REVIEW ARTICLE

CARBAMATE INSECTICIDES IN THE CZECH REPUBLIC: HEALTH AND ENVIRONMENTAL IMPACTS

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Summary
Carbamates neurotoxins are a group of compounds acting as pseudo-irreversible inhibitors of an enzyme acetylcholinesterase and butyrylcholinesterase. The compounds are well known as pesticides. Some of them, such as rivastigmine, pyridostigmine, and neostigmine, can be used as drugs for Myasthenia gravis or Alzheimer disease as well. The present review is aimed to summarize the basic facts about carbamate pesticides. Legislative aspects, e.g. law No. 326/2004 Coll., in the Czech Republic and examples of accidental exposures to carbamates in the country are provided, too. Other general provisions are determined by the EU, in particular by the Directive 91/414/EHS. The European legislative is discussed, too. Finally, examples of accidental exposures are introduced in the study.

Key words: Acetylcholinesterase; Butyrylcholinesterase; Pyridostigmine; Pesticide toxicity; Persistence; Carbofuran

INTRODUCTION
Pesticides are a group of compounds which are toxic to pests. From the pesticides group, insecticides are toxic to insect. Application of insecticides is not privileged for agricultural purposes only. They are used for extirpation of vectors as well. Fighting malaria by organophosphorus, carbamate, and pyrethroid insecticides can be shown as an example [1]. N-alkyl and N,N-dialkyl carbamates inhibiting cholinesterases, especially acetylcholinesterase (AChE), are a group of toxic compounds suitable for application as insecticides and drugs. The first found carbamate inhibitor of AChE was a secondary metabolite physostigmine from African plant Physostigma venosum (Fabaceae) seeds called ordeal bean of Old Calabar [2]. In the further text, there is discussed an application of carbamate insecticides. Aside from this, the compounds have importance as drugs for Myasthenia gravis treatment, e.g.: pyridostigmine and neostigmine and also for Alzheimer’s disease treatment: rivastigmine [3,4]. Structures of the mentioned compounds are depicted as figure 1.

Carbamate insecticides are relatively harmless for vertebrates due to poor penetration through a blood brain barrier [5]. On the other hand, it can pollute environment and become a problem when it reaches water supplies which, for example, was the case, reported by Tarig et al. [6] for carbosulfan. The environmental impact is yet not well comprehended because of low understanding of some side effects.
Especially, genotoxicity of carbamate insecticides should be further investigated because some authors pointed out chromosomes damage and micronuclei formation [7]. The present review is focused on application of carbamates used for agricultural purposes and their environmental impact. Their persistence in nature and adverse toxic effects are discussed throughout. In this paper, actual situation in the Czech Republic is reported beside the common facts about carbamates toxicity and environmental effects.

![Figure 1](image1.png)

Figure 1. Structures of carbamates applicable as drugs.

**Molecular and biological effect of toxic carbamates**

Carbamate insecticides are pseudo-irreversible inhibitors of AChE. They react with serine in esteratic part of the enzyme active site in a process called carbamylation [8]. Unlike the organophosphorus irreversible inhibitor, carbamate moiety can be split from serine hydroxyl spontaneously by hydrolysis process [9]. Carbamates are not simply passing through a blood brain barrier. E.g. pyridostigmine do not pass into a central nervous system in healthy individuals; however, it can penetrate through the barrier under stress conditions [10]. Due to the limited action on a central nervous system, peripheral nerves’ AChE is the main target of carbamate insecticides. As AChE in peripheral nerves of a cholinergic system becomes inhibited, hyper-stimulation of nicotinic and muscarinic acetylcholine receptors follows because neurotransmitter acetylcholine is not converted into acetic acid and choline. The neuromuscular blocks result in upper respiratory tract collapsibility and impair muscle activation [11]. Principle of cholinergic transmission and site of carbamates action is depicted in figure 2.

![Figure 2](image2.png)

Figure 2. Scheme of cholinergic neurotransmission; used abbreviations: ACh – acetylcholine, AChE – acetylcholinesterase, nAChR – nicotinic acetylcholine receptor, mAChR – muscarinic acetylcholine receptor.
Toxicity of carbamates can be based also on another mechanism than inhibition of AChE. Insecticide carbofuran can be used as an example of an insecticide with dual action. It is used in many countries and it is popular for its efficacy. Carbofuran is a potent inhibitor of both AChE and butyrylcholinesterase (BChE). Rats which were orally exposed to carbofuran have maximum inhibition of blood cholinesterases 0.5–1 hours after an exposure [12]. After one day, the cholinesterases activity turns into the original level indicating low toxicity burden for accidentally exposed vertebrates. Carbofuran is metabolized by cytochromes P450 into 3-hydroxycarbofuran. The oxidation of carbofuran by cytochromes P450 leads to activation of the detoxification pathways and elevated metabolism of testosterone [13]. As hormone balance is altered, endocrine disruption follows. Goad et al. [14] proved that rats exposed to carbofuran had significantly decreased testosterone level and increased progesterone, cortisol, and estradiol levels. The scale of the hormonal imbalance exceeds the effect on cholinesterases. In course of carbofuran adverse effects, it is banned in many countries including the European Union countries and Canada. In the countries where carbofuran is banned, less toxic carbamates (see later) are preferred.

**Fate of carbamates in environment**

In environment, pesticides are degraded by number of physical, chemical and biological factors. Their decomposition is mostly done by solar radiation (photolysis), temperature (evaporation), moisture (hydrolysis), atmospheric oxygen (oxidation) on the surface of microorganisms in soil or plants, etc. Places where we can often meet pesticides in the environment are soil and water. Movement of pesticides in soil can take place by two ways: diffusion or flow of water. The water can serve not only as a transport medium in soil body, but can also contaminate groundwater and surface water.

In general, under optimal conditions, the rate of degradation is influenced mainly by their physico-chemical properties, while environmental factors come into the forefront when environmental conditions turn into extremes. For that reason, it is not possible to evaluate the transport of pesticides in soil in a complex way.

Most studies agree that the sorption and/or desorption of pesticides in soil is mainly related to a content and a quality of humus [15]. Non-ionic pesticides or pesticides with slightly ionic character are attracted to humus mainly by hydrophobic bond [18-20].

Potential of immobilization (filtration, P) in the soil profile can be expressed by equation [21]:

\[
P = F(S)
\]

where: S = precipitation

We can assume that the intensity of sorption is influenced by organic matter by 66% and by clay fractions of texture by 33%. However, soil organic matter is relatively heterogeneous, therefore the sorption properties depend not only on its quantity, but quality as well. Overall, we can express filtration index (If) / immobilization of organic xenobiotics in soil by the following equation [21]:

\[
If = F(S) \times [0.66 F(J) \times F(C) \times F(H) + 0.33 F(J) \times F(P)]
\]

where: rainfall (S), soil organic matter: quantity (C) and quality (Q), depth humus horizon (H), clay content (J), soil depth (P).

The potential immobilization in soil, as shown by some experiments [22-26], can be also affected by the soil reaction. Sorption capacity of soil increases with the decreasing pH. A pesticide is dissociated in aqueous solution and exists mainly in the form of anions. There are repulses by the negatively charged surfaces of soil particles and colloids and a pesticide becomes mobile in the soil environment.

In general, the rate of intensity of pesticide sorption and its decomposition is lower when the rainfall intensity is higher or when the terrain slope is steeper in a given territory. This increases the potential for horizontal and vertical transport with a higher risk of surface or groundwater contamination.

**Overview of past and current carbamate pesticides**

**Desmedipham** (CAS No: 13684-56-5) is used as a herbicide on strawberries, spinach and beets. Bioaccumulation potential is medium with a half-life in soil from 8 to 34 days. According to the WHO classification, it is recognized as potentially dangerous. It is quite toxic to aquatic organisms. Toxicity: median lethal dose (LD50) for rat 7625 mg per kg, median lethal concentration (LC50) for fish 1.7 mg per liter (trout), 1.88 mg per liter (daphnia). It is contained in preparations of e.g. Betanal, DMP, Demifan, Duofan, Synbetan (D, D Forte, Duo Mix).
**Fenoxycarb** (CAS No: 79127-80-3). It is used as an insecticide for apple trees, plum trees and grapes. Bioaccumulation potential is medium with a half-life in soil from 25 to 31 days. According to the WHO classification it is recognized as potentially dangerous (ADI Acceptable Daily Intake) 0.03 mg per kg and day. It is very toxic to aquatic biota, it might be carcinogenic. Toxicity: LD50 for rat 10,000 mg per kg, LC50: 1.6 mg per liter (trout), 10.3 mg per liter (carp), daphnia 0.201 mg per liter. It is contained in preparations: Insegar (25WG, 25WP).  

**Methiocarb** (CAS No.: 2032-65-7). This solid is poorly soluble in water. It is used as an acaricide, insecticide, molluscicide and repellent (birds) for potatoes, barley, beet and vegetables. Bioaccumulation potential is low with a half-life in soil from 0.25 to 1.4 days. However, according to WHO classification it is listed as highly hazardous (Ib), ADI from 0.013 to 0.02 mg per kg and day. Toxicity: LD50 for rat 0.060 g per kg (orally), or from 0.060 to 0.135 g per kg (Ben Dyke et al. 1970). Percutaneous LD ranges from 0.035 g per kg [27] to more than 2 g per kg [28]. LC50 is 0.019 mg per liter (Daphnia) [21]. It is contained in preparation of Mesurol.  

**Phenmedipham** (CAS No: 13684-63-4). Used as a herbicide for beet. Bioaccumulation potential is medium with a half-life in soil from 18 to 25 days. According to the WHO classification it is recognized as potentially dangerous (U). ADI is 0.03 mg per kg and day. Toxicity: LD50 for rat 8000 mg per liter, LC50 fish (trout) 1.71 mg per liter. It is contained in the following preparations: Synbetan, Tandem, Mix, Largo, Betasana, Betanal etc. Very often it is found in sediments.  

**Pirimicarb** (CAS No: 23103-98-2). It is a solid which is badly soluble in water. Currently it is used as an insecticide for common crops like potatoes, corn, fruit trees and vegetables. Bioaccumulation potential is low with a half-life in soil from 7 to 234 days. According to the WHO classification it is listed as moderately hazardous (ADI 0.035 mg per kg per day). Toxicity: LD50 rat 143 mg per kg, 29.31, LC50 fish 79 mg per liter (trout), 10.3 mg per liter (carp). It is contained in the following preparations: Pirimor 50WG and Trigger Tribute.  

**Propamocarb** (CAS No: 24579-73-5). It is a solid which is soluble in water. It is used as a fungicide for vegetables (celery, cabbage, cauliflower, tomato, etc.). Half-life in soil is from 10 to 30 days. Toxicity: EC50: Daphnia 106 mg per liter, LD50 for rat 8.6 g per kg (oral), Mouse from 1.6 to 2.0 g per kg. No response: 2 year rat dietary application of 0.04 g per kg. It is contained in preparation of Previcur.  

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Figure 3. Structures of carbamate insecticides.
Some legislative aspects

In the Czech Republic, the use of pesticides and pesticide substances is regulated mainly by Law No. 326/2004 Coll. related to phytosanitary care. The act defines the use of pesticides and their approval. Before usage of pesticides which are not approved to be used in the Czech Republic there must be a registration of the product. Registration is time limited.

Other general provisions are determined by the EU, in particular by the Directive 91/414/EHS. Other relevant laws / regulations in relation to pesticide substances are:

- Decree No 400/2006 Coll. which determines maximum permitted levels of residues of pesticides in various types of food and food ingredients.
- Decree No. 252/2004 Coll. which determines the health requirements for drinking and hot water and the frequency and range of drinking water controls. The maximum allowed level of pesticides in drinking water is 0.1 mg per liter respectively. 0.5 mg per liter in total. Only pesticides which are likely to be in the source are determined. The limit value of 0.5 mg per liter refers to the total sum of the individual identified and quantitatively detected pesticides.
- Directive 98/8/ES22 concerning the placing of biocide products on the market
- Regulation (EC) No 2377/9023 which determines a procedure of the European Community for the establishment of maximum residue limits of veterinary medical products in food of animal origin.
- an important factor to ensure the objectivity of pesticides monitoring is the application of suitable sampling methods. In 2003, Directive 2002/63 came into force. The directive solves purposes of pesticide residua monitoring, the problem of plant and animal origin samplings.

Accidental exposures

It is estimated that approximately 3000000 people are exposed to effects of organophosphates or carbamates every year worldwide, which leads to up to 300000 deaths [29,30]. In the United States in 2008, there were more than 8000 exposures by these compounds reported with less than 15 deaths [31]. In China, pesticide poisoning, and especially organophosphates, causes estimated 170000 deaths every year. Virtually, all of these are the result of suicides [29]. Because of the increased use and availability of pesticides, the poisoning by organophosphates and carbamates is relatively high, mainly in developing countries.

Probably the first major case of human poisoning by organophosphates occurred in 1930s in the U.S. during the prohibition, when thousands of people in the American South and Midwest became intoxicated by "medical" alcohol "Ginger Jake," which contained fake Jamaican ginger. Instead of ginger extract, it contained tricresyl phosphate (TOCP), which caused partially reversible neurological damage [32] resulting in characteristic way of walking. Approximately 30–50 thousands people were exposed. In Europe and Morocco, there was TOCP poisoning recorded caused by contaminated food and cooking oil.

In the Czech Republic, known poisonings in human is e.g. accidental poisoning of preschool age children by organophosphate insecticides [33]. The authors describe a case of a 33-month old boy with severe impairment of consciousness and hypotonia caused by organophosphates intoxication. Based on the results of toxicological examinations, the treatment with atropine in clinical symptoms of atropinization was started as well as treatment by obidoxime with a positive result.

Impacts of carbamate and organophosphate pesticides on ecosystems (either water or terrestrial) are considered to be serious. In recent years carbofuran has been the most common cause of wild birds prey poisoning in the Czech Republic. Illegal use of this pesticide was a fairly widespread method of fighting against unwanted animals (foxes, martens, etc.). However, the lethal doses of this compound for birds are about 10 times lower compared to mammals. Examples of "use" of these products in the Czech Republic from the last decade are the following:

- April 15, 2011 Smrk na Morave (district Trebic) sea eagle, the expected poisoning
- April 11, 2011 Hradistsko Dacice (District Jindrichuv Hradec) sea eagle, carbofuran poisoning confirmed
• February 4, 2011 Trebsko (District Pribram), a dog, poisoning confirmed
• March 25, 2011 Hory u Predina (District Trebic) raven (2 individuals) and fox confirmed carbofuran poisoning
• July 2, 2011 Starec (District Trebic), dog, carbofuran poisoning confirmed
• October 10, 2010 Milicin (District Benesov) eagle (female), confirmed carbofuran poisoning
• April 3, 2010 Dobroviz (District Prague West) buzzard, confirmed carbofuran poisoning
• March 1, 2010 Komorany-4 (District Prague 4) cats poisoning in the urban part of Prague Komorany. After 10 minutes, the cat began to feel dizzy, it had cramps, it died the next day despite an immediate intervention of a veterinarian. The analysis showed carbofuran poisoning.
• November 23, 2009 Dvur Kralove nad Labem (District Trutnov) two dogs poisoned in the garden of the village, laboratory evidence of carbofuran.
• September 29, 2009 at Olbramovice Votice (District Benesov) young female golden eagle from discharges in Beskydy.

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REFERENCES


