Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 145 (2015) 198–202

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

Partitioning of organophosphorus pesticides into phosphatidylcholine small unilamellar vesicles studied by second-derivative spectrophotometry

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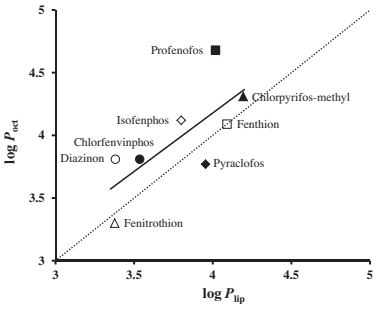
HIGHLIGHTS

- Derivative spectra of pesticides in liposome suspensions showed isosbestic points.
- Partition coefficients (K_ps) of 8 pesticides were determined as a lipophilic index.
- K_p values reflected the chemical structure difference of the pesticides.
- There was no linear relationship between the log K_p and log $P_{octanol}$ values.
- K_p is a good index to predict the bioaccumulation of organophosphorus pesticides.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 February 2014
Received in revised form 16 February 2015
Accepted 17 February 2015
Available online 3 March 2015

Keywords:
Organophosphorus pesticide
Partition coefficient
Liposome
Second-derivative spectrophotometry

ABSTRACT

In order to quantitatively examine the lipophilicity of the widely used organophosphorus pesticides (OPs) chlorfenvinphos (CFVP), chlorpyrifos-methyl (CPFM), diazinon (DZN), fenitrothion (FNT), fenthion (FT), isofenphos (IFP), profenofos (PFF) and pyraclofos (PCF), their partition coefficient (K_p) values between phosphatidylcholine (PC) small unilamellar vesicles (SUVs) and water (liposome–water system) were determined by second-derivative spectrophotometry. The second-derivative spectra of these OPs in the presence of PC SUV showed a bathochromic shift according to the increase in PC concentration and distinct derivative isosbestic points, demonstrating the complete elimination of the residual background signal effects that were observed in the absorption spectra. The K_p values were calculated from the second-derivative intensity change induced by addition of PC SUV and obtained with a good precision of R.S.D. below 10%. The K_p values were in the order of CPFM > FT > PFF > PCF > IFP > CFVP > FNT ⩾ DZN and did not show a linear correlation relationship with the reported partition coefficients obtained using an *n*-octanol–water system (R^2 = 0.530). Also, the results quantitatively clarified the effect of chemical-group substitution in OPs on their lipophilicity. Since the partition coefficient for the liposome–water system is more effective for modeling the quantitative structure–activity relationship than that for the *n*-octanol–water system, the obtained results are toxicologically important for estimating the accumulation of these OPs in human cell membranes.

Introduction

The development and spread of synthetic pesticides have greatly contributed to an increase of food production worldwide.

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http://dx.doi.org/10.1016/j.saa.2015.02.061
1386-1425/

One family of synthetic pesticides in particular, the organophosphorus pesticides (OPs), have been widely used for agricultural and domestic purposes in the control of insect pests. Given their widespread use, these agents have very high potential for human exposure and uptake, and they have been shown to affect most organs in the human body. In general, the molecular mechanism of the acute toxicity of OPs has been shown to involve the powerful inhibition of acetylcholinesterase. In addition, due to their lipophilic nature, OPs accumulate in the membranes of living cells and can thereby cause chronic toxicity. Therefore, it is crucial to investigate the interaction between OPs and biomembranes in order to obtain fundamental information related to their toxic effects.

The *n*-octanol–water partition coefficient (log P_{oct}) is widely used as a simple model system to evaluate the toxicity and bioconcentration factors (BCFs) of pesticides [1–3], although the *n*-octanol–water system has some thermodynamic differences from the actual uptake by biota due to the use of *n*-octanol as the model biological phase [4]. In fact, in the quantitative structure–activity relationship (QSAR) studies of drugs, it has been suggested that the partition coefficients obtained for the lipid bilayer vesicles (liposomes)-water system are more effective than log P_{oct} [5–7]. Accordingly, previous studies have examined the partitioning of pesticides [8] and endocrine disrupting compounds [9] between lipid vesicles and water. However, in these studies, the partition coefficients of chemicals in the liposome–water system were determined by equilibrium dialysis and filtration techniques [10–12], which require difficult separation procedures that may disturb the equilibrium states of chemicals between the lipid bilayer membranes and water. In contrast, since derivative spectrophotometry can eliminate the effect of background signals caused by the light-scattering of vesicles [13], it has been successfully employed to determine the liposome–water partition coefficient without disturbing their equilibrium condition, e.g., second-derivative spectrophotometry has been applied to determine the partition coefficients (K_ps) of psychotropic phenothiazines [14–18], benzodiazepines [19,20], anti-inflammatory steroid drugs [21], non-steroidal anti-inflammatory drugs (NSAIDs) [22], and several other drugs [23,24] between the phosphatidylcholine (PC) small unilamellar vesicles (SUVs) and water.

In this study, we determined the K_ps of eight OPs, chlorfenvinphos (CFVP), chlorpyrifos-methyl (CPFM), diazinon (DZN), fenitrothion (FNT), fenthion (FT), isofenphos (IFP), profenofos (PFF) and pyraclofos (PCF), between PC SUV and water by using second-derivative spectrophotometry.

Methods and materials

Calculation of molar partition coefficients

The molar partition coefficient (K_p) of an OP between PC SUV and water was defined as follows [14,25]:

$$K_p = \frac{([OP_m]/[OP_t])/[L]}{([OP_w]/[OP_t])/[W]} \quad (1)$$

where $[OP_m]$ and $[OP_w]$ represent the molar concentrations of OP in PC SUV and water, respectively, and $[OP_t] = [OP_m] + [OP_w]$, and $[L]$ and $[W]$ are molar concentrations of PC in SUV and water (55.3 mol L^{-1} at 37 °C), respectively.

If the background signal effect based on PC SUV is eliminated in the second-derivative spectra, the derivative intensity difference (ΔD) of OP before and after the addition of PC SUV at a specific wavelength is proportional to the concentration of OP in PC SUV. As described in a previous paper [14], the following Eq. (2) can be derived from Eq. (1):

$$\Delta D = \frac{K_p \Delta D_{max}[L]}{[W] + K_p[L]} \quad (2)$$

where ΔD_{max} is the maximum ΔD value assuming all OPs are partitioned in PC SUV. The values of K_p and ΔD_{max} can be calculated from the experimental values of $[L]$ and ΔD by applying a non-linear least-squares calculation to Eq. (2) [14]. The calculation was performed using a laboratory-made Visual Basic program on a personal computer.

Chemicals and reagents

CFVP, CPFM, DZN, FNT, FT, IFP, PFF and PCF (Fig. 1) were purchased from Supelco Inc. (Bellefonte, PA, USA). The buffer used was 50 mmol L^{-1} NaCl-10 mmol L^{-1} 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes buffer, pH 7.4, 37 °C). L-α-PC (egg yolk) of 99% purity was supplied as a 2% (w/v) chloroform solution from Avanti Polar-Lipids Inc. (Alabaster, AL, USA) and used without further purification after the purity of PC was confirmed by thin-layer chromatography.

Preparation of PC SUV

Appropriate amounts of the PC stock solution were mixed and dried by using a rotary evaporator and then a vacuum pump. To the residue, 5 ml of the buffer was added to yield a PC concentration of ca. 40 mmol L^{-1}, and the mixture was vortexed to produce multilamellar vesicles. Then SUV was prepared by the sonication method as previously reported [14].

Measurement of the mean diameter and zeta potential of PC SUV

The SUV size distribution was determined by a dynamic light scattering method using a submicron particle analyzer (Nicomp Model 380; Particle Sizing Systems, Santa Barbara, CA, USA) [15]. The zeta potential was measured by ZEECOM ZC-3000 (Microtec Co., Ltd., Chiba, Japan), which was based on the principle of electrophoresis.

Phosphorus determination

The exact PC concentration in SUV suspensions was calculated from phosphate analysis according to the phosphovanadomolybdate method [26].

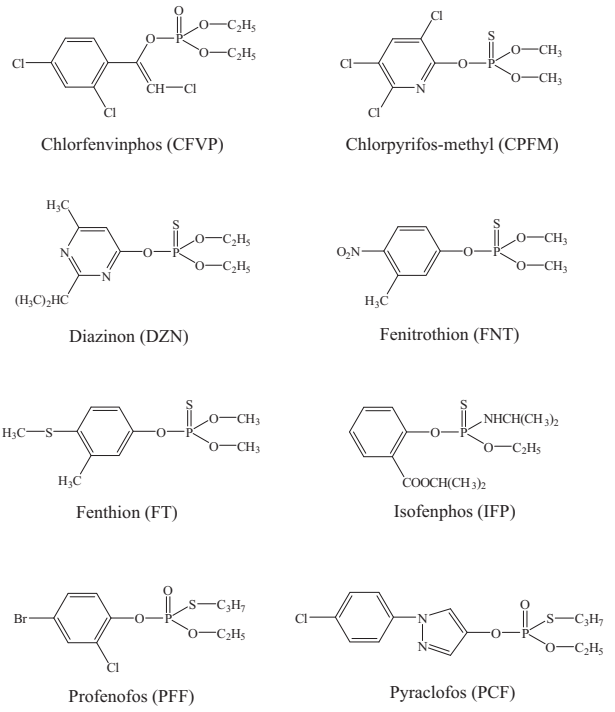

Fig. 1. Chemical structures of the eight organophosphorus pesticides studied.