International Journal of Biological Macromolecules 57 (2013) 38–44

Contents lists available at SciVerse ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

The effect of bromfenvinphos, its impurities and chlorfenvinphos on acetylcholinesterase activity

Bożena Sosnowska[a], Bogumiła Huras[b], Anita Krokosz[c], Bożena Bukowska[a,*]

[a] University of Łódź, Faculty of Biology and Environmental Protection, Department of Biophysics of Environmental Pollution, Poland
[b] Institute of Industrial Organic Chemistry, Warsaw, Poland
[c] University of Łódź, Faculty of Biology and Environmental Protection, Department of Molecular Biophysics, Poland

ARTICLE INFO

Article history:
Received 12 December 2012
Received in revised form 11 February 2013
Accepted 12 February 2013
Available online 7 March 2013

Keywords:
Human acetylcholinesterase
Erythrocytes
Bromfenvinphos
Chlorfenvinphos
Impurities

ABSTRACT

The aim of this work was to examine the effect of two organophosphorous compounds i.e. bromfenvinphos (BFVF) and chlorfenvinphos (CFVF) possessing acaricidal and insecticidal properties, on the activity of human erythrocytes acetylcholinesterase (AChE, EC 3.1.1.7). Moreover, the effect of five bromfenvinphos production impurities on AChE activity was studied. The erythrocytes were incubated with the compounds studied in the concentrations range from 0.05 to 250 μM for 1 h. The organophosphorous compounds studied in low concentrations increased K_m value but they did not change V_{max} value (competitive inhibition). Higher concentrations of the compounds studied decreased V_{max} value and increased K_m value, what revealed a mixed type of AChE inhibition by these xenobiotics. Basic significance in AChE activity inhibition has the type of halogen in vinyl group. Chlorfenvinphos (insecticide) exhibited stronger enzyme inhibition than bromfenvinphos. CFVF and dibromo-bromfenvinphos possessed the lowest K_i and K_i' values among all the compounds studied.

The presence of Cl atom (chlorfenvinphos) instead of Br atom (bromfenvinphos) considerably increases antiesterase activity of the individual compound. Three impurities like 2,4-dichlorophenacyl bromide, 2,4-dichlorophenacylidene bromide and 2,4-dichlorophenacylidyne bromide did not induce any statistically changes in AChE activity. Two impurities of bromfenvinphos such as: dihydro-bromfenvinphos and dibromo-bromfenvinphos revealed significant effect on the AChE activity, which may be connected with the presence a phosphate group in these substances. It was proven that an increase in antiesterase activitiy of the compounds studied corresponded with the increase in the number of Br atoms at carbon of their vinyl group: dibromo-bromfenvinphos > bromfenvinphos > dihydro-bromfenvinphos.

1. Introduction

Bromfenvinphos – (*E,Z*)-*O,O*-diethyl-*O*-[1-(2,4-dichlorophenyl) -2-bromovinyl] phosphate (BFVF) is organophosphorous insecticide and acaricide, which has been used both in plant protection and as a veterinary drug. Its LD_{50} has a values ranging from 51.7 to 63.7 mg/kg [1]. In veterinary, bromfenvinphos has been used for control of ectoparasites in breeding cattle [2] and also against *Varroa destructor* [3]. *V. destructor* are an external parasitic mite, which causes honeybee (*Apis cerana* and *Apis mellifera*) disease called varroosis. Bee colony attacked by *V. destructor* cannot survive without human help and the most effective way to faith with this mite was the usage of insecticides [4].

Bromfenvinphos is manufactured in the Institute of Industrial Organic Chemistry in Poland. It was effectively and commercially (as Apifos preparation) used against *V. destructor* and many research works have proven high efficiency of Apifos in mites elimination, which was estimated from 95.32 to 99.8% [5,6]. BFVF is very soluble in fats, thus it may infiltrate to honey bee products, e.g. beeswax, honey and bee pollen, which may endanger the potential consumers [7]. Despite that BFVF has been used from many years, not all of the impurities have been determined. Due to UE directive 91/414/EWG, registration of the pesticides requires full identification and toxicological research for all of its impurities. Up to now, more than 10 impurities, which are formed during synthesis of this compound have been discovered [8].

The chlorfenvinphos – (*E,Z*)-*O,O*-diethyl-*O*-[1-(2,4-dichlorophenyl)-2-chlorovinyl] phosphate, an insecticide was used in agriculture and in controlling of pests. Due to its high neurotoxicity, the use of chlorfenvinphos (CVFV) has been banned

Abbreviations: AChE, acetylcholinesterase; BFVF, bromfenvinphos; CFVF, chlorfenvinphos; Ach, acetylcholine; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid).

* Corresponding author at: Pomorska Str. 141/143, 90-236 Łódź, Poland. Tel.: +48 42 635 44 53; fax: +48 42 635 44 73.
E-mail address: bukow@biol.uni.lodz.pl (B. Bukowska).

0141-8130/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijbiomac.2013.02.011

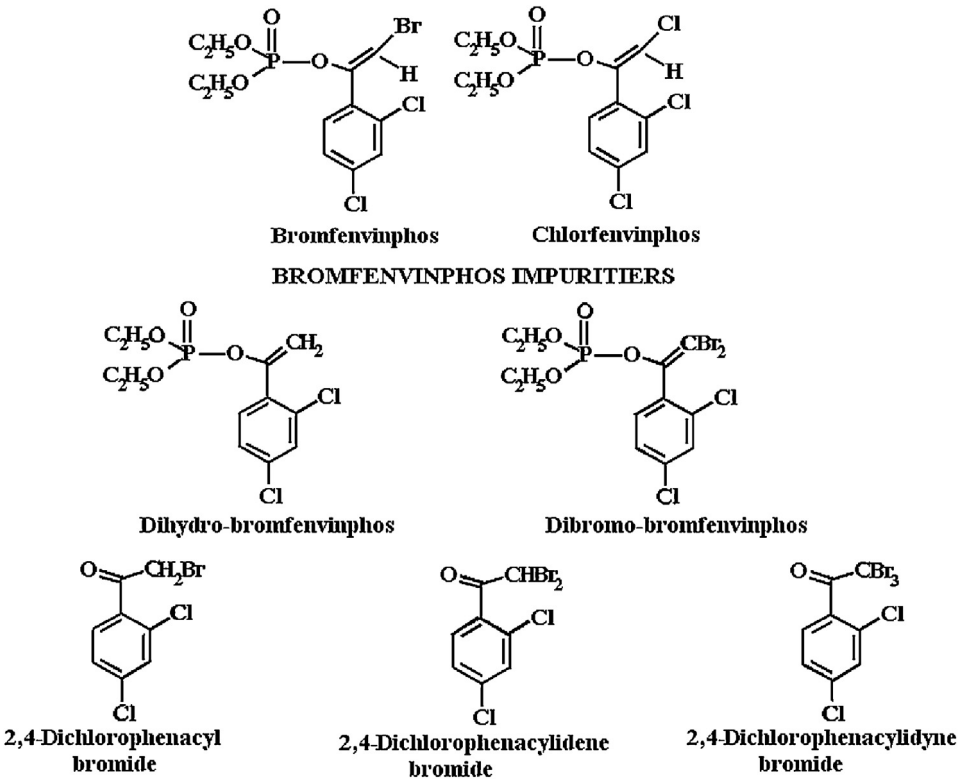

Fig. 1. Bromfenvinphos (BVFV), chlorfenvinphos (CVFV) and BVFV impurities: dihydro-bromfenvinphos, dibromo-bromfenvinphos, 2,4-dichlorophenacyl bromide; 2,4-dichlorophenacylidene bromide and 2,4-dichlorophenacylidyne bromide [20].

in many countries (for example in the USA) for 1991 [9]. In spite of the fact that chlorfenvinphos is harmful to human health, it is still used in some countries, for example in Australia or Switzerland.

Acetylcholineasterase (AChE E.C.3.1.1.7) is present in sensory innervated tissues and it functions in the central and peripheral neuron system to terminate nerve signal transduction at the neuromuscular junction by rapid hydrolysis of the acetylcholine (ACh) receptor released into the cholinergic synaptic cleft [10].

AChE is also found in human red blood cells and may be regarded as a model of AChE in the nervous system. The measurement of erythrocytes AChE activity reflects dynamics of inhibitors adsorption as well as various disturbances in the nervous system. Erythrocyte AChE inhibition has been used as a peripheral surrogate biomarker for the activity of centrally acting AChE inhibitors in the treatment of Alzheimer's disease [10]. The correlation between AChE inhibition in blood and its inhibition in target tissues has been shown [10–12]. The determination of AChE activity in blood is of a great consideration in the diagnosis of poisonings caused by reversible and irreversible inhibitors of this enzyme including pesticides [13–15].

The function of human AChE activity located in the erythrocytes membrane is not well known to this cells. Therefore, it is evidenced what consequences for the structure and function of the erythrocytes can be connected with inhibition of its activity [16].

The activity of AChE is regarded as the most sensitive and accurate indicator of poisoning with organophosphorous compounds [9,17,18]. The erythrocyte acetylcholinesterase activity have a long history of use in monitoring both workers at risk of organophosphorous pesticide (OP) exposure and in investigating accidental exposures to OPs [19].

We investigated the effect of bromfenvinphos, chlorfenvinphos and five of BFVF impurities: *O,O*-diethyl *O*-[1-(2,4-dichlorophenyl) vinyl] phosphate (dihydro-bromfenvinphos); *O,O*-diethyl *O*-[1-(2,4-dichlorophenyl)-2,2-dibromovinyl] phosphate (dibromo-bromfenvinphos); 2-bromo-2′,4′-dichloroacetophenone (2,4-dichlorophenacyl bromide); 2,2-dibromo-2′,4′-dichloroacetophenone (2,4-dichlorofenacylidene bromide); and 2,2,2-tribromo-2′,4′-dichloroacetophenone (2,4-dichlorophenacylidyne bromide) on erythrocytes membrane acetylcholinesterase activity since there is no data on the action of these compounds on AChE activity in human (Fig. 1, [20]).

We have analyzed the impact of the chemical structure of investigated xenobiotics on inhibition of AChE activity and compared toxicity of chlorfenvinphos and bromfenvinphos on AChE activity, which differ in the type of one substituent (CVFV has Cl and BFVF has Br) as well as compared toxicity of BFVF and their impurities.

2. Experimental

2.1. Materials

The investigated compounds e.g., bromfenvinphos (purity 99.9%), chlorfenvinphos (purity 99.8%), dihydro-bromfenvinphos (purity 99.5%), dibromo-bromfenvinphos (purity 99.6%), 2,4-dichlorophenacyl bromide (purity 98.6%), 2,4-dichlorophenacylidene bromide (purity 98.2%), 2,4-dichlorophenacylidyne bromide (purity 98.9%) were synthesized in the Institute of Industrial Organic Chemistry, Warsaw, Poland.

Human erythrocytes were isolated from human blood taken from healthy donors in the Blood Bank of Łódź, Poland. The erythrocytes were centrifuged (3000 rpm/min) and washed twice with phosphate-buffered saline (PBS; 150 mmol L^{-1} NaCl, 1.9 mmol L^{-1} NaH_2PO_4, and 8.1 mmol^{-1} Na_2HPO_4, pH 7.4).

The red blood cells of 5% hematocrit were incubated at 37 °C with bromfenvinphos, its impurities and chlorfenvinphos for 1 h. The compounds were dissolved in DMSO, and then 2 μL of the compound solutions in DMSO were added to the erythrocytes at a final volume of 1.2 mL (the above concentration of DMSO (<0.2%)