Fatal aldicarb poisoning case report: The first case in horse

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ABSTRACT

Aldicarb is a carbamate pesticide used in agriculture. In Brazil, aldicarb is illegally used as a household rodenticide with a widespread risk of poisoning. The granular formulation is very attractive for the deliberate poisoning of animals. A few cases of aldicarb poisoning have been described in large domestic animal. The authors present a fatal intoxication case with aldicarb in a Quarter Horse breeder with five years old of high economic value. He was found lying on the floor, unconscious, and when the veterinarian evaluated his vital functions, he already dead. The necropsy was performed, but did not observe any indication of the cause of death, being collected material for conducting toxicological tests. The toxicological analysis was done in a liver sample. It was initially done a screening technique by thin layer chromatography and subsequently used the technique of high performance liquid chromatography coupled with a photodiode array detector for the confirmation findings, and finally a gas chromatography coupled with mass spectrometry analysis. The toxicological analysis results allowed us to conclude that death occurred due to an aldicarb acute intoxication. This case shows the importance of toxicological analysis as a tool for elucidating the cause of death of the animal.

Keywords: intoxication, carbamate pesticide, toxicological analysis, chromatography.

INTRODUCTION

Aldicarb (2-methyl-2(methylthio)propanal o-[(methylamino)-carbonyl] oxime) is a carbamate pesticide used in agriculture as insecticide and nematicide. This carbamate ester is manufactured since 1965 and is sold under the tradename, Temik. In Brazil, aldicarb is illegally used as a household rodenticide with a widespread risk of poisoning.

The granular formulation of aldicarb is very attractive for the deliberate poisoning of domestic animals and wildlife [1] and is known to be highly toxic to mammalian, with LD50 oral 0.49 a 1.41 mg/kg [2,3]. It has also been involved in many fatal poisonings in humans [4,5,6].

Only a few cases of aldicarb poisoning have been described in large domestic animals such as horses. [4,7]. The authors present the first fatal case of poisoning detected in the Laboratory of Toxicological Diagnosis (LADTOX) - School of Veterinary Medicine and Animal Science – University of São Paulo.

MATERIALS AND METHODS

Case history

It was reported that a highly valued five-year old breeder quarter horse was found lying on the floor of his stall. Upon inspection of the horse's vital signs, a veterinarian pronounced him dead. During the necropsy, no obvious indications of the cause of death were observed. Biological samples were collected for toxicologic testing.
On December 12, 2012 material related to this case was received in the LADTOX. The sample was wrapped in an isothermal container with ice packs, containing inside plastic jar with tissue sample. The information provided on the label was related to the chain of custody and identified the contents of jar with a liver sample 8.3 g from necropsy of the one Quarter Horse with five years of age that died in.

Thus, according to routine laboratory, it was initially done a screening technique by thin layer chromatography (TLC) and subsequently used the technique of high performance liquid chromatography coupled with a photodiode array detector (HPLC-DAD) for the confirmation findings in the screening and finally a gas chromatography coupled with mass spectrometry (GC-MS) analysis in order to make the report irrefutable.

**Chemicals, Reagents, and Instruments**

Aldicarb, aldicard sulfone, aldicarb sulfoxide, carbofuran and carbofuran 3- hidoxi were obtained from Sigma-Aldrich; acetonitrile and methanol of HPLC-grade from Merck; magnesium sulfate, sodium chloride from Merck; chloroform, ether, dichlormethane, acetone and n-hexane of analysis-grade from Tedia; water was purified by a Milli-Q system obtained from Millipore®; TCL plates with a stationary phase form silica gel activated C60 from Merck; chromatographic column C18 Shimpack® (15 x 4.5 cm, 5μm) and capillary column DB-5 30 meters; chromatographic tanks and other glassware for extraction and filtration.

It was used the liquid HPLC-DAD – Shimadzu, Proemience model, and GC-MS – Perking Elmer Clarus 600 capillary column model. Stock solution (1 mg/ml) was prepared in acetonitrile and stored at -80°C. Standard working solution was prepared from stock solution.

A pen sonicator, balance and syringes needles and filters Millipore® also used.

**Extraction**

It was applied a modified QuEChERS extraction technique for separation and quantification of the pesticides. Thus, it was used 5.0 g sample of liver, which was homogenized with a pen sonicator and then extracted with ether:chloroform the ratio 1:1; after this, the extract was re-suspended with acetonitrile and filtered with filter paper with 1 g anhydrous sodium sulfate the liquid phase was separate. The organic solvent was evaporated at room temperature. This extract was re-suspended in 1.0 ml acetonitrile and used for chromatographic analyses.

**Analytical Techniques**

The analyses was conducted using a screening method (TLC) and two others different physical and chemical methods for confirmation.

For the screening, the extract was applied to a plate chromatographic until to deplete the solvent. The mobile phase was n-hexane:acetone in the ratio 80:20. After complete elution and drying the plate, it was revealed with chromogenic reagent iodine platinum. The possibility of positivity was adopted when the extract presented the same Rf of aldicarb standard.

After TLC analysis, an aliquot of the same extract was analyzed by HPLC-DAD with a validated methodology in the LADTOX. In this methodology, the aldicarb has a retention time of 14.5 minutes ± 0.2 (acceptable range of 5%) with a maximum absorption at 246 nm. In the analysis performed by HPLC-DAD were also simultaneously analyzed metabolites of aldicarb, aldicarb sulphoxide, aldicarb sulphone, carbofuran and carbofuran 3-hidoxi. The confirmatory analysis was done in the Núcleo de Toxicologia Forense do Instituto Médico Legal (IML), São Paulo – SP, Brazil, using a GC-MS with a capillary column DB-5 30 m.

**RESULTS**

The TLC indicated that the sample tested contained the same Rf (0.284) of aldicarb standard (Figure 1). In the HPLC-DAD, the calibration curve of aldicarb in liver samples was linear over a concentration range of 10 – 2000 μg/ml, with a correlation coefficient of 0.998. The detection limit (LOD) of aldicarb was 1 μg/ml.

The sample of horse liver showed 15 μg/g of aldicarb and was positive for aldicarb sulfone and aldicarb sulfoxide, but negative for carbofuran and carbofuran 3- hidoxi (Figure 2).

The presence of aldicarb was confirmed by GC-MS (Figure 3).

![Figure 1](image-url) The thin layer chromatography (TLC) of the sample of horse liver (in replicate: S1 and S2) tested contained the same Rf (0.284) of aldicarb standard. White sample of horse liver (in replicate: S3 and S4). Samples were chromatographed on C18 reversed-phase thin-layer plates, developed with n-hexane:acetone in the ratio 80:20 and it was revealed with reagent iodine platinum.

**DISCUSSION**

Accidental poisoning by aldicarb in horses are extremely uncommon, being found only two cases reported in the literature [4,7], since the horses have the behavior of grazing high (only the top of the vegetation). In these papers it was not quantified the aldicarb and metabolites presence in tissue sample of horse. On the other hand, Proença et al. [8] reported a human fatal aldicarb poisoning that the toxicological analysis revealed the following toxic
concentrations of aldicarb: liver (0.80 μg/g), blood (6.2 μg/ml), stomach (48.9 μg/g), kidney (8.10 μg/g), heart (6.70 μg/g) and urine (17.50 μg/ml).

It is known that in the animal's body the kinetics of absorption and distribution of aldicarb is rapid, which makes it extremely lethal in some cases. After being readily absorbed by any of the routes of exposure (oral, dermal, respiratory), aldicarb is initially oxidized to aldicarb-sulfoxide and a portion of it is slowly degraded to aldicarb-sulfone before being hydrolyzed to non-cholinergic metabolite [3]. These metabolites are eliminated 80 a 90% by the urine. These metabolites have been reported as indicators of aldicarb exposure, although they are stable for up to two days in live or dead animals [6,7].

The fatal case of this horse may reflect the hepatic metabolism or the phenomenon of post-mortem redistribution of the aldicarb. Since the enzymes involved in the processes of aldicarb metabolism are found in the microsomal fraction of the liver homogenate [11] and liver is the primary organ of metabolism, these facts would explain the high levels of aldicarb and the metabolites found in the liver of horses. In addition, Ponder and Jones [9] suggest that there is postmortem drug diffusion along a concentration gradient, from high concentration sites in solid organs (as lung and liver), which may be responsible for elevated levels of toxicant in highly irrigated organs.

Ponder and Jones [9] suggests that there is a post-mortem diffusion of drugs along a concentration gradient, from sites of high concentration in solid organs, into the blood with resultant artefactual elevation of drug levels in blood. Highest drug levels were found in central vessels such as pulmonary artery and vein, and lowest levels were found in peripheral vessels such as subclavian and femoral veins.

In the present study the aldicarb presence in liver sample was confirmed by three different techniques: TLC, HPLC-DAD and CG-MS, following international recommendations. In fact, The Forensic Toxicology Laboratory Guidelines (2006), [10] recommends, as a general matter of scientific and forensic principle, that the detection or initial identification of drugs and other toxins should be confirmed whenever possible by a second technique based on a different chemical principle. In addition, where possible, the confirmatory (second) test should be more specific than the first test for the target analyte.

Although these techniques have criteria for analytical validation according to international guidelines and Brazilian legislation, the analysis were performed only in the liver sample of the animal. It would be desirable to have collected samples of other tissues during necropsy, but this was not done. This fact shows the importance of communication between the veterinarian and the forensic analyst, allowing confirmation of the finding in other matrices.

Finally, this case shows the importance of toxicological analysis as a tool for elucidating the cause of death of the animal, since no clinical signs or necropsy findings was detected on the horse, allowing the discovery of the cause of death of the animal only when the toxicology was performed.

The toxicological analysis results allowed us to conclude that death occurred due to an aldicarb acute intoxication. This case shows the importance of toxicological analysis as a tool for elucidating the cause of death of the animal.
**Figure 3.** The gas chromatography coupled with mass spectrometry (GC-MS) of the sample of horse liver. A) Standard of aldicarb, the red arrows represent the peak for aldicarb. B) Sample of horse liver, the red arrows represent the peak for aldicarb.

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**REFERENCES**


