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Evaluation of acute toxicity of europium–organic complex applied as a luminescent marker for the visual identification of gunshot residue

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ABSTRACT

The growing concern about the health of police officers and other people who use guns, constantly exposed to a rich atmosphere of heavy metals, such as lead, barium, and antimony that are key elements detected in gunshot residue (GSR), has increased the production of ammunition that is free of these metals (clean range or environmental ammunition). In this perspective, a new europium-based photoluminescent complex ($Eu(PIC)_3(NMK)_3$, where PIC is picric acid and NMK is *n*-methyl-caprolactam) was tested as an optical marker for GSR identification in conventional ammunition used in a .38 revolver and a .40 pistol. However, information on the toxicity of this substance is not reported in the literature. This study aims to assess the acute toxicity of $Eu(PIC)_3(NMK)_3$, using the protocol 423 recommended by the Organization for Economic Cooperation and Development (OECD). Toward that end, the behavior of female wild type mice (*Mus musculus*, C57BL/6) was observed for 14 days and biochemical tests of liver function markers (AST, ALP, GGT, and albumin) and renal function markers (creatinine, urea, and protein concentrations) were performed. The experiments were begun at a marker dose of 50 mg kg^{-1}, increasing until the dose reached 2000 mg kg^{-1}. The marker for $Eu(PIC)_3(NMK)_3$ showed a median lethal dose (LD_{50}) of 1000 mg kg^{-1}, which is classified as category 4 in the globally harmonized system of classification and labeling of chemicals scale; therefore, it was considered to be of average toxicity. This GSR marker proved to be less toxic than the inorganic components found in conventional ammunition (especially Pb), thus justifying its promising use in forensic ballistics.

1. Introduction

The elements lead (Pb), barium (Ba), and antimony (Sb) are the major chemical markers detected in the gunshot residue (GSR) of conventional ammunition that is used in firearms [1–4]. The GSR from firearm is defined as environmental microcontaminants. These types of residues as well as other metals are considered to contain non-essential elements that can compete with essential elements, inhibiting their functions due to their electronic configurations and their common properties, which enable them to be easily absorbed, distributed, and bioaccumulated. Thus, accumulation of these chemical markers in the human body is a major health risk and can lead to a variety of pathologies [5–26]. The growing concern about the health of police officers and other people who use firearms that are constantly exposed to Pb, Ba, and Sb that are present in GSR [27], has increased the production of ammunition that is free of these metals (clean range or environmental ammunition), subsequently making it impossible to determine the authorship of the gunshots by using GSR analysis [28–30]. Thus, the use of ammunition that does not contain metals, such as Pb, but instead contains photoluminescent markers, is an alternative solution to the problem of chronic exposure to toxic elements, thereby contributing to the investigation of crimes involving firearm use [30–34].

Destefani et al. [30] showed that high photoluminescence complex $Eu(PIC)_3(NMK)_3$, where PIC is picric acid and NMK is n-methyl-caprolactam, can be applied as an optical marker for identifying GSR concentrations greater than 25 mg in ammunition used in short-barreled firearms like the .40 revolver. The visual detection of markers at the crime scene investigation can be made using a lamp in the UV region with a $\lambda = 395$ nm. In general, 1 g of marker is enough to load 40 ammunitions [30]. However, data on the safety of this compound and

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other analogous have been little reported in the literature [35]. It is necessary therefore to conduct toxicological tests to evaluate its toxicity (i.e., median lethal dose, LD_{50}) and compare it with the toxic effects of the heavy metals that are present in GSR generated by conventional ammunition in order to determine its harmful effect on human health. This study aims to assess the acute toxicity of $Eu(PIC)_3(NMK)_3$ using protocol 423, as recommended by the Organization for Economic Cooperation and Development (OECD). The behavior of wild type mice (*Mus musculus*) was observed for 14 days and biochemical tests of liver function markers (AST, ALP, GGT, and albumin) and renal function markers (creatinine, urea, and protein concentrations) were performed.

2. Experimental methods

The acute toxicity of the compound was evaluated using protocol 423, as recommended by the OECD. The experiments were performed at the Animal Experimentation Laboratory for Pharmacological Analysis at the University of Vila Velha.

2.1. Synthesis of complex $Eu(PIC)_3(NMK)$

The compound analyzed in the acute toxicity test was the photoluminescent complex $Eu(PIC)_3(NMK)_3$, which has as ligands picric acid (PIC) and *n*-methyl-caprolactam (NMK) with the molecular formula $Eu(C_6H_2N_3O_7)_3 \cdot 3C_7H_{13}NO$. The synthesis of $Eu(PIC)_3(NMK)_3$ was performed in three steps: **I)** synthesis of hydrated basic carbonate of europium (III) ($[EuCO_3(OH) \cdot xH_2O]$); **II)** synthesis of hydrated europium (III) picrate ($[Eu(PIC)_3 \cdot xH_2O]$), and **III)** synthesis of europium (III) picrate complex with *n*-methyl-caprolactam ($[Eu(PIC)_3(NMK)_3]$) [30].

2.2. Materials, reagents and instrumentation

Ultrapure 65% nitric acid (HNO_3) (Merck, Germany), ultrapure water (1 + type, with resistivity = 18.2 MΩ cm) prepared by a reverse osmosis system (Purelab Ultra Mk2, United Kingdom) and hydrogen peroxide, H_2O_2 (Cromoline Química Fina, Brazil) were used for preparation and extraction of the samples for inductively coupled plasma optical emission spectrometry (ICP-OES) analysis. All reagents and solvents were used as received. A multi-element stock solution (1000 μg L^{-1}) of europium standard (Sigma Aldrich, Switzerland) was prepared to build a calibration curve. All standard solutions were acidified with 2% HNO_3 (*v*/*v*).

An ICP OES instrument (Perkin Elmer, Optima 7000, USA) was used for the Eu quantification. The instrument contained a Meinhard concentric pneumatic nebuliser and a cyclonic nebuliser chamber attached to a peristaltic pump. Both are used to introduce the samples into the plasma. An axial view and the following spectral line was used: Eu 412.970 nm. For the preparation step, a microwave was used (CEM Model Xpress, CEM Corporation, Matthews, NC, USA) [2–4].

Biochemical analyses were performed using a semi-automatic biochemical analyzer (LabmaxPlenno-Labtest). The biochemical parameters measured were urea (Labtest® cod. 104.2, linearity ≥ 300, sensitivity of 0.051–0.061 mg dL^{-1}, colorimetric method, 600 nm), creatinine K (Labtest® cod.96.2, linearity of 0.2–12 mg dL^{-1}, sensitivity of 0.583–0.648 mg dL^{-1}, colorimetric method, 510 nm), total protein (Labtest® cod.99.1, linearity > 14 g dL^{-1}, sensitivity of 0.017–0.019 g dL^{-1}, colorimetric method, 345 nm), albumin (Labtest® cod.19.1, linearity > 6 g dL^{-1}, sensitivity of 0.0091–0.0099 g dL^{-1}, colorimetric method, 630 nm), aspartate aminotransferase (AST, Labtest® cod.109.2, linearity ≥ 400 U L^{-1}, sensitivity of 01.430–1.943 U L^{-1}, cinetic UV-IFCC method, 340 nm), gamma glutamyltransferase (GGT, Labtest® cod.105.2, linearity ≥ 700 U L^{-1}, modified Szasz method, 405 nm), alanine-aminotransferase (ALT, cod.108.2, linearity ≥ 400 U L^{-1}, sensitivity of 01.469–2.029 U L^{-1}, cinetic UV-IFCC method, 340 nm), and alkaline phosphatase (ALP, Labtest® cod.79.4, linearity ≥ 1500 U L^{-1}, colorimetric assay, 405 nm).

2.3. Experiments with animals

The experiments with wild type mice (*M. musculus*, C57BL/6) were performed in accordance with the ethical principles established by the Brazilian College of Animal Experimentation (COBEA, 1991), after approval by the Ethics Committee on Animal Use of the Federal University of Espírito Santo (CEUA/UFES, Protocol 026/2014). The animals were between 8 and 12 weeks old at the beginning of the experiments, with a mean weight of 20.0 ± 2 g; they were kept in polypropylene cages with a maximum of three animals per cage. They were housed in humidity and temperature controlled room with a light–dark cycle of 12 h. They had access to water and food *ad libitum*. The animals were provided by the Experimental Monitoring Laboratory of the Biological Practices Complex–University of Vila Velha.

2.4. Experimental protocol

A week before starting the protocol, three animals were housed per cage with free access to water and food (Probiotério feed, Moinho Primor SA, São Paulo, Brazil) *ad libitum*. The animals were fed food and had access to water throughout the experiment, except for the 3 to 4 h of fasting they were subjected to prior to the administration of the compound and 1 to 2 h after administration. After the fasting period of 3 to 4 h, time (t) = 0, the animals were weighed. The substance, $Eu(PIC)_3(NMK)_3$, was administered as a single dose by gavage using a stomach tube or a suitable intubation cannula (gavage).

Three animals were used for each step (n = 3, control group, n = 3, test group), each dose was tested in duplicate (except the 2000 mg kg^{-1} dose, as specified by protocol 423). Since there was no data available about the toxicity of the test substance, the starting dose for the test group was 50 mg kg^{-1} of body weight and the control group received the vehicle (drinking water) orally, in an amount equal to the average volume (0.2 mL) of the compound, both in a single dose. After treatment, the animals were closely observed for 4 h and then daily for 14 days; any behavioral changes that occurred were noted. After the 14th day, any deaths that occurred were recorded to calculate the LD_{50}, and all of the surviving animals were anesthetized with sodium thiopental 120 mg kg^{-1} intraperitoneally; then, thoracotomy was performed. Blood was collected by cardiac puncture in the right ventricle and it was added to a tube without the addition of an anticoagulant. The samples were then used for biochemical tests of liver function markers (albumin, aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP], and gamma-glutamyl transferase [GGT]) and renal function markers (creatinine, urea, and total protein). The animals were then euthanized and their kidneys and livers were extracted.

Additionally, the weight of each animal was monitored during the experiments and at the end of the 3 to 4 hour fasting period as well as after seven and fourteen days of administration of the studied doses. After all of the reported procedures were completed, the animals were frozen and all medical waste was collected according to the required protocol.

Fig. 1 presents the detailed and chronological scheme denoting all the procedures performed with the animals for each administered dose.

The results observed in the experiments with mice were individually recorded for each animal, showing for each experiment the number of animals used, the number of animals presenting signs of toxicity, the number of animals found dead during the test, and the number of animals that were euthanized after 14 days. The description and the time course of the toxic effects and the reversibility and the necropsy findings were also documented.

To interpret the LD_{50} data in human, it was performed the translation of LD_{50} dose from animal studies to human, using the body surface