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Determination of genotoxic effects of methidathion alkaline hydrolysis in human lymphocytes using the micronucleus assay and square-wave voltammetry

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ABSTRACT

The interaction of pesticides with environmental factors, such as pH, may result in alterations of their physicochemical properties and should be taken into consideration in regard to their classification. This study investigates the genotoxicity of methidathion and its alkaline hydrolysis by-products in cultured human lymphocytes, using the square-wave voltammetry (square wave-adsorptive cathodic stripping voltammetry (SW-AdCSV) technique) and the cytokinesis block micronucleus assay (CBMN assay). According to the SW-AdCSV data the alkaline hydrolysis of methidathion results in two new molecules, one non-electro-active and a second electro-active which is more genotoxic than methidathion itself in cultured human lymphocytes, inducing higher micronuclei frequencies. The present study confirms the SW-AdCSV technique as a voltammetric method which can successfully simulates the electrodynamics of the cellular membrane.

1. Introduction

The biological activity of a pesticide is influenced by its physical and chemical properties. Once a pesticide is introduced into the environment, its physicochemical parameters and processes, such as pH, redox potential, UV light, ionic strength, adsorption/desorption and transformation determine its fate [1–3]. The assessing of the impact of a particular pesticide and its products degradation on human health is difficult due to speciation of pesticides. The mechanism by which the pesticides exert their toxic effects on mammals has only been characterized for a few compound groups [1]. The genotoxic and mutagenic activities of certain agro-pharmaceuticals have been studied both with in vitro and in vivo systems, using indicators of genetic damage such as micronuclei (MN), single cell gel electrophoresis (SCGE), chromosomal aberrations (CA) and sister chromatid exchanges (SCE) [4–10]. The cytokinesis block micronucleus assay (CBMN assay) in human lymphocytes, developed by Fenech and Morley [11], uses cytochalasin-B, an inhibitor of actin polymerization, to prevent cytokinesis without blocking nuclear division [12–13]. As a result, binucleated (BN) cells are produced, which are scored for the presence of MN [12,14–15].

Methidathion ($C_6H_{11}N_2O_4PS_3$) (*S*-2,3-dihydro-5-methoxy-2-oxo-1,3,4-thiadiazol-3-ylmethyl *O*,*O*-dimethylphosphorodithioate), Schematic 1, is a non-systemic organophosphorous pesticide intended to control insects and arachnoids in a wide range of cultivations. It was first registered in 1972 and is characterized as a Class I Toxic Compound (Highly Toxic) and as a Restricted Use Pesticide (RUP) by US Environmental Protection Agency [16]. Data from 1987 to 1997 indicate an average domestic use of approximately 241.000 lb, of active ingredient, per year [16–18]. Its LD_{50} ranges between 25 to 48 mg/kg for rats and 25 to 70 mg/kg for mice [19]. Methidathion and its derivatives are still in use, regardless their toxicity. This is due to their low persistence in the environment and their high effectiveness.

At alkaline pH, methidathion undergoes non-reversible hydrolysis [20]. In Schematic 2 a detailed study of the effect of pH on methidathion that has been made by Eto [21] is presented. The cleavage of the phosphoro-sulfur bond "*P*-*S*" results into two by-products. According to stripping analysis theory [22], the first product (Schematic 2) is a non-electroactive phosphono-compound, while the second product is a highly electro-active sulfhydryl-species (marked "*H*-*SR*"). Molecular structures with "*H*-*SR*" electroactive groups can react with sulfur-containing enzymes and coenzymes blocking their catalytic activity [23–24]. Enzyme inhibition may also occur by complex formation of the active "*H*-*SR*" group with metal ions of metal-containing enzymes [24–25]. In addition, the "*H*-*SR*" species can also act as a chelating agent

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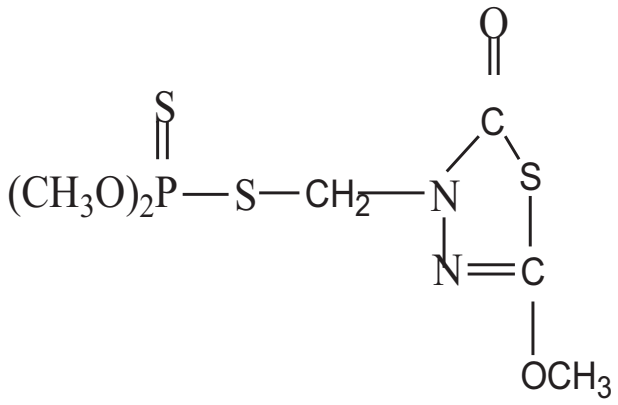


Schematic 1. Molecular structure of methidathion.

for various metal ions [M^{n+}] such as Fe^{2+}, Mn^{2+}, Cu^{2+}, Ni^{2+}, Hg^{2+} and Bi^{3+} to form coordination complexes [25–28]. The toxicity is significantly enhanced if M^{n+}-SR complex can act as a fungicide, such as in the case of ziram: Zn^{+2}-$(SR)_2$ [25–27].

Especially, the interaction of the S-containing molecules with Hg^{2+} ions has drawn the continuous attention of scientists over the past 50 years. The pioneering works of Kolthoff and Stricks [29] have contributed to the fundamental understanding of the physical chemistry of the interaction of cysteine/cystine with Hg [29]. The reaction of cystine at the Hg-electrode was comprehensively discussed by Heyrovský et al. [30]. Gregg and Tyler [31] paved the way for the polarography of dithiocarbamates compounds. Several studies have examined the electrochemical behavior of the cystine and cysteine on mercury electrodes and confirmed the considerable influence of pH, supporting electrolyte as well as of the concentrations of cystine/cysteine on the double layer properties at the mercury/solution interface [28,32–34].

A common denominator in their action is the cell membrane activity. Their adsorption on the biological membranes can determine the surface activity of these molecules [24,35]. In a more general context, surface adsorption is widely recognized as a regulatory physicochemical mechanism for the interaction of pesticides with surfaces such as biological membranes [36], or the hanging mercury drop electrode surface (HMDE) [37].

Nowadays, among the various electrochemical techniques, the Electrochemical impedance spectroscopy (EIS) [38] and the square cathodic stripping voltammetry (SW-CSV) using a HMDE [22,39] can be used to determine the electroactive groups of molecular structures as well as to simulate electrodynamically the adsorptive ability of cellular membranes [2,40].

More precisely, the EIS is an in-situ non-invasive analytical technique for characterizing electrochemical systems [38,41]. This technique measures the impedance of the concerned electrochemical system over a range of frequencies. The EIS technique has been used for investigating membrane microstructure, membrane fouling and immediately changes that occurred by osmosis process on the zeolite membrane surface [41] and catalytic activity of cysteine and cystine on the electroreduction of Bi(III) ions [28].

On the other hand, the SW-AdCSV technique [39] has been widely used in environmental and biological samplings [2,42] to obtain important information about the fate of agro-pharmaceuticals near the cellular membranes. Its function is based on the application of a fixed voltage between −100 mV and −800 mV, targeted at the redox of the electroactive species, such as dimethyldithiocarbamate pesticides, that have been adsorbed on its surface [37]. Electrons flowing between the adsorbed electroactive species and the electrode surface are expressed in current (measured in nanoamperes) that corresponds with these species in solution [39]. Electroactive species move towards the electrode surface by diffusion, while they are adsorbed via electrostatic interactions [39,43–44]. In previous works, the HMDE has been widely used as a model surface for the cellular membrane in order to study the adsorption of the drug mitomycin [45], of polyphenolic molecules with radical activity [46] and of imidacloprid [2] on the electrode surface.

Additionally, the HMDE has been employed for the analytical determination of dithiocarbamate pesticides, including ziram and thiram. They were addressing topics such as the polarographic determination of thiram [47], the analytical determination of ziram by anodic stripping voltammetry [48] and the analytical measurement of thiram by cathodic stripping voltammetry [49]. A detailed study of the thermodynamics of adsorption for two representative dimethyl-dithiocarbamate pesticides on the HMDE showed a cleavage of the disulfide S—S bond of the thiram near the HMDE at around −550 mV and a strong adsorption of the dithiocarbamate products onto electrode surface [37,50].

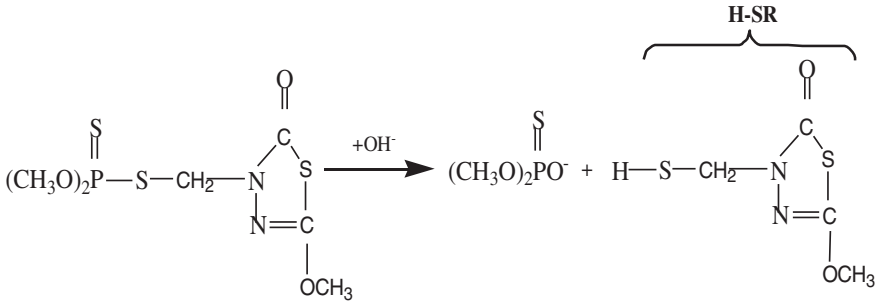
According to the above, the methidathion upon its application in the field undergoes gradual alkaline hydrolysis, depending on soil's pH (Schematic 2). So far, the effect of methidathion alkaline hydrolysis and its by-products have not yet been tested for their possible side effects.

In the present study, the SW-AdCSV voltammetric technique using a HMDE as simulator of cellular membrane and the CBMN assay were used in order to study the potential genotoxic effects of methidathion and its hydrolysis by-products in cultured human lymphocytes.

2. Materials and methods

2.1. Reagents and solution

All the experiments were performed with analytical grade chemicals. Methidathion (Riedel-de Haën 45572, purity > 99.7%, CAS-Number: 950-37-8) was used without further purification. Stock solution of methidathion (Water Solubility: 240 mg/l) was prepared with ultrapure Milli-Q water at a concentration of 50 μg/ml [18] at



Schematic 2. Reaction scheme on methidathion hydrolysis by Eto [21].