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Prikazi slučaja / Case reports

FATAL DINOSEB INTOXICATION: CASE REPORT

FATALNO TROVANJE DINOSEBOM: PRIKAZ SLUČAJA

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Ključne reči
trovanje dinosebom; HPLC-PDA; GC-MS

Abstract

Dinoseb, or 2,4-dinitro-6-sec-butylphenol, is a selective non-systemic herbicide. It is marketed as an oil formulation or ammonium salt. It is extremely toxic to humans and animals. It is yellowish substance. This report presents a case of accidental poisoning a 48-year-old man with dinoseb. The objective of this work was identification dinoseb in biological samples, by liquid chromatography method with photodiode array detection (HPLC-PDA) and confirmed technique of gas chromatography mass spectrometry (GC-MS).
A 48-years-old man, consumed uncqoun amount of commercial liquid containing dinoseb. He thought it yellowish liquid was juice. About several hours later the patient was admitted to intensive care unit. All the applied treatment measures remain without effect and lethal outcome were declared. Based on history, clinical examination and committed, it was found that the patients had acute intoxication substance from the group of dinitrophenol (dinoseb), severe degree, which was manifested by foregoing a typical clinical picture. Despite all the measures applied intense treatment there was a fatal outcome.
The determination of dinoseb in the biological *postmortem* samples was achieved by HPLC-PDA on the Symmetry®C8 column, with mobile phase acetonitrile : sodium dihydrogen phosphate. Dinoseb was isolated using liquid-liquid extraction method.
Toxicological analysis using HPLC-PDA method, in a blood was identified in concentration of 24.51 mg/L. Concentration in urine was 38.71 mg/L, and the kidney 1.24 mg/kg. Cause of death was attributed of acute dinoseb intoxication.

INTRODUCTION

Dinoseb is an herbicide in the dinitrophenol family.(1) The IUPAC name is 2-*sec*-butyl-4,6-dinitrophenol and its molecular formula is $C_{10}H_{12}N_2O_5$ (Figure 1). Dinoseb is a selective non-systemic herbicide. It is marketed as an oil formulation or ammonium salt. Dinoseb is effective in the control of many broadleaf weeds in such crops as cereals, seedling alfalfa and peas and is also used for pre-emergence control of annual weeds in beans, peas and potatoes and for control of runners and suckers in raspberries and strawberries.(2)

Technical dinoseb has a melting point between 38 and 42°C and is therefore a solid or liquid, depending on the ambient temperature. Dinoseb is orange solid (pure compound) or orange-brown solid (technical product, 95-98% pure). The pure compound is very soluble in water (52 g/L at 20°C) and is soluble in most organic solvents.(3) Dinoseb has a vapor pressure of 10 Pa at 20°C.(4) Molar mass is 240.2 g/mol.

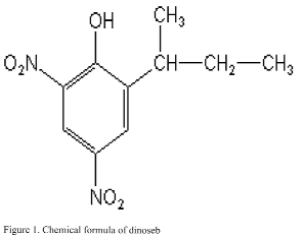

Figure 1. Chemical formula of dinoseb

Figure 1. *Chemical formula of dinoseb*

Dinoseb is well absorbed by both oral and dermal routes. A number of poisoning incidents including fatalities have been reported for people ingesting concentrated dinoseb or exposed dermally in occupational settings.

Symptoms of acute poisoning include vomiting, pain and swelling of the eyes, deteriorated vision, headache,

malaise, lassitude, sweating, anorexia, pain in the chest and abdomen, excessive thirst, insomnia, loss of weight, generalized yellow staining of the skin and shortness of breath.[5] Personality changes in affected individuals have also been documented. Dinitrophenol compounds used as weight-reducing agents in the 1930s induced cataracts. Dinoseb is very toxic to humans and is believed to act by uncoupling oxidative phosphorylation.

Juthberg and Brismar [6] studied the effects of 2, 4-dinitrophenol (DNP) on metabolic inhibition on membrane potential and ion conductance. They concluded that 2, 4-DNP had specific effects on the plasmalemma in rat astrocytes; these effects may be due to an opening of calcium channels. Ravesloot and Rombouts [7] suggest that 2, 4-DNP-induced toxicity may involve activation of ATP-sensitive K+ channels. Wu et al. [8] reported increased ATP-sensitive K+ channel activity in pituitary GH3 cells treated with 2,4-DNP. Hudman et al. [9] investigated the basis for DNP-induced increase in cytoplasmic calcium in rat cardiac myocytes. Their results indicated that the increase in cytoplasmic calcium occurs in two phases. The first phase appears to result from the release of mitochondrial calcium due to mitrochondrial depolarization. The second phase appears to be the result of a progressive release of calcium from the sarcoplasmic reticulum following depletion of intracellular ATP. Ribeiro et al. [10] probed actomyosin interactions with 2, 4-DNP in an effort to understand chemo-mechanical coupling in muscle contraction.

Ribeiro et al. (2005) characterized DNP as an inhibitor of actin-myosin interactions. Han et al [11, 12] demonstrated that 2, 4-DNP is an uncoupler of oxidative posphorylation. Han et al. (2008b) indicated that 2,4-DNP, which induced reactive oxygen species (ROS) and reduced GSH content, inhibited the growth of human lung cancer cells via cell cycle arrest at G1 phase and apoptosis.

Politi et al (2007) used liquid chromatography-mass spectrometry to analyze biological fluids from a fatal poisoning case. They tentatively identified two possible conjugated DNP metabolites: 2-amino-4-nitrophenol glucuronide and 2, 4-dinitrophenol glucuronide.

Case report

R.M., 48 years old male, presented to the emergency department (ED) of Military Medical Academy (MMA), one hour after unintentional ingestion of approximately one mouthful of unknown, light yellow colored liquid, thinking that it was juice. Immediately after ingestion he developed nausea and vomited. At the arrival in ED gastric lavage was performed, activated charcoal administered and patient was admitted to Intensive Care Unit (ICU) of Clinic of Emergency and Clinical Toxicology MMA. At the admission he was conscious, agitated, with signs of tachypnoea, body temperature 38,7 C, sweaty skin, yellow colored skin of chin and palms, yellow spots on the clothes as well as sclera yellowness and has complained of fatigue. Blood pressure and heart rate were 170/85 mmHg and 120/min., $SatO_2$ 96%. Initial laboratory testing showed leucocytosis 15.6, blood glucose 12.8 mmol/L, potassium 5.4 mmol/L, without any other laboratory abnormalities. Chemical and toxicology analysis detected dinoseb, pesticide from the group of dinitrophenols, in the gastric content and blood sample, as well as in the sample of ingested liquid. Immediately after admission he became somnolent, and few minutes after he was in respiratory and cardiac arrest and had vigorous muscular rigidity. Despite the all intensive care treatment measures applied, patient died eighty minutes after admission.

During external examination of the deceased, a yellowish discoloration of the skin on the palms and hairs of the right temporal region was observed. An autopsy showed hyperemia affecting the organs, including the brain, as well as pulmonary edema. Histopathological examination of the organs showed pervascular microhemorrhage in the brain, microhemorrhage of the heart muscle and pancreas so as fatty liver alteration.

Chemical and toxicological analyses of blood, urine and kidney found pesticide (Dinoseb). Based on chemical and toxicological analyses and the autopsy report, death was violent and occurred due to pesticide poisoning.

MATERIALS AND METHODS

Materials:

Men, 48- years old, to understand the reason for his sudden death performed the autopsy and toxicological analysis. His autopsy performed at the Institute of Forensic Medicine and Pathology, Military Medical Academy, Belgrade. Toxicological analysis was performed to the Department of Toxicological Chemistry. The commercial pesticide (unknown liquid, jealous colored), blood, urine and tissue samples of the patient, were submitted by the Institute of Forensic Medicine and Pathology, MMA. Biological samples are stored until analysis at a temperature of -200C.

The analytical standard of dinoseb was obtained from Sigma-Aldrich Chemical Company (St Louis, MO, USA). Ether, methanol, n-hexane and acetonitrile were purchased from Merck Company (Darmstadt, Germany); deionized water was purified using the Milli-Q system (Millipore, Bedford, MA, USA). All other reagents were of analytical grade. Reagents of mobile phase sodium dihydrogen phosphate, which were used for analysis, were obtained from Sigma-Aldrich Chemical Company (St Louis, MO, USA).

Methodology:

The determination of dinoseb in the biological samples was achieved by means of high performance liquid chromatography (HPLC), and photodiode array detection (PDA). The Alliance Waters 2695 Separations Module was used, consisting of a Waters 2996 Photodiode Array detector. System was with Toxicological library.

Dinoseb was isolated using a liquid- liquid extraction with ether, at pH 4.0. Separation was achieved on the Symmetry® C8 column, with mobile phase acetonitrile : sodium dihydrogen phosphate 50 mM, with pH 3.6, in the gradient mode. Retention time for dinoseb was 24.31 min.

Gas chromatography–mass spectrometry (GC-MS) was confirmed technique in this poisoning case. Following extraction, the dry residue was reconstituted in n-hexane and analyzed using GC-MS. Analyses of biological materials and analyses was performed using gas chromatography-mass spectrometry (Trace GC Ultra, ITQ 900 MS, Thermo Sceincific) with NIST library. Column TG-5 MS, 30 m x 0.25 mm, film layer 0.25 μm. Injector temperature was 200 °C. Transfer line temperature was 200 °C. Column temperature

program: column start temperature was 50 °C in duration of 1 min, than the column was heated up to 300°C with temperature elevation of 10°C/ min. MS detector: detection range m/z 50-650. Retention time for dinoseb was 17.31 min.

RESULTS AND DISCUSSION

Toxicology-chemical analysis of unknown liquid, blood, urine and body tissue samples, proved the presence of pesticides dinoseb, which was determined by liquid chromatography coupled with Photodiode Array detection (PDA) and confirmed technique was gas chromatography with mass spectrometry (GC-MS).

Results of toxicological analysis are shown in Table 1.

Table 1: *Results of toxicological analysis in postmortem samples*

Sample	Dinoseb
Blood	24,5 mg/L
Urine	38,7 mg/L
Kidney	1,2 mg/kg
Brain	not detect
Liver	not detect

Identification of dinoseb in the commercial product, which was send as unknown liquid, also was verified through HPLC-PDA and GC-MS method. Blood was examined for ethanol and methanol by GC-FID with headspace. No opiates, alcohol, and other drugs were detected.

In this manuscript we discussed the clinical and forensic imaging related to dinoseb intoxcation. Toxicological-chemical analysis using HPLC-PDA and GC-MS method, in a sample of blood, urine and in all other autopsy samples was identified dinoseb,

Hsiao et al. [14] described a laboratory method that allowed *postmortem* determination of the

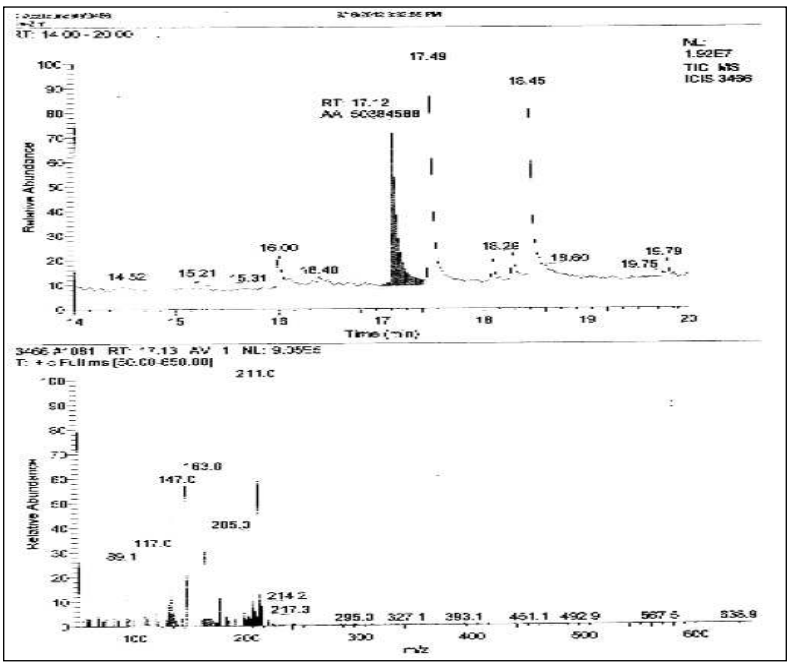

Figure 3. *Show a chromatogram of the dinoseb in the postmortem blood with full-scan mass spectrum of dinoseb. Retention time for dinoseb was 17.12 min.*

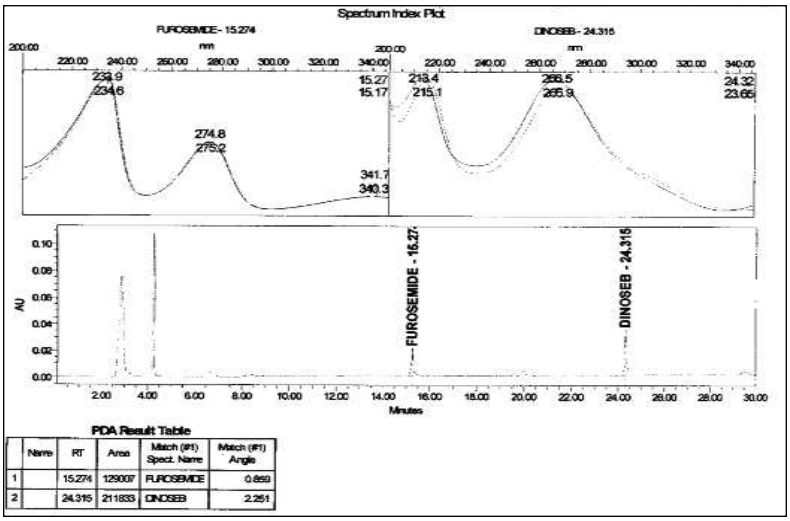

Figure 4*. Show a chromatogram of dinoseb in the postmortem blood with UV spectrum of dinoseb. The retention times were 24.31 min. The total chromatographic run time was 30.0 min.*

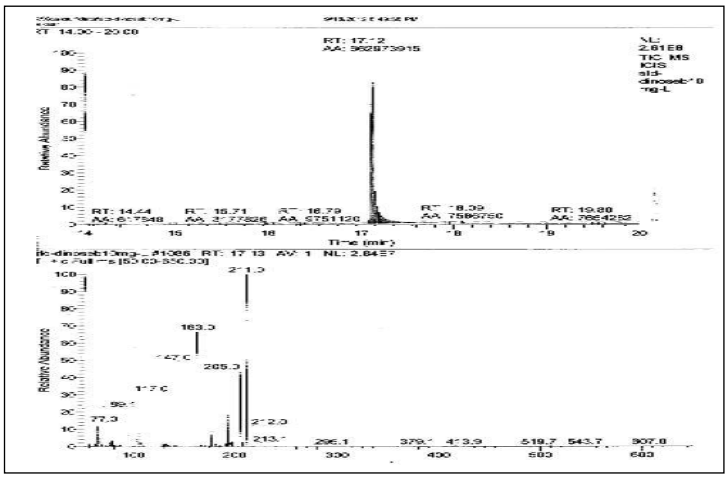

Figure 2*. Show a chromatogram of the standard of dinoseb in the concentration 10 mg/L with full-scan mass spectrum of dinoseb. Retention time for dinoseb was 17.12 min.*

DNP concentration in serum using ultraviolet-visible spectrophotometry. The DNP concentration in the samples was determined from a plot of concentration vs. the absorbance generated from DNP standards.

Macnab and Fielden [15] report on a farm child who became comatose and who was admitted to a hospital. Additional clinical signs and a search of the farm indicated that the child likely had been exposed to dinitrophenol (DNP) from an open container of the herbicide Dinoseb (2-*sec*-butyl-4, 6-dinitrophenol). No dose estimate was provided.

Case studies are available of persons ingesting DNP in suicide attempts or to lose weight. Hsiao et al. [14] reported the death of a17-year-old girl who died just 10 hours after she ingested between 12 and 15 diet pills (estimated DNP concentration of 192 mg/pill) in a suicide attempt. The calculated serum concentration of DNP was 315 mg/L. Before death, the girl's symptoms

included uncontrolled fever, agitation, combativeness, and mental status diminution. An autopsy revealed „profound" pulmonary edema, yellow staining of the skin and organs, and hepatic necrosis. McFee et al. [16] reported the case of a 22-year-old man who, to lose weight, ingested an unknown quantity of 2, 4-DNP. He died 16 hours later. No blood concentrations were reported. Miranda et al. [17] reported the deaths of two persons who died after they ingested unknown quantities of DNP. The blood concentrations of 2, 4-DNP at the time of admission for the two patients were 36.1 and 28 mg/L, respectively.

CONCLUSION:

Dinoseb is a selective non-systemic herbicide. This report presents a case of accidental poisoning a 48-year-old man with dinoseb. The objective of this work was identification dinoseb in biological samples, by liquid chromatography method with photodiode array detection (HPLC-PDA) and gas chromatography–mass spectrometry (GC-MS).

A 48-years-old man, intentionally consumed commercial liquid containing dinoseb. About three hours later the patient was admitted to intensive care unit. Subject died three hours after. Cause of death was attributed of acute dinoseb intoxication.

The determination of dinoseb in the biological samples was achieved by HPLC on the Symmetry®C8 column, with mobile phase acetonitrile: sodium dihydrogen phosphate. Dinoseb was isolated using the liquid-liquid extraction method.

Toxicological analysis using HPLC-PDA method, in a blood was identified dinoseb in concentration of 24.51 mg/L. Concentration in urine was 38.71 mg/L. When examining post mortem material (the organs), the highest concentrations were measured in the kidney 1.24 mg/kg. Dinoseb was not detected in brain and liver. Dinoseb confirmed with GC-MS technique. The identification of dinoseb in the commercial product, which was send as unknown liquid, also was verified through HPLC-PDA and GC-MS method.

For the more precise determination of the dinoseb concentration in biological material, especially when the analyses of the judicial and medical importance are concerned, the chosen technique is the gas chromatography with the mass spectrometry detector. By application of this technique the presence of dinoseb in the analyzed samples is confirmed.

Based on chemical and toxicological analyses and autopsy report, death is violent and occurred due to pesticide poisoning.

Sažetak

Dinoseb, 2,4-dinitro-6-*sec*-butilfenol, je selektivni, nesistemski herbicid. Na tržistu se može naći u formulaciji ulja ili amonijumove soli. Izuzatno je toksičan za ljude i životinje. To je supstanca žute boje. U radu je prikazan slučaj akutnog trovanja dinosebom. Cilj rada bio je identifikacija i kvantifikacija dinoseba u uzorcima biološkog materijala, metodom tečne hromatografije sa PDA detektotom (HPLC-PDA), a potvrdna tehnika bila je gasna hromatografija sa masenom spektrometrijom (GC-MS).

Muškarac, star 48 godina, slučajno je popio nepoznatu količinu komercijalnog preparata herbicida, koja sadrži dinoseb. Mislio je da je žuta tečnost u flaši sok. Nekoliko sati kasnije, pacijent je primljen na odeljenje intenzivne nege. Sve primenjene mere bile su bez efekta i proglašen je smrtni ishod. Na osnovu istorije i obavljenog kliničkog pregleda, utvrdeno je da je pacijent imao akutno trovanje jedinjenjem iz grupe dinitrofenola (dinoseb), teškog stepena, sa tipičnom kliničkom slikom.

Određivanje dinoseba u biološkim postmortem uzorcima, postignuto je HPLC-PDA metodom na Symmetry®C8 koloni, sa mobilnom fazom acetonitrile : natrijum dihidrogenfosfat. Dinoseb je izolovan metodom tečno-tečne ekstrakcije. Prisustvo dinoseba potvrđeno je metodom gasne hromatografije sa masenom spekrometrijom (GC-MS). Toksikološkim analizama HPLC-PDA metodom, u uzorku *postmortem* uzorku krvi, detektovan je dinoseb u koncentraciji od 24,51 mg/L. Koncentracija u urinu bila je 38,71 mg/L, a u bubregu 1,24 mg/kg. Uzrok smrti bio je akutno trovanje dinosebom.

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