ORIGINAL ARTICLE

Incidental poisoning of animals by carbamates in the Czech Republic

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Summary

Illegal poisoning of wildlife and domestic animals is a worldwide issue. The carbamates primarily used as pesticides are often misused for such a purpose. In this study, 181 birds, mammals and baits were analysed over the period 2004–09 for possible intoxication by carbamates. Intoxication by carbamate carbofuran was diagnosed in 89 cases, and in another 19 cases (nine Wild Boars and 10 Bisons) intoxication with another carbamate – methomyl – was proven. Incidental ingestion of the marten bait was the main cause of intoxication. Although the distribution of carbofuran was prohibited in 2007, no decline in the number of intoxicated animals in the following two years was detected. New cases of intoxication by carbofuran are anticipated in the future until all remaining stock is expended.

Key words: pesticide poisoning; organophosphorous inhibitors; forensic medicine; acetylcholinesterase

INTRODUCTION

Pesticide poisoning of non target organisms is a worldwide issue and many cases are described (Roberston et al. 1992, Hornfeldt and Murphy 1997,

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Xavier et al. 2002, Berny and Gaillet 2008). Carbamate compounds are a very important group of cholinesterase inhibitors and intoxications caused by these compounds represent the majority of pesticide poisoning among animals. According to present knowledge, carbofurane (2,3-dihydro-2,2-dimethyl-7-benzofuranylmethylcarbamate) is the most frequently encountered carbamate as a cause of intoxication. Its LD_{50} is ranging from 3–19 mg.kg⁻¹ of body weight in many animal species (Wang et al. 2007).

Toxicologically, carbamates belong to the group of acylating (carbamylating) acetylcholinesterase (AChE) inhibitors which inhibit AChE by carbamoylation of the serine hydroxyl group in the enzyme active site. Moreover, this inhibition leads to the accumulation of acetylcholine in the gap junction causing hyperstimulation of cholinergic receptors. "Overdose" with acetylcholine is followed by overstimulation of the receptors and finally it ends with a cholinergic crisis followed by muscarine, nicotine and central nervous signs, i.e. miosis, hypersecretion of exocrine glands, bradycardia, tonic and clonic convulsions (O'Malley 1997).

Although carbamate pesticides are less toxic than organophosphorus pesticides, many cases of animal poisonings caused by these agents are reported with increasing frequency (Balcom 1983, Elliot et al. 1996, Mineau et al. 1999, Wobeser et al. 2004).

In this study, we summarize the results from the analysis of 89 cases of positively tested birds (White-tailed Eagle, Golden Eagle, Common Buzzard, Rough-legged Buzzard, Hooded Crow, Common Raven, Common Magpie, Grey Partridge) and mammals (dog, Red Fox, Otter, Wild Boar, European Polecat, Marten) during the period 2004–09 poisoned by carbofuran and 19 cases of animals (Wild Boar, Bison) intoxicated with other carbamate methomyl (methyl N {[(methylamino)carbonyl] oxy}ethanimidothioate).

MATERIAL AND METHODS

In this retrospective study, 181 cases of suspected intoxications from January 2004 to December 2009 were analysed. In these 181 cases, 59.0% were tissue samples (mostly liver), 31.0% were gastric content and 9.8% were baits (mostly eggs, meat and meat products). Baits had been laid in forests and parks, or thrown into private gardens.

White-tailed Eagle, Golden Eagle, Common Buzzard, Rough-legged Buzzard, Hooded Crow, Common Raven, Common Magpie, Grey Partridge, dog, Red Fox, Otter, Wild Boar, European Polecat, Marten, Bison were among the intoxicated animals.

Necropsy and histopathology were performed on entire animals (six dogs and three otters) delivered with a no longer *post mortem* interval than 24 hours.

The analysis was conducted with the available chromatographic techniques at the State Veterinary Institute in Jihlava Czech Republic, using tissue samples (liver), solid (bait, undefined material) or liquid samples (gastric content). Samples were extracted by petroleumether and after evaporation on the rotary vacuum evaporator and dissolution in isooctane were analysed by the gas chromatography method with a nitrogen-phosphorous detector (Lee and Westcott 1983).

RESULTS

The numbers of examined samples and positive cases in each year of the study are given in Fig. 1. The presence of carbofuran could be verified in a total of 89 samples. This means that there were approximately half (49.2%) of the samples positive out of 181 in 6 years. In 2004 only three samples were examined. Two of these three samples originally from birds of prey were positive for carbofuran - a White-tailed Eagle (Haliaeetus albicilla) and a Rough-legged Buzzard (Buteo lagopus). In 2005, the percentage of positive samples was 45.5% (10/25), as in 2007 and 2008 [2007 44.0% (11/25), 2008 46.2% (18/39)]). In 2006, the percentage of positive samples was somewhat higher [67.9% (36/53)], whereas some decline was obvious in 2009 [30.8% (12/39)]). Birds of prey were the most common victims of carbofuran (Fig. 2) – 22 Buzzards (Buteo sp.) and 8 endangered Eagles (Haliaeetus sp., Aquila sp.) were illegally poisoned during the studied time frame. Also dogs (n=12), Otters (n=10, Lutra lutra) and Corvids [n=10; Common Raven (Corvus corax)], Common Magpie (*Pica pica*)] and Hooded Crow (*Corvus cornix*)] were frequently involved. Other species, such as small carnivores [Marten (Martes sp.), European Polecat (Mustela putorius)], Stoat (Mustela erminea)] or birds [Grey Partridge (Perdix perdix)] rarely show signs of intoxication by carbamates.

In 2007 and 2008, 19 samples were positively tested for another carbamate – methomyl (methyl *N*-{[(methylamino)carbonyl]oxy}ethanimidothioate). Nine samples were taken from Wild Boars (*Sus scrofa*), another ten samples were collected from Bison (*Bison* sp.) from a private farm in south Bohemia. Up to November 2008 a total of a 37 Bison died supposedly poisoned by methomyl (not all animals were investigated).

Necropsy revealed dried saliva around the oral cavity, congestion of the organs, and haemorrhagic necrosis of the small gut. Histopathology showed congestion of the kidney, liver and lung, granular dystrophy of liver and haemorrhagic necrosis of the small intestine.

DISCUSSION

Carbamate intoxication is the most common form of pesticide poisoning of animals in Europe and the US (Berny 2007, Wang et al. 2007). The most common route of exposure to pesticides is an ingestion of poisoned insects, carcasses, or grains intentionally treated with pesticides for bait (Reece and Handson

Novotný et al.: Incidental poisoning of animals by carbamates



Fig.1. No. of samples analysed in the State Veterinary Institute Jihlava tested for carbofuran (black columns) and number of positive cases (gray columns).



Fig.2. Distribution of carbofuran intoxicated samples. Birds of prey were the victims of carbofuran in most cases.

1982). Organophosphorous and carbamate pesticides present also a potential threat for humans mainly because of their wide use in agriculture. Thousands of intoxication cases in humans are recorded every year especially in developing countries (Jayaratnam 1990).

According to many studies, birds of prey are common victims of carbofuran poisoning (Balcom 1983, Elliot et al. 1996, Wobeser et al. 2004) and this was confirmed in our study. This could be caused by secondary poisoning when the raptor consumes a primary victim with unassimilated remains of pesticide in its alimentary tract (Dietrich et al. 1995, Hill 1995, Mineau et al. 1999, Berny 2007), but it is also possible that some of the baits are directed at birds of prey.

Compared to the high number of intoxicated birds we found only sporadic cases of small carnivore intoxication (except otters), although martens or foxes are thought to be the main target object of poisoners. This could be caused by the hidden behaviour of carnivores, so the real number of intoxications is expected to be much higher.

Also domesticated animals such as pets, mainly dogs, and livestock are at risk of being poisoned (Wang et al. 2007). The consumption of bait could be accidental during walks outdoors but there are also some cases of intentional poisoning (Novotný et al. 2003).

Although the use of carbofuran was prohibited by law in the Czech Republic on 13th December 2008 and its sale was stopped on 13th December 2007, new cases of intoxication by carbofuran are still expected in the future until all stocks are expended.

In organophosphorous and carbamate pesticide investigation, analysis of the brain and blood cholinesterase (ChE) activity is widely used as a diagnostic technique (Worek et al. 1999 Zdarova Karasova et al. 2009), but gas chromatography was used in this study. However, diagnostic interpretation of ChE data always requires knowledge of the normal value for each species (Blakley and Skelley 1988), and it may be confounded as well by changes in ChE levels caused by post mortem decomposition (Wobeser et al. 2004) and post mortem reactivation of carbamylated ChE (Hill 1989).

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