The Use of a Daphnia magna Bioassay for Rapid Screening of Acute Intoxications with Insecticides in Dogs and Cats

H. De Bosschere1,*, K. Baert2, R. Ducatelle1 and P. De Backer2
1Department of Pathology, Bacteriology and Poultry Diseases, Laboratory of Veterinary Pathology, 2Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Ghent, Salisburylaan 133, B-9820 Merelbeke, Belgium
*Correspondence: E-mail: hendrik.debosschere@rug.ac.be


ABSTRACT

Sudden death due to acute insecticide intoxications occurs frequently in dogs and cats. The absence of characteristic lesions at autopsy often renders post-mortem diagnosis dependent on the analysis of samples taken from the carcase at autopsy. In the present study, a bioassay utilizing Daphnia magna was proposed and tested as a rapid screening method for acute intoxications in dogs and cats. The bioassay was shown to be highly sensitive for detecting carbamate and organophosphate insecticides in the stomach contents. Generally, the mean survival time of the water fleas in the control group was 5.17 h (SD = 1.24) and in the intoxicated group 1.32 h (SD = 1.49), during a 6 h observation period. If a cut-off is set at 4 h, this Daphnia bioassay gave 5.5% false negative results and 18.2% false positive results, using the results of toxicological analyses as a gold standard.

Keywords: cat, carbamate, Daphnia magna, diagnosis, dog, organophosphate, poisoning, sudden death, stomach contents, survival time, waterflea

Abbreviations: HPLC, high-performance liquid chromatography; LSD, least significant difference; TLC, thin-layer chromatography; UV, ultraviolet

INTRODUCTION

Organic insecticides used in agriculture, especially carbamate and organophosphate insecticides, are an important source of intoxication in dogs and cats (Delaunois et al., 1997; De Bosschere et al., 1999) as well as in humans (Borowiak, 1995; Wagner, 1997). Uptake of these chemicals usually results in acute intoxication and sudden death. However, a wide spectrum of other toxic substances may also cause sudden death (Humphreys, 1988). Moreover, animals may die suddenly from causes that have nothing to do with toxins. Sudden death without premonitory signs usually gives rise to very few, if any, specific lesions found at autopsy. The finding of suspicious materials, discoloration or a foul smell from the contents in the stomach may be helpful in establishing a diagnosis of intoxication. When these typical findings are lacking, however, the post-mortem diagnosis of acute intoxications becomes almost exclusively dependent on further analysis of samples taken from the carcase at autopsy. According
to Humphreys (1988), toxicological analysis should be considered only when there is sound evidence that the animal has been poisoned and where at least the group of compounds involved has been established. Unfortunately, in many cases presented for autopsy, neither the case history nor the clinical signs observed ante mortem provide this evidence.

In ecotoxicity analyses, the presence or absence of a toxic pollutant is often screened in bioassays using test organisms such as *Artemia salina*, *Brachionus caliciflorus*, *Photobacterium phosphoreum* or *Daphnia magna* (Calleja et al., 1994). *Daphnia magna* is one of the most popular organisms used to test for environmental pollution or contamination with pesticides (WHO Working Group, 1986; van Wijngaarden et al., 1989; Schmuck et al., 1994; Guilhermino et al., 1996; Kikuchi and Wakabayashi, 1997; Maund et al., 1997; Diamantino et al., 1998).

The presence of insecticides, such as carbamate esters (WHO Working Group, 1986) and organophosphates (Galli et al., 1994; Kikuchi and Wakabayashi, 1997; SosakSwiderska et al., 1998; Sturm and Hansen, 1999), in various substrates has already been tested for using *Daphnia magna*. Khangarot and Ray (1998) used these waterfleas as a simple, inexpensive screening method for compounds of potential mammalian toxicity, which otherwise have to be tested for in time-consuming, ethically unacceptable and expensive, mammalian test models (rat and mouse).

The purpose of the present study was to assess the possibilities of using a *Daphnia magna* bioassay as a screening method for the detection of carbamate and organophosphate insecticides in the stomach contents of dogs and cats that have been presented for autopsy with a suspicion of intoxication.

**MATERIALS AND METHODS**

**Samples**

Samples of the stomach contents were collected from 18 dogs and 27 cats presented for autopsy with a history of sudden death, without premonitory signs and with a suspicion of intoxication. In some cases, a brief episode of vomiting, salivation and nervous signs was noted shortly prior to death. Control samples of stomach contents were selected from 20 dogs and 13 cats that had died due to old age, infections, metabolic diseases or trauma.

**Daphnia bioassay**

The waterfleas were collected in a pool and housed in tanks at room temperature under continuous illumination. Five grams of the stomach contents of the intoxicated and control animals were mixed with tank water in a 50 ml container. After 15 min, the solution was filtered using a funnel and a cotton-wool plug. Then 10 adult waterfleas were put in the filtrate. The survival time of the waterfleas was recorded over 6 h using viability and swimming capability as parameters.