

A case of a brown bear poisoning with carbofuran in Croatia

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Abstract: We provide the first known documentation of a fatal brown bear (*Ursus arctos*) poisoning with carbofuran (an acetylcholinesterase inhibitor) in Croatia. Diagnosis using liquid chromatography–mass spectrometry confirmed the presence of high concentrations of carbofuran in liver and kidney tissue (12.650 and 2.695 ppm, respectively). These measurements, combined with the very small distance between poisonous baits and the brown bear carcass, provided the basis on which we concluded that the animal consumed a lethal dose of carbofuran and succumbed to acute poisoning soon thereafter. We believe this mortality was caused by the illegal placement of this poison probably to eliminate perceived pests, such as jackals (*Canis aureus*) or foxes (*Vulpes vulpes*). Our documentation may help identify similar cases and raise awareness of the risks posed by illegal poisons for non-target species, particularly scavengers such as bears.

Key words: brown bear, carbofuran, Croatia, gas chromatography–mass spectrometry, liquid chromatography–mass spectrometry, poisoning, *Ursus arctos*

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The brown bear (*Ursus arctos*) population in Croatia is managed as game, and currently poaching does not seem to be a serious problem. However, poaching control remains one of the toughest challenges in wildlife conservation. In the cases when the prime motivation of poacher is to generate

cash income or to provide protein requirements (e.g. using meat), the problem is at least understandable (e.g., bush-meat hunting in Africa; Coad et al. 2010, Lindsey et al. 2011a,b). In contrast, poisoning destroys nearly all value of the animal and of its remains. The only motivation of such a poacher may be the elimination of an unwanted competitor (Berny 2007, Guitart et al. 2010). Poisons also claim the non-target victims (Frkovic et al. 1987, Mineau 2005, Berny 2007). Bears often scavenge for food and thus are vulnerable to consumption of poisoned baits (Frkovic et al. 1987, Wobeser et al. 2004, Guitart et al. 2010).

Poisons were used legally in Croatia after the Second World War in the wolf (*Canis lupus*) extermination campaigns, until they were banned in 1970s (Frkovic et al. 1992). About 9% of total bear mortality in 1946–85 has been attributed to accidental poisoning (Frkovic et al. 1987). Since then, almost no incidents of bear poisoning have been registered. Huber et al. (2008) estimated the brown bear population in Croatia to be between 600 and 1,000 individuals. Later, Kocijan and Huber (2008) reported an estimate of 1,000 bears obtained using DNA sequenced from scat samples collected over 3 study areas (Kocijan and Huber 2008). This population size seems to be approaching the social acceptance level, but so far continues to be tolerated (Majić et al. 2011).

Carbofuran belongs to the group of carbamates, esters of carbamic acid. It is an insecticide and nematocide used in crop protection. Worldwide, numerous cases have been recorded of poisoning of wild and domestic animals, as well as humans (Antoniou et al. 1996, Tataruch et al. 1998, Mineau et al. 1999, Srebočan et al. 2003, Wobeser et al. 2004, Satar et al. 2005, Recena et al. 2006, Hernandez and Margalida 2008, Venkataramanan et al. 2008, Jozsef et al. 2009, Jung et al. 2009, Guitart et al. 2010), which was the main reason for banning the use of this substance in the European Union. In Croatia, use has been prohibited since 2007.

In this paper, the first proven record of fatal poisoning in brown bear in Croatia is presented, although the bait was probably not targeted toward bears. Our objectives are to provide documentation that may help identify similar cases and to raise awareness of the problem of carbofuran poisoning in bears and other wildlife.

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Study area and methods

On 19 April 2010 in the hunting ground “Sveto brdo XIII/29” adjacent to the Velebit Nature Park near the natural water hole called Ivanjska Lokva, the carcass of a jackal (*Canis aureus*) was found in the evening hours (about 1800 hours) by a woman returning from a hiking tour. A number of hiking trails originate from the vicinity of Ivanjska Lokva, and the carcass was visible from the one that leads to the Sveto brdo peak. She informed the authorities of Velebit Nature Park.

The following day, on 20 April 2010, the ranger from the Nature Park investigated the site and discovered the carcass of a brown bear about 200m from the water hole. On 21 April the first author (SR), accompanied by police officers, visited the site. There were no visible injuries on the bear carcass; however, a vomited watery substance was found on the animal's front legs. The investigation team also discovered small piled concentrations of meat, bones, and dark blue compact granules at 3 locations near the water hole; these appeared to have been left as attractants, and their condition suggested that they had been placed subsequent to most recent rain event, or a maximum of 6 days prior to discovery of the bear carcass. The veterinarian (SR) who performed the autopsy found the carcass to be bloated and characterized by moderate autolysis and congestion of organs. No outside or inside traumatic lesions were found on the body, and there was no evidence of post-mortem scavenging. Liver and kidney tissue samples and the entire ligated stomach were taken. All baits found near the water hole were also collected. Frozen tissue samples and baits were sent to the police forensic laboratory “Ivan Vučetić” where all the analyses were performed. The jackal carcass had been removed prior to arrival of the investigation team; thus, no sampling could be conducted.

Laboratory methods

A small amount of liquid bluish content was found in the stomach. We assayed for the presence of carbofuran in stomach contents as well as in dark blue compact granules found on baits using gas chromatography–mass spectrometry (GC–MS).

Concentration of carbofuran in kidney and liver tissue was estimated using liquid chromatography–mass spectrometry (LC–MS) on Agilent 1100 LC/MSD instrument (Agilent Technologies, Santa Clara, California, USA) with SunFire C18 Column;

3.5 μ m, 4.6 x 150 mm (Waters Corporation, Milford, Massachusetts, USA). The 1.90 g of tissue sample was homogenized, and carbofuran was extracted with 19 mL of ethanol. The LC conditions were as follows: oven temperature 40°C, flow rate 0.6 mL/min, mobile phase acetonitril: H₂O(NH₄Ac1 g/L) (23:77), volume injector 20 μ L, retention time (Rt) for carbofuran 15.1 min. The ionization mode was positive. The interface and MSD conditions were as follows: dry gas (N₂) 12 L/min, gas temperature 350°C, gas pressure (N₂) 2.41 bar (35 psi), spray capillary voltage 3000 V, ion transfer voltage 90 and SIM mode (222 m/z). The concentration of carbofuran in the sample was calculated using calibration curve for carbofuran standard. Because of the presence of autolysis, our investigation did not include histopathological analyses and we depended entirely for our conclusions on the chemical assays.

Results

GC–MS confirmed the presence of carbofuran in both baits and stomach contents. This indicated that the dark blue compact granules found in the baits and stomach contained factory-prepared carbofuran. In addition, LC–MS of kidney and liver tissue indicated mean measured carbofuran concentrations of 2.695 and 12.650 ppm, respectively (Fig. 1).

Discussion

Carbofuran is classified by the US Environmental Protection Agency (EPA) in group I according to toxicity. The acute oral LD₅₀ for rats (*Rattus norvegicus*), mice (*Mus musculus*), dogs (*Canis familiaris*), and Japanese quail (*Coturnix japonica*) are 8, 14.4, 15, and 2.5–5 ppm, respectively (Tomlin 2000). The mechanism of toxic action of carbofuran is reversible inhibition of the acetylcholinesterase enzyme (AChE) with consequential accumulation of acetylcholine, which causes excessive synaptic neurotransmitter activity in the parasympathetic (cholinergic) nervous system and at neuromuscular (nicotinic) sites. This usually results in an acute onset of clinical signs categorized into 3 syndromes: muscarinic (vomiting, diarrhea, salivation, lacrimation, myosis, dyspnea, bradycardia); nicotinic (muscle tremor and twitching, paresis progressing to paralysis); and central (behavioral changes, hyperactivity, seizures, depression). Not all poisoned

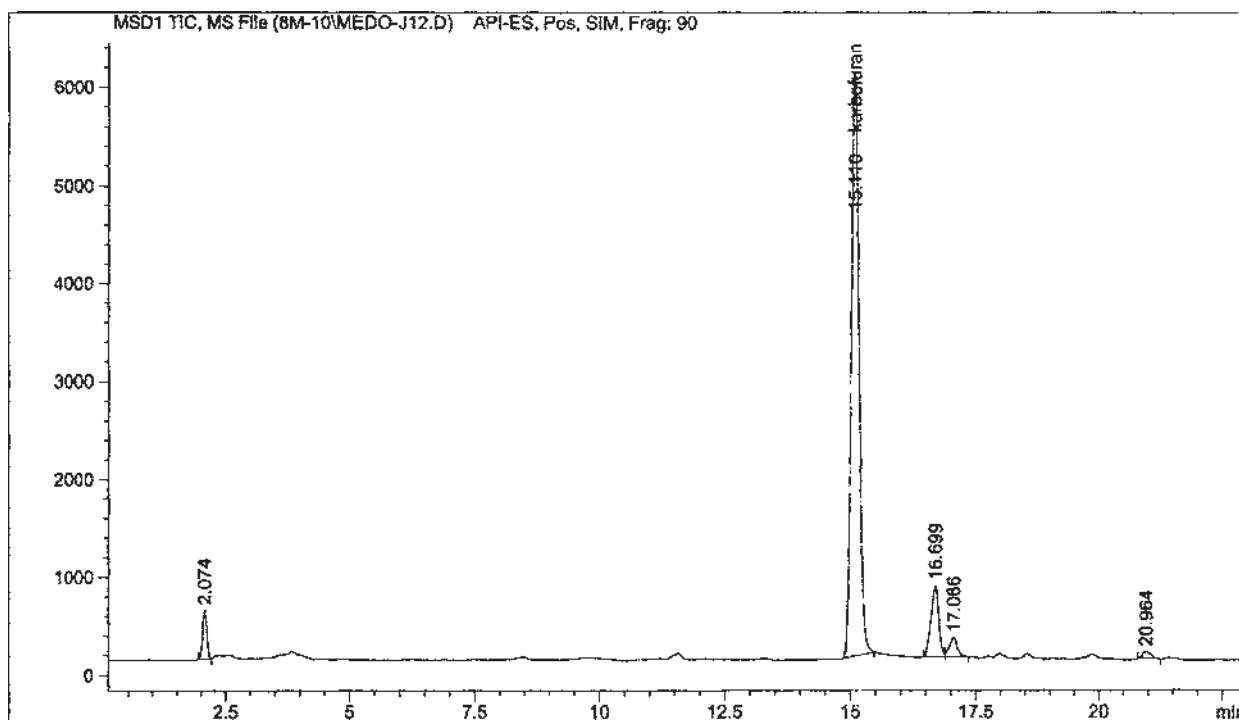


Fig. 1. Chromatogram for the liver sample from a brown bear found in April 2010 in Croatia (LC–MS technique). The retention time (Rt) or peak for carbofuran was at 15.1 minutes (x-axis). Intensity of the peak (y-axis) shows the carbofuran concentration in the sample.

animals necessarily show all clinical signs; i.e., there is considerable variation in presentation and symptoms among the individual cases of poisoning. Pathomorphological changes in acute carbamate poisoning with AChE inhibition are quite unspecific, and in most cases, death is primarily due to respiratory failure (Antoniou et al. 1996, Tomlin 2000). No specific gross pathology and histopathology changes could be found, mostly because the time was too short for them to develop. Treatment in domestic animals includes the administration of emetics (if the animal has not vomited and can vomit) and adsorbent and atropine (Gupta 2007, Srebočan and Srebočan 2009). However, to be effective this treatment must be timely, which can rarely be implemented for wildlife.

There is no acceptable level of carbofuran in body tissues; it is a strong poison not normally present. The concentrations of carbofuran measured in liver and kidney samples were very high (12.650 and 2.695 ppm, respectively). Antoniou et al. (1996), reported 0.53 ppm of carbofuran in liver tissue of a fox, and 0.33 ppm in liver tissue and 0.11 ppm in kidney tissue of a black vulture (*Aegypius monachus*).

The high concentrations, together with the very small distance between the poisonous baits and the brown bear carcass, allowed us to conclude that the animal consumed a lethal dose of carbofuran and succumbed to acute poisoning soon thereafter. Although we lack histopathology results, we believe our conclusions remain strong. We speculate that the jackal may have been similarly poisoned.

Similar cases of dog poisoning by chlorpyrifos (organ phosphorous insecticide) and carbofuran occurred in 2002 in the Split area, and in a griffon vulture (*Gyps fulvus*) by methomyl (insecticide of carbamates group) on the island of Cres. In both cases, poisons were illegally used to eliminate pests, in the first case jackals and foxes, and in second case wild boars (*Sus scrofa*; Srebočan et al. 2003, Sabočanec et al. 2005). Before banning carbofuran in 2007, it was easily accessible at agricultural stores in Croatia. Apparently, some people still possess old reserves. This is the first proven record of a poisoned bear in Croatia, although the bait was probably not targeted toward bears. The fact that carbofuran is no longer available in markets assures us that extensive future use is unlikely. The police have made charges

against the alleged perpetrator, who remains unknown to us.

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