through this availability, the last decades have seen an increase of fatal intoxications. Websites selling them are regularly closed by French public authorities and Interpol, but unfortunately it seems insufficient.

**Keywords** 2,4-dinitrophenol (DNP) · Clenbuterol · Cause of death · Bodybuilder · Forensic toxicology

## Introduction

Athletes, particularly bodybuilders are known to misuse various substances to enhance their physical performances, most frequently androgenic anabolic steroids and their synthetic derivatives, but also drugs acting on the β-adrenergic system, or enhancing lipolysis (1). Combination of drugs is also frequently seen. Death from toxic effects are often reported in medical literature, but rarely occurred in a man this young. We present here the case of a fatal combination of 2,4-dinitrophenol and clenbuterol intake, in a 17-year old.

## Case report and main autopsy findings

We report the death of a 17-year-old man, with no medical history. He was found sitting in an armchair at his home, wearing only his underpants. His place was tidy, without any sign of struggle. The outside temperature was cold (between 3° and 6 °C) but the windows were open and a cooling fan was on. Many substances known to be used by bodybuilders were found at his place: a sachet labelled (clenbutérol 50 μg/cp) with 8 tablets remaining of the 50 contained, a sachet labelled (2,4-dinitrophénol 100 mg/cp) with 21 tablets remaining of the 50 contained, a vial containing a colourless liquid labelled (testostérone énanthate 300 mg/ml), a vial containing a yellow liquid labelled (trenbolone énanthate 300 mg/ml) and several products (tablets or powder) assumed to contain protein and creatine. Injection supplies were also found at his place.

The forensic post-mortem examination was performed 24 h after the discovery of the body. It revealed a yellowish skin colour located mostly on the hands and trunk, nonspecific signs of asphyxia (cyanosis, pulmonary edema and congestion) without any sign of violence or injection sites.

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Macroscopic examination of the heart was unremarkable, it weighted 350 g. The lungs were mildly congestive and weighted 515 g on the right side and 420 g on the left one. Standard samples were collected for toxicological analysis (blood, gastric content, bile, urine, lungs, liver) and histological examination (brain, lungs, heart, liver, spleen, kidneys).

### Toxicological analysis

A toxicological standardised screening via gas chromatography/mass spectrometry (CG/MS) and high-pressure liquid chromatography/photodiode array detection (HPLC/DAD) tested negative for various medicines and drugs, carboxyhemoglobin, ethanol, acetone, isopropanol and methanol in femoral vein blood, cardiac blood and urine.

Specific toxicological examinations were performed by gas chromatography/tandem mass spectrometry (CG/MS/MS) and liquid chromatography/tandem mass spectrometry (LC/MS/MS) looking for various anabolic agents and 2–4-dinitrophenol (DNP) in samples taken at autopsy (femoral vein blood, cardiac blood, urine and gastric content) and in the products found in the deceased's apartment.

Since these substances were found close to the body, clenbuterol and DNP were specifically tested by LC-MS/MS in blood and urine. Briefly, clenbuterol was extracted from 1-ml fluid (blood or urine) in the presence of testosterone-d3 used as internal standard, with Toxivial® type A (Interchim, Montluçon, France). Transitions *m/z* 277.0 > 132, 277.0 > 167 and 277 > 203 (quantification ion) were used for quantification of clenbuterol. DNP was extracted from 0.1 ml of fluid (blood or urine) in the presence of lidocaine used as internal standard, with acetonitrile. Transitions *m/z* 182.9 > 109, 182.9 > 152.9 and 182.9 > 136.9 (quantification ion) were used for quantification of DNP.

According to Peters et al. (2) who recommended that a limited method validation is sufficient for the analysis of rarely encountered analytes or in publications of case reports, the validation of the method was limited to the determination of linearity, limit of quantification and precision in blood. Linear ranges were 0.5–10 ng/ml and 0.5–50 μg/ml for clenbuterol and DNP, respectively. Limits of quantification (LOQ) were defined as lowest calibrator. Precision (within-run evaluation) of the method, determined at three levels (0.5, 2 and 10 ng/ml for clenbuterol and 0.5, 20 and 50 μg/ml for DNP), yielded acceptable relative standard deviations lower than 10% and lower than 20% at the LOQ (10 replicates per level). Testosterone was tested like clenbuterol on the same extract.

Results of toxicological analysis are shown in Table 1.

### Histology

All samples were fixed in 4% formaldehyde prior to processing and embedding in paraffin. Sections were stained with haematoxylin and eosin (H&E). Histological examination of the heart only revealed occasional foci of vascular congestion in the connective tissue surrounding coronary arteries. The architecture of the myocardium was normal, and there was no sign of coronary artery disease or of myocardial infarction. The lungs were oedematous and congestive, with some areas of alveolar haemorrhage. Mild congestion was also observed in the centrolobular region of the liver as well as in both kidneys. There was no histological sign of any underlying disease.

## Discussion

Four anabolic agents and DNP were found in the autopsy samples. Dehydroepiandrosterone (DHEA) is an endogenous steroid prohormone, produced mostly in the adrenal glands (3). Excessive levels of DHEA cause virilization via its conversion into testosterone, and it is often used as a doping agent. Testosterone is an endogenous anabolic steroid, which promotes secondary sexual characteristics such as an increase of muscle mass. In our case, DHEA levels were slightly higher than those usually reported in medical literature (4) but testosterone levels were lower than expected (4) and urinary testosterone/epitestosterone ratio was 3. When above 4, this ratio is considered suspicious for the use of testosterone or any precursor molecule of testosterone such as DHEA (5). Even if testosterone enanthate was found at the residence of the deceased, these results are not in favor of a recent intake. Such low levels of testosterone could be explained by injections of trenbolone, a synthetic analog of testosterone. It has been demonstrated that trenbolone reduces serum testosterone level, most likely through pituitary or hypothalamic feedback inhibition (6). Trenbolone was initially used in veterinary medicine to promote muscle growth in cattle and is known to be used by athletes as a performance enhancing drug. Compared with medical literature, levels of trenbolone in our case were low, indicating an administration within days before his death (7). Also found in our case, clenbuterol is a β2 agonist, initially used in human and veterinary medicine as a bronchodilator. As it also enhances lipolysis and increases lean muscle mass, it is used by bodybuilders and athletes (8). Many toxic effects have been reported, including electrolyte disturbances (hypokalemia mostly), ST segment changes on electrocardiogram, sinus tachycardia and even myocardial infarction by coronary vasospasm (9) (10) (11) (12). Compared with literature, clenbuterol levels were high in our case. In ten healthy volunteers, after a single 40-μg clenbuterol oral dose, peak plasma concentrations of 0.133 ± 0.031 ng/ml occurred within 2.6 ± 0.9 h (13). In a case of clenbuterol cardiac toxicity, a 25-year-old male ingested 4500 μg of clenbuterol and had a clenbuterol serum level at 2983 ng/ml roughly 1 h after ingestion, he recovered in 36 h with

**Table 1** Results of specific toxicological analysis

| Sample | Substance (s) | Quantification |
|---|---|---|
| Femoral vein blood | Dehydroepiandrosterone (DHEA) | 17 ng/ml |
| | Testosterone | < 1 ng/ml |
| | Trenbolone | 0.8 ng/ml |
| | Clenbuterol | 9 ng/ml |
| | 2–4-dinitrophenol | 31 μg/ml |
| Cardiac blood | DHEA | 39 ng/ml |
| | Testosterone | < 1 ng/ml |
| | Trenbolone | 0.5 ng/ml |
| | Clenbuterol | 8 ng/ml |
| | 2–4-dinitrophenol | 49 μg/ml |
| Urine | DHEA | 16 ng/ml |
| | Testosterone/epitestosterone | 3 |
| | Trenbolone | 74 ng/ml |
| | Clenbuterol | 25 ng/ml |
| | 2–4-dinitrophenol | 42 μg/ml |
| Gastric content | Negative | |
| Sachet labelled (clenbutérol 50 μg/cp) | Clenbuterol | |
| Sachet labelled (2,4-dinitrophénol 100 mg/cp) | 2–4-dinitrophenol | |
| Vial containing a colourless liquid labelled (testostérone énanthate 300 mg/ml) | Testosterone enanthate | |
| Vial containing a yellow liquid labelled (trenbolone énanthate 300 mg/ml) | Trenbolone enanthate | |

supportive therapies (8). In another case of clenbuterol toxicity (pulmonary edema and respiratory failure with full recovery), the assumed inhaled dose is unknown but clenbuterol was found in serum at 6 ng/ml and in urine at 874 ng/ml (14). Such high levels of clenbuterol could be explained by a high-ingested dose, but also by clenbuterol accumulation as it was described in literature (13). Lastly, DNP was found at high concentrations in blood and urine. DNP is a metabolic poison, acting at the mitochondria level by uncoupling oxidative phosphorylation. General effects on the body are linked to uncontrolled thermogenesis and include tachycardia, diaphoresis and hyperthermia, which may result in death (15) (16). As it speeds up the metabolic rate, it was widely used in the twentieth century for weight loss, but reports of complications and death appeared and the US food and drug administration labelled it as (extremely dangerous and not fit for human consumption) in 1938 (16). Recently, reports of intoxication concern mostly bodybuilders and intentional overdose (16). Deaths have been reported with DNP, but few of them benefited from toxicological analyses. Toxic and lethal doses of DNP are still discussed even if the acute administration of 20 to 50 mg/kg seems to be commonly lethal to humans. Daily doses of 5 to 8 mg/kg are advised by DNP enthusiasts to bodybuilders (17). DNP concentrations in our case are consistent with chronic intakes resulting in death as described in the literature. Two cases of death following chronic absorption were described (dose absorbed unknown) and DNP was found in whole blood at 36.1 μg/ml and 28 μg/ml (18). In another case, the absorption of 12.3 g of DNP over 44 days lead to the death of an obese man, and post-mortem analyses found a DNP concentration of 4.24 μg/ml in cardiac blood, 21.6 μg/ml in peripheral blood and 95.3 μg/ml in urine (19). In cases of death following voluntary acute overdose, DNP blood levels can rise as high as 23 mg/dl (230,000 ng/ml, with 72.25 g being the assumed ingested dose) (20). Yellow discoloration of the skin is frequently observed in DNP intoxication and was noted at autopsy in our case. Given the circumstances in which the body was found, we believe the cause of death to be fatal hyperthermia from DNP intake. Clenbuterol participation is likely, presumably through electrolyte disturbances and tachycardia (12). Clenbuterol has also been described to cause ischemic injuries, most likely through vasospasm and demand ischemia but in our case, histologic examinations of the heart did not show such lesion (12).

We do not know when the deceased started taking these substances, but given what was found at his place, his total intake might have been 2.9 g of DNP and 2.1 mg of clenbuterol.

The use of DNP, clenbuterol and over (diet aid) are widely discussed by bodybuilders on specialized website and they exchange tips and recipes. Although these substances are

illegal, many people like this young man acquire them easily via the Internet. This resulted in an increase of lethal intoxications since the beginning of the twenty-first century (19).

## Conclusion

We report the case of a young bodybuilder with no significant past medical history, who ordered online DNP, clenbuterol and other substances, likely to enhance his physical performance. Toxicological analysis showed high levels of clenbuterol and DNP, confirming an intoxication suspected on the context (open windows, cooling fan on) and on the autopsy findings (yellow discoloration of the skin). As the patient did not have any history of suicidal behavior, an involuntary chronic intoxication is most likely. This trend of misuse amongst bodybuilders results in an increase of fatal intoxications, mostly in young and previously healthy man, and awareness should be raised in this community. Widely used amongst young people, discussion forums could be used for such prevention, as well as social media. As it was discussed in other health issues, public health organizations could reach out to public via Twitter to better communicate on the risks of using these types of products (21).

### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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