10th INTERNATIONAL SCIENTIFIC CONFERENCE
ON DEFENSIVE TECHNOLOGIES
OTEH 2022
Belgrade, Serbia, 13 – 14 October 2022

DIMETHOATE AND OMETHOATE HYDROLYSIS IN AQUEOUS SOLUTIONS AND THE ASSESSMENT OF THEIR NEUROTOXIC EFFECTS

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Abstract: *Organophosphates are widely used nowadays. They have applications as pesticides, drugs, plasticizers, flame retardants or chemical warfare agents. Their acute toxicity is ascribed to the inhibition of acetylcholinesterase, a key enzyme in the transmission of nerve impulses in animals. Their toxic effects manifest by acetylcholine accumulation in the nerve synapses and can lead to paralysis or death. Dimethoate, a systemic and contact organophosphate insecticide, has been registered for use since 1962. Its oxo-analog omethoate also can be found in the environment due to oxidation. Under environmental conditions, dimethoate and omethoate undergo chemical transformations and decomposition. However, systematic data about dimethoate and omethoate hydrolysis are scarce. We systematically analyzed dimethoate and omethoate hydrolysis under different pH conditions and estimated their neurotoxic effects. Dimethoate and omethoate hydrolyzed fast in alkaline aqueous solutions (half-lives 5.7±1.4 and 0.89±0.21days) but were stable in acidic solutions (half-lives 124±18 and 104±9 days). The toxicity of these pesticide solutions decreases over time, indicating that more toxic products were not formed.*

Keywords: *dimethoate, omethoate, pH stability, toxicity.*

1. INTRODUCTION

Dimethoate (DMT, Figure 1a) is an organophosphorus pesticide (OP) with contact and systemic action. It is in use against many insects in the agriculture and the housefly's control. DMT is known for its moderate toxicity to mammals. The US EPA classified it as a possible human carcinogen based on tumor occurrence in exposed mice [1]. Like other OPs, the acute toxicity of DMT is caused by its inhibition of acetylcholinesterase (AChE) [2]. The oxidation of DMT leads to the formation of its oxo-analog, omethoate (OMT, Figure 1b), which is more toxic to acetylcholinesterase than the respective parent compound. Besides it is one of the metabolites of DMT, OMT may also be found in the environment due to different oxidizing agents in water and soil [2]. Therefore, it is important to have a thorough understanding of the environmental fate of DMT and its analog OMT to mitigate the negative impacts on the environment and their non-target species.

Due to the high water solubility and low soil persistence of DMT, its potential to runoff into surface waters and leach into groundwater is high [3]. At the same time, it is not expected to adsorb to suspended solids and sediment [4].

The most important degradation pathways of DMT in the environment are hydrolysis, photolysis, and microbiological degradation [3]. The photocatalytic oxidation and microbial metabolism of DMT often have OMT as the final product, which is not desirable due to its extreme toxicity [5]. On the other hand, hydrolytic degradation is the main inactivating pathway of DMT in the environment and typically gives no OMT as the final product [6]. The hydrolysis of DMT is mostly dependent on the temperature and pH [7].

Thus, the aim of the present work is to investigate the kinetics of DMT and OMT hydrolysis in aqueous media in the pH range from 3 to 9 and explain the observed trends. In addition, the present work thoroughly addresses the evolution of toxicity of DMT and OMT solutions over time, which is essential for tailoring efficient strategies for DMT and OMT removal from water. Toxicity is assessed using AChE inhibition measurements to provide a broader context of the work and fill the identified gap in the literature. Namely, AChE inhibition is a common biomarker for environmental monitoring [8] which targets the overall eco-neurotoxicity of cholinesterase-inhibiting compounds.

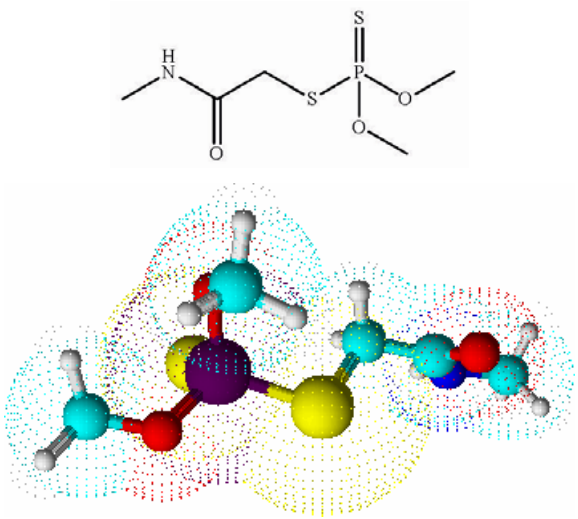

Figure 1a. The structure of DMT.

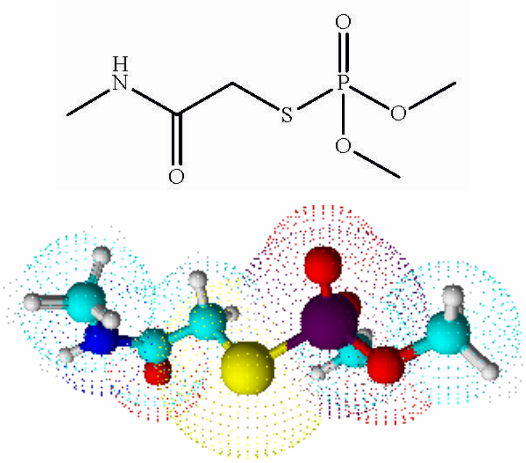

Figure 1b. The structure of OMT.

2. EXPERIMENTAL

2.1. Chemicals

Acetylcholinesterase from electric eel (AChE), acetylthiocholine iodide (ASChI), and 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) were purchased from Sigma-Aldrich St. Louis, MO, USA. Potassium-hydrogen phosphate ($K_2HPO_4 \cdot 3H_2O$) and acetonitrile were purchased from Merck KgaA, Germany. DMT and OMT (>98% purity) were purchased from Pestinal®, Sigma-Aldrich, Denmark. The pesticide working solutions were prepared by diluting the 1×10^{-1} mol dm^{-3} stock solutions in water. The pesticide stock solutions were held in the refrigerator until used. All chemicals were used without further purification. Deionized water was used throughout.

2.2. Stability of dimethoate and omethoate in aqueous buffer solution

The stability of DMT and OMT on different pH was investigated in 50 mmol dm^{-3}phosphate buffer solution (made using deionized water) with pH ranging from 3 to 9. The solutions of 1×10^{-4} mol dm^{-3} OPs were incubated at a temperature of 25 °C and 35 °C in a laboratory orbital shaker-incubator (Orbital Shaker-Incubator ES-20, Grant-bio) for 10 days. The concentrations of investigated OPs were measured as described below in aliquots taken at relevant time points. In addition, the decomposition of 1×10^{-4} mol dm^{-3} DMT and OMT is analyzed in spiked tap water samples in order to link the results with a realistic scenario. All the measurements were done in triplicate, and the uncertainties were propagated using the least significant differences test (LSDs) at a 95% significance level.

2.3. UPLC analysis

For measuring the concentration of DMT and OMT, Waters ACQUITY Ultra Performance Liquid Chromatography (UPLC) system, coupled with a tunable UV photodiode array (PDA) detector controlled by the Empower software, was used. Chromatographic separations were run on an ACQUITY UPLC™ BEH C18 column with the dimensions 1.7 µm, 100 mm × 2.1 mm (Waters). DMT and OMT solutions were analyzed under isocratic conditions with a mobile phase consisting of 10% acetonitrile and 90% water (v/v). The eluent flow rate was 0.25 cm^3 min^{-1}, and the injection volume was 5 mm^3. Optical detection for both OP was done at 200 nm. Under described conditions, retention times of DMT and OMT were (2.65±0.05) min and (1.12±0.05) min, respectively. DMT and OMT concentrations in the analyzed samples were determined using the linear calibration curves constructed using standard pesticide solutions in a wide concentration range. The described method was previously optimized and cross-validated using the in-house developed protocols and, as such, used in this and our previous works on DMT/OMT determination.

2.4. Neurotoxicity of DMT and OMT solutions over time

AChE inhibition measurements were performed to follow and quantify changes in the toxicity of DMT and OMT and investigate if there are any transformations of OPs into more toxic forms upon hydrolysis at different pH. These transformation products could exert harmful effects at concentrations below the detection limits of UPLC. AChE activity was assayed according to modified Ellman's procedure. The method is described in detail in our previous work [9], and here we provide a description for completeness. The *in vitro* experiments were performed by exposure of 0.5 IU commercially purified AChE from electric eel to OP solutions obtained in adsorption experiments at 37 °C in 50 mmol dm^{-3} PB pH 8.0 (final volume 0.650 cm^3). The enzymatic reaction was started by adding acetylthiocholine-iodide (ASChI) in combination with DTNB as a chromogenic reagent and allowed to proceed for 8 min until stopped by 10% sodium dodecyl sulfate (SDS). The enzymatic reaction product, thiocholine, reacts with DTNB and forms 5-thio-2-nitrobenzoate, whose optical absorption was measured at 412 nm. It should be noted that in these measurements, the enzyme concentration was constant and set to give an optimal spectrophotometric signal. Physiological effects

were quantified as AChE inhibition given as:

$$inhibition_{AChE} = 100 \times \frac{A_0 - A}{A_0} \quad (1)$$

where A_0 and A stand for the AChE activity in the absence of OP and the one measured after the exposure to a given OP. DMT solutions of initial concentration 1×10^{-4} mol dm^{-3} were left in phosphate buffers (pH ranging from 3 to 9, 25 °C and 35 °C) and in tap water for ten days to monitor the toxicity of the spontaneous hydrolysis products.

3. RESULTS AND DISCUSSION

3.1. Stability of dimethoate and omethoate in aqueous buffer solution and decomposition over time

The concentration of DMT and OMT was monitored in tap water and phosphate buffers with pH ranging from 3 to 9, as described in Section 2.2. for 10 days using UPLC analysis.

The time dependence of DMT concentration is presented in Figure 2. It was shown that the spontaneous concentration decay at 25ºC (Figure 2a) and 35 ºC (Figure 2b) over time is rather fast in buffers with neutral and alkaline pH and tap water (pH 6.5). On the other hand, in buffers with acidic pH, the decrease of OPs concentrations was also noticeable but at a lower rate. Different rate orders for the hydrolysis process were checked. However, in all the cases, the decay of DMT concentrations fitted the best to the exponential one and followed the pseudo-first-order kinetics.

Hence, the hydrolysis rate constants (k_h) were obtained by direct fitting the experimental data into the equation:

$$C_t = C_0 e^{-k_h t} \quad (2)$$

where C_t and C_0 are the remaining OPs concentrations at a given time (t) and the initial OPs concentration.

The results presented in Figure 2 show that with increasing pH value, i.e., the basics of the solution increase the degradation efficiency nonlinearly. The half-life of dimethoate with a concentration of 1×10^{-4} mol dm^{-3} at pH 9 at 25 ° C is 8 days, and at 35 °C, it is 1.5 days, while in 9 days, it decomposes to (3.5±0.5)% of initial concentration. An increase in temperature of 10 °C accelerates degradation three times.

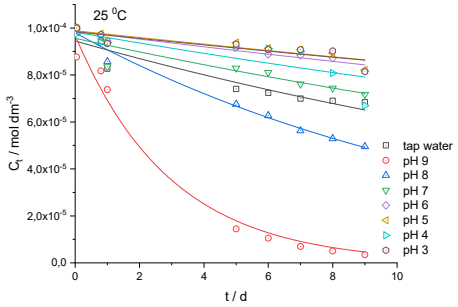

Figure 2a. Dependence of DMT concentration over 10 days at pH from 3 to 9 (25 °C).

Less efficient degradation to pH 8 at 35 ° C shows that the half-life is 8.5 days, which means that reducing the pH by 1 increases the degradation length by 3.5 times. It applies to reducing the pH from 9 to 8. For other pH changes, the degradation takes more time.

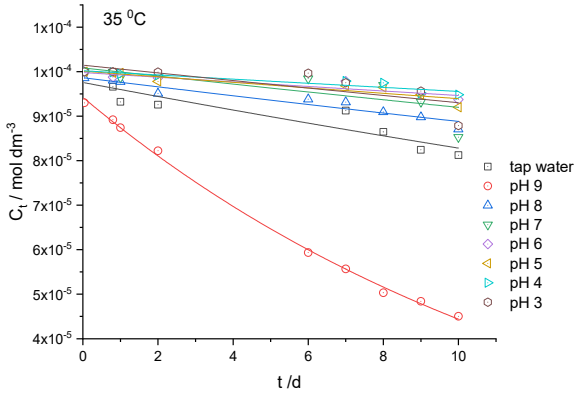

Figure 2b. Dependence of DMT concentration over 10 days at pH from 3 to 9 (35 °C).

The obtained hydrolysis rate constants for DMT and OMT were further used to determine the half-life ($t_{1/2}$) of these OPs under the given experimental conditions. As a result, the half-lives were estimated as:

$$t_{1/2} = \frac{\ln 2}{k_h} \quad (3)$$

The results are presented in Figure 3.

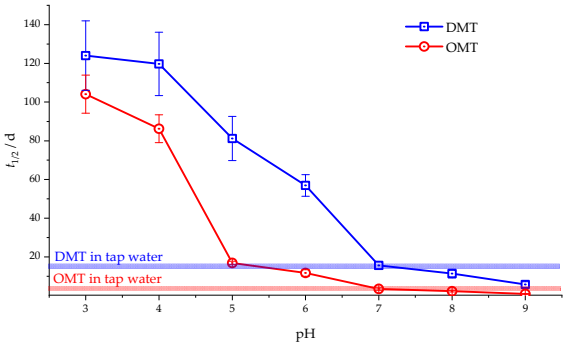

Figure 3. Estimated half-lives ($t_{1/2}$) for DMT and OMT in aqueous solutions as a function of pH (25 °C). The half-lives for spiked tap water are indicated using horizontal lines.

3.2. Neurotoxicity of DMT and OMT solutions over time

In this work, we are primarily interested in reducing DMT solutions toxicity and confirming that no OMT is formed, while the hydrolysis products are not specifically identified. The toxicity of DMT solutions was estimated via the AChE inhibition test as described in Section 2.4. The aliquots for AChE inhibition testing were taken at the start and after 1, 2, 6, and 10 days. The results are given in Figure 4. Toxicity measurements data showed that there is no formation of more toxic products during hydrolysis under the given experimental conditions in all cases.

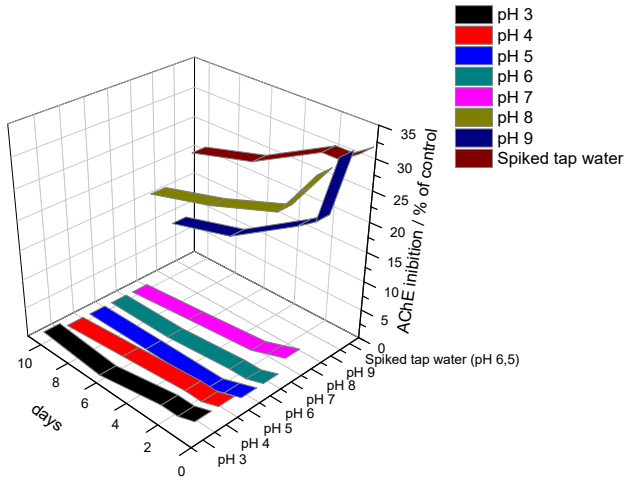

Figure 4. Toxicity of DMT solution over 10 days at pH from 3 to 9. The initial concentration of DMT was 1×10^{-4} mol dm^{-3} (25 °C)

5. CONCLUSION

DMT and OMT hydrolyze at different rates, and OMT hydrolysis is faster than the hydrolysis of DMT. During hydrolysis, there is no accumulation of OMT, which is much more toxic than DMT. As the rate constants of DMT and OMT hydrolysis rapidly increase with pH in alkaline media, it is suggested that alkaline hydrolysis is a suitable way to remove DMT from water. Namely, in contrast to some cases of microbial degradation and photocatalytic oxidation, alkaline hydrolysis does not lead to the accumulation of more toxic products during the degradation process. Hence, if alkaline hydrolysis is used for DMT (and OMT) removal, no special care should be taken to monitor the degradation process as the risk for the formation of toxic products is minor. The matrix effects in tap water were found to have a negligible impact on DMT and OMT hydrolysis rate, so the presented data can be safely used to estimate DMT and OMT half-lives in contaminated water. Toxicity measurements data showed that there is no formation of more toxic products during hydrolysis under the given experimental conditions in all cases. Moreover, the toxicity data can be used to evaluate acute toxicity upon water contamination, measured as the AChE inhibition. The estimations for longer periods (beyond ten days) can be done using the combination of the stability data and the AChE inhibition curve for DMT. However, the results should be used with care, and it is suggested that general systematic work on OPs stability assessment is needed.

Acknowledgments

The research was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

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