Chapter 7

Toxic Effects of the Organophosphorus Insecticide Fenthion on Growth and Chlorophyll Production Activity of Unicellular Marine Microalgae *Tetraselmis suecica*: Comparison between Observed and Predicted Endpoint Toxicity Data

Maria C. Vagi, Andreas S. Petsas, Maria D. Pavlaki, Niki M. Smaragdaki and Maria N. Kostopoulou

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.72321

Abstract

This chapter provides the results of a laboratory ecotoxicological study conducted to assess the acute toxicity of the organophosphorus pesticide fenthion toward the marine microalgal species *Tetraselmis suecica*. Bioassays were performed, and algal densities and chlorophyll pigments fractions were measured in the exponential phase after 96 h of exposure to fenthion. Two quantitative structure activity relationships (QSARs) were used to estimate the toxicity of 13 primary metabolites and degradation products of fenthion toward the selected organism; the first was based on the use of the n-octanol/water partition coefficient, whereas the second was based on the solubility of the compound in water. Results revealed that fenthion can have marked effects on the growth and photosynthesis of the target primary producers of marine ecosystems *T. suecica*. The parent pesticide toxicant was found not toxic to the tested algal species up to 1.00 mg L^{-1}, while higher treatment concentrations not only affected algal densities and significantly decreased specific growth rate values (μ) ($p < 0.05$) but also decreased the contents of photosynthetic pigments. The comparison between the observed and the predicted toxicity values of the parent compound fenthion indicated that the predictive capability of the QSARs applied can be considered highly satisfactory. Consequently, both QSAR models were used for the prediction of toxicity data of fenthion's principal metabolites and degradation products.

Keywords: fenthion, *Tetraselmis suecica*, toxicity test, ecotoxicology, pigment biomarker, QSARs

1. Introduction

Fenthion (*O,O*-dimethyl *O*-(4-(methylthio)-*m*-tolyl) phosphorothioate) is a contact and stomach systemic organophosphorus pesticide, used as a wide-spectrum insecticide for numerous crops against many suckings and biting pests. It was developed in 1960 and first commercialized by Bayer Agriculture in the USA as an insecticide/acaricide for mosquito and insect control that is commercially available worldwide in several formulations [1].

In Greece, only one formulation of fenthion was registered by Bayer CropScience Hellas, with the trade name LEBAYCID 50% EC (containing 50% w/v fenthion as the active ingredient), which is classified as dangerous for humans, but is not classified for aquatic organisms [2]. This insecticide is extremely effective in controlling the major insects infecting olives, such as the olive fruit fly *Bactrocera oleae (Dacus)*, the olive kernel borer or olive moth *Prays oleae*, the black scale *Saissetia oleae (Olivier)*, and *Margaronia* sp. and other masticatory insects. Although *B. oleae* is considered the most serious insect, all aforementioned insects are widely distributed in the Mediterranean region and occur on olives at population densities causing important economic losses. Therefore, fenthion was for many years one of the most commonly used pesticides in Greek territory and in the Mediterranean area generally [2].

The available information on the production and use of pesticides in general and hence of organophosphates as well is limited, fragmentary and in some cases unreliable [3]. On the basis of the limited information received from the Mediterranean countries, fenthion was one of the important compounds used during the 1980s and 1990s among other organophosphorus pesticides [3]. According to data provided by the Greek Ministry of Rural Development and Food, it appears that the quantities of fenthion that were used for agricultural purposes during the years 1983, 1984, 1985, 1986, 1987, 1988, and 1989 in Greece were 216,892; 409,139; 24,359; 197,843; 87,787; 160,433; and 213,514 tons of active ingredient, respectively [3].

Since June 2007, fenthion is no longer approved by the Greek Ministry of Rural Development and Food because of an excess number of poisoning-related events and ecotoxicology effects on nontarget organisms (Greek Ministry Decision, Register Number 122914–27/4/2005, 2005), apart from its 120 days of exceptional authorization (from May 1, 2009 to August 31, 2009) in accordance with Art. 8(4) of Directive 91/414/EEC for the treatment of olive trees against *Dacus oleae* (Greek Ministry Decision, Register Number 128569–11/5/2009c IN, 2009).

Although fenthion was developed as a safe pesticide because it is not easily converted to the possibly highly toxic oxon derivative (fenthion oxon) in mammalian species, however according to relative literature, many of its metabolites were detected in various plants, animals, and environmental matrices [4–7]. Kitamura et al. demonstrated that the in vivo metabolism of fenthion in fish leads to the formation of two metabolites, fenthion sulfoxide and fenthion oxon [4], while other studies proved that fenthion and its oxidation products were accumulated in fish [5]. Oxidation products of fenthion, including fenthion oxon, were also detected in house mosquitoes exposed to fenthion [6]. It has also been reported that fenthion was converted to fenthion oxon in the aqueous environmental bodies [7]. On the contrary, the toxicity and the metabolism of this organophosphorus insecticide have not been extensively studied in aquatic microspecies, such as microalgae.

http://dx.doi.org/10.5772/intechopen.72321

Microalgae are important inhabitants of aquatic ecosystems, where they fulfill critical roles in primary productivity, nutrient cycling, and decomposition. Detrimental effects of pesticides on algae may have subsequent impacts on higher trophic levels [8]. It has been well established that changes in the macromolecular composition of phytoplankton species or shifts in community composition can affect the growth rate of zooplankton grazers [9]. Unquestionably, aquatic environments receive direct and indirect pesticide inputs, inevitably exposing microorganisms to pesticides. Millions of pounds of active pesticide ingredients are applied in coastal watersheds each year, and in addition, pesticides may affect marine inhabitants via spills, runoff, and drift [10].

Toxicity data involving ecotoxicology of fenthion toward nontarget microorganisms are limited. Most studies have focused on microbial degradation and biotransformation of fenthion rather than impacts on natural microbial populations and communities. Furthermore, studies of fenthion effects on soil microbes are far more common than studies of toxicology assessments in aquatic environments. Published data regarding marine or estuarine microorganisms are even scarcer [11].

The aims of the present survey were (i) to assess the acute toxicity of the organophosphorus insecticide fenthion toward nontarget aquatic microorganisms, such as marine algae, (ii) to investigate the possibility of using the parameter of chlorophyll pigments as biomarkers of exposure to fenthion, (iii) to compare the observed and predicted endpoint toxicity data and evaluate the predictive capability of two QSARs based on physicochemical properties of target organic toxicant (n-octanol/water partition coefficient and water solubility), and (iv) to predict the toxicity of 13 principal metabolites and degradation products of fenthion toward the selected marine microalgae.

2. Materials and methods

2.1. Organism and culture conditions

Tetraselmis is a genus of a marine, motile, green phytoplankton (Prasinophyceae) that has very high lipid levels and also contains natural amino acids that stimulate feeding by other marine animals [12]. For this reason, it is used as a food source for feeding marine crustaceans, especially shrimp and mollusks. *Tetraselmis suecica* (Kylin) Butcher [12] (formerly known as *Platymonas suecica*, by Kylin, [13]) is a free-living, flagellate species that was initially isolated from seawater of the English and Swedish coasts, but later research has suggested that it is probably cosmopolitan [14]. The unicellular marine microalga *T. suecica* that is used in the bioassays of the present study is a strain of phytoplankton that is commonly cultivated in shellfish husbandries [15] and has been routinely cultivated by our laboratory [16]. This species was chosen because it is easy to be cultivated [15], and its response in toxicity tests is highly reproducible [16].

Unialgal cultures of the species were maintained in liquid *f*/2 growth medium as recommended by Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP) [17]. More specifically, the selected strain of microalga was cultured in natural seawater, which had been

filtered through a 0.45 μm Millipore filter, autoclaved at 121°C for 20 min, and enriched with several nutrients, trace metals, and vitamins. The final concentration of each component in *f*/2 growth medium was $NaN0_3$, 0.882 mM; $NaH_2PO_4 * H_2O$, 36.2 μM; $N\alpha_2SiO_3 * 9H_2O$, 0.106 mM; $FeCl_3 * 6H_2O$, 11.7 μM; $Na_2EDTA * 2H_2O$, 11.7 μM; $CuSO_4 * 5H_2O$, 39.3 nM; $Na_2MoO_4 * 2H_2O$, 26.0 nM; $ZnSO_4 * 7H_2O$, 76.5 nM; $CoCl_2 * 6H_2O$, 42.0 nM; $MnCl_2 * 4H_2O$, 0.910 μM; thiamine HCl (vit. B_1), 0.296 μM; biotin (vit. H), 2.05 nM; and cyanocobalamin (vit. B_{12}), 0.369 nM. Stock solutions of these components were autoclave-sterilized except in the case of vitamins which were filter-sterilized by passing through a Millipore filter (0.45 μm) before supplementing to the growth medium. Salinity of the seawater was 35.0 ± 0.1%, and the initial pH of the cultures was 8.0 ± 0.1 [18].

One hundred milliliters of inoculated growth medium *f*/2 with *T. suecica* at a cell density of 1×10^5 cells mL^{-1} was contained in 250 mL flasks with air-permeable stoppers. The cultures were incubated under continuous illumination with cool-white fluorescent lights emitting a radiant energy of 4300 Lux equivalent to 12.9 W m^{-2}. Temperature was maintained stable in a temperature-controlled growth chamber (Snijders Scientific B.V., The Netherlands), at 20.0 ± 0.3°C. The test vessels containing the cultures during the course of the experiments were gently shaken by hand once per day in order to keep the cells in free suspension, to facilitate CO_2 mass transfer from air to water, and in turn to reduce pH shift. Hence, variations in pH during the 96 h of incubation were within the limit of ±1.0 unit. All glassware and mediums used were previously sterilized by autoclaving at 121°C for 20 min, and all handlings were made under aseptic conditions so as to avoid contamination from bacteria or other species of algae [16].

2.2. Test chemicals, reagents, and standards

The tested compound fenthion was an analytical grade (purity >99.5%), obtained from Dr. Ehrenstorfer-Schäfers (Augsburg, Germany) and used without further purification. Pure fenthion is a colorless, almost odorless liquid, while technical product of fenthion (95–98% pure) is a brown oily liquid with a weak garlic odor. Data for other physiochemical properties of fenthion, taken from reference [19], include melting point, 7°C; boiling point, 87°C at 0.01 mmHg; vapor pressure, 1.4 mPa at 25°C; water solubility, 55 mg L^{-1} (at 20°C and pH = 7); log $K_{ow,}$ 4.84; and M_r, 278.34. **Figure 1** shows the chemical structure of the target compound.

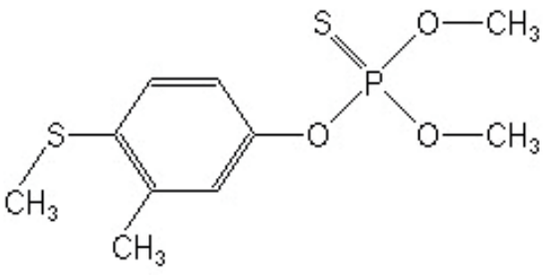

Figure 1. Chemical structure of fenthion.

Due to low water solubility of the tested substance, acetone was used for the preparation of its stock solutions. Hence, acetone was used as the carrier solvent of the compound to the bioassays, since previous experiments proved that this solvent up to a final concentration of 0.5 μL mL^{-1} in *f*/2 medium did not affect the growth rate of the tested algae [16]. Stock standard solutions of fenthion (1000 and 10,000 mg L^{-1}) were prepared by dissolving the required amounts in acetone (HPLC grade) and were stored under refrigeration.

Pesticide-grade organic solvents such as acetone, hexane, methanol, and dichloromethane were purchased from Pestiscan (Labscan Ltd., Ireland). Organic-free water was prepared with a Milli-Q/Milli-Ro system (Millipore Corp., Bedford, USA). Other chemical reagents and solvents used were of HPLC grade and procured from Merck (Merck, Germany).

2.3. Procedure for the study of the stability of fenthion

The stability of fenthion in seawater was determined under the experimental conditions employed for the incubation of the cultures. Therefore, parallel experiments were performed without algae using all six test concentrations that were chosen for the toxicity treatments (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg L^{-1}) and prepared in 0.45 μm GF/F-filtered natural seawater. Triplicate samples were prepared in 250 mL borosilicate flasks, and each contained 100 mL of pesticide solution. At various time intervals (0, 24, 48, 72, and 96 h, as for the toxicity tests), 5 mL aliquots of the aqueous reaction solutions were withdrawn for analysis.

Fenthion concentrations were quantitatively confirmed by gas chromatography analysis after liquid–liquid extraction of the fortified aqueous samples with hexane (2 × 5 mL). Organic extracts were dried over anhydrous sodium sulfate, and 1 μL of the extracts was injected into gas chromatographic system (Hewlett-Packard 5890) equipped with a nitrogen-phosphorus detector (GC-NPD). A 30 m × 0.32 mm i.d. × 0.25 film thickness fused silica-bonded phase capillary column (MDN-5, Supelco, USA) was used for the chromatographic separation of target analyte and oven temperature programmed at 150°C for 3 min; increased from 150–170°C at 20°C min^{-1}; then increased from 170–190°C at 2°C min^{-1}; after that increased from 190–250°C at 15°C min^{-1}; and was held to 250°C for 15 min. Helium was used as the carrier gas at constant flow of 1.2 mL min^{-1} during GC analysis. Injection technique was on column. Detector's temperature was 280°C, while hydrogen and air were used as NPD's airs with flows of 3.5 and 110 mL min^{-1}, respectively.

2.4. Acute toxicity test and pesticide treatment

Bioassays were performed according to the OECD Guideline 201 for testing the effects of chemicals on alga growth inhibition test [18], with some modifications. Cells in the exponential phase of growth were collected from stock cultures (called as pre-cultures and incubated under the previously mentioned conditions) and for this reason used as the inoculum. The initial algal density in each one of the experimental treatments was of 1×10^5 cells mL^{-1} [16].

The experimental design and test conditions were identical for all replicates performed. Each chemical bioassay included the below-described treatments: a control (C) containing no pesticide; a control containing acetone as carrier solvent of the organic toxicants, in concentration

0.05% (C + A); and various toxicant exposure concentrations of fenthion (in mg L^{-1}), following the results of preliminary range-finding experiments conducted for the tested compound previously [16]. Algae were exposed to the concentration series of 0.50, 1.00, 1.50, 2.00, 2.50, and 3.00 mg L^{-1} of fenthion, respectively. Each treatment contained three replicate flasks. The environmental conditions during the experiments were the same as the growth conditions stated in paragraph 2.1.

Cell densities were assessed daily (after 0 h, 24 h, 72 h, and 96 h of incubation) by microscope counting using an improved Neubauer hemocytometer. Lugol solution was added to the samples (ratio of lugol/culture media, 1/10 v/v) to prevent the natural movement of *T. suecica* cells. Specific growth rate (μ), which is the proportional rate of increase in cell density per unit of time, was calculated for each treatment sample according to Eq. (1):

$$\mu = \frac{\ln N_t - \ln N_o}{t - t_o} \tag{1}$$

where t_o is the time of test start (t_o = 0 d) and t is the time of test termination (t = 4 d), while N_o and N_t are the initial and final cell densities at times t_o and t, respectively [20]. Inhibition of algal growth as a reduction in specific growth rate, relative to control cultures grown under identical conditions (%I), was estimated from the relationship (2):

$$\%\text{Inhibition} = \%I = \frac{\mu_{control} - \mu_{pesticide}}{\mu_{control}} \times 100 \tag{2}$$

where $\mu_{pesticide}$ is the algal growth rate in the presence of the tested compound and $\mu_{control}$ is the growth rate in the untreated control. The EC_{20} and EC_{50} values (pesticide concentration required to cause a 20 and 50% reduction in growth, respectively) were calculated using linear regression analysis of transformed pesticide concentration as logarithm data versus percentage inhibition. Furthermore, the no-observed-effect concentration (NOEC) was defined as the highest tested concentration of fenthion below which no reduction in reproduction was observed after a 96-h exposure period, while the lowest-observed-effect concentration (LOEC) was defined as the lowest tested concentration of fenthion at which reduction of algal growth was observed after a 96-h exposure period. The maximum acceptable toxicant concentration (MATC) was defined as a hypothetical threshold concentration that was calculated as the geometric mean between NOEC and LOEC concentrations.

The contents of acetone-soluble chlorophyll pigments, chlorophyll-a (Chl_a), chlorophyll-b (Chl_b), and chlorophyll-c (Chl_c), contained in 10 mL of culture medium at the end of incubation (96 h), were determined according to the spectrophotometric method described in detail by Strickland and Parsons [21].

2.5. Prediction of toxicity values of primary metabolites and degradation products of fenthion: Quantitative structure activity relationships (QSARs)

Two structure-toxicity relationships have been proposed by Vagi [22] for the growth inhibition of the marine microalga *T. suecica* exposed to various organophosphates such as dimethoate, parathion methyl, parathion ethyl, and its oxidative metabolite paraoxon ethyl. These are described by Eqs. 3 and 4:

$$\log(1/EC_{50}) = 0.5415 \log P_{OW} - 2.6499, \text{ with correlation coefficient } R^2 = 0.9689 \quad (3)$$

$$\log(1/EC_{50}) = -0.6367 \log S + 0.5338, \text{ with correlation coefficient } R^2 = 0.9094 \quad (4)$$

where P_{OW} is the n-octanol/water partition coefficient and S is the solubility of the compound in water at 20°C in mg L^{-1}. P_{OW} characterizes the lipophilicity of the molecule and quantifies its tendency to partition between water and suspended solids, its partitioning and uptake into biota (bio-concentration) as well as its adsorption to sediments; thus, log P_{OW} is considered to be a parameter describing the kinetics of uptake of chemicals from water. On the contrary, S value encodes quantitative information on the hydrophilicity of the compound and comprises the inclination of the chemical to remain into the aqueous phase.

Since the experimental determination of log P_{OW} and S values can be impractical and time-consuming, accurate and straightforward methods for the determination of this important property are available. As it is well known, computational chemical methods, which only require the chemical structure of the molecule, are one of the most famous and useful approaches to estimate several physicochemical properties such as log P_{OW} and S values. In the absence of a complete set of reliable experimental values and in order to obtain homogeneous values, the hydrophobicity and hydrophilicity of degradation products of parent compound fenthion, expressed as log P_{OW} and S values, respectively, were calculated according to the available scientific prediction methods provided by Virtual Computational Chemistry Laboratory (VCCLAB) by using the ALOGPS 2.1 ++ logP/logS calculation software program [23].

2.6. Data reliability and statistical analysis

Independent experiments were repeated three times, and each sample (treatment and/or control culture) was repeated three times. Mean values ± standard deviations (SD) are shown in the figures, and tables are presented in this chapter. Data collected were calculated as percentages, and arcsine was transformed (arcsine $\sqrt{x}$) and analyzed using one-way analysis of variance (ANOVA). Variances were considered equal ($p > 0.05$) based on Kolmogorov–Smirnov test for homogeneity of variance. The highest concentration of toxicant demonstrating no effect as compared to the controls was estimated by Dunnett's test for statistical significance ($p > 0.05$) with SPSS software program.

3. Results

3.1. Fenthion stability

Experimental data of the present study concerning the stability of fenthion in 0.45 μm GF/F-filtered seawater during 96 h of exposure to illumination of cool-white fluorescent lights (4300 Lux or 12.9 W m^{-2}) and at 20.0 ± 0.3°C are summarized in **Table 1**.

Treatment concentration (mg L^{-1})	Remaining pesticide (%) over time				
	0 h	24 h	48 h	72 h	96 h
0.50	98.88	96.64	95.74	92.76	91.95
1.00	98.55	97.17	95.81	94.47	93.14
1.50	97.48	96.94	95.54	93.33	93.25
2.00	98.23	97.65	95.09	94.87	92.79
2.50	100.76	97.75	96.02	95.18	93.62
3.00	102.98	99.36	97.45	94.63	92.89

Values are the percentage (%) of the nominal concentration remaining and represent the means of triplicate tests.

Table 1. Fenthion loss in 0.45 μm GF/F-filtered seawater during 96 h of illumination of cool-white fluorescent lights (4300 Lux or 12.9 W m^{-2}) and at 20.0 ± 0.3°C.

It must be mentioned that the loss of the target organophosphorus toxicant from solutions over 96 h was similar in all three replicates (data not shown), and the mean values of triplicates are presented. It is obvious that the initial test concentrations (0 h) were between 97.48 and 102.98% of the nominal concentrations, while after 96 h of exposure (under 4300 Lux and at 20 ± 0.3°C), concentration of fenthion in seawater had reduced to between 91.95 and 93.62% of the nominal concentrations. This percentage of loss (less than 10%) is in accordance with the condition set by the OECD for the validity of the test that requires no more than 20% of the test chemical to be lost during the conducted toxicity test [18].

3.2. Toxicity of fenthion on growth of marine alga *T. suecica*

Algae exhibit several responses to toxicants, including growth inhibition and stimulation and morphological and physiological changes [24]. During the performance of present bioassays, the algal cells changed morphologically when treated with fenthion and observed under the optical microscope. Changes in cell shape, color, and size were observed as some cells became darker in color and in other cases were swollen as well. Moreover, many cell divisions were found abnormal because when the material cell divided, the descendant cells remained attached and the daughter cells were not separated. Thus, usual algal aggregations could also be observed. The above phenomenon indicated that fenthion could have been a potential of mutagenic effects on *T. suecica*. This was consistent with the phenomena observed by Li et al. while studying the effect of the synthetic pyrethroid cypermethrin on *Scenedesmus obliquus* [25], but to our knowledge, it has not been reported as a toxic effect of any other organophosphorus pesticide on algal toxicity tests.

Cultured in different concentrations of fenthion, the algal growth curves of T. suecica are shown in **Figure 2(a–f)**. The results contained in these charts indicated that cells in fenthion-treated medium grew slower than those in control group.

Furthermore, the mean specific growth rates (μ) of target alga when exposed to the range of concentrations of the tested toxicant for the three replicated bioassays conducted are summarized

http://dx.doi.org/10.5772/intechopen.72321

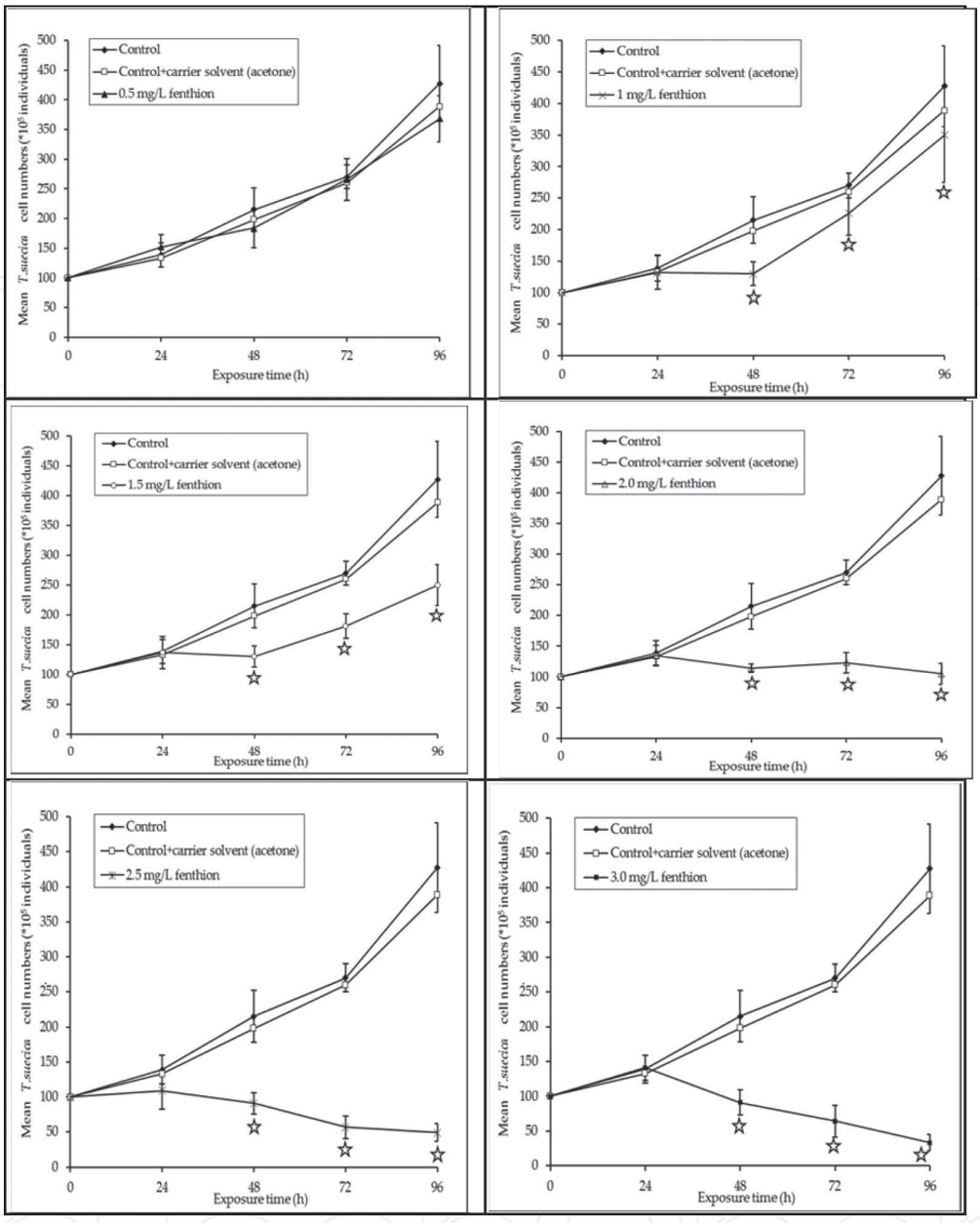

Figure 2. Effects of different concentrations of fenthion on growth of *Tetraselmis suecica*. [error bars represent standard deviations of three replicates, ☆significantly different as compared to the controls ($p < 0.05$)].

in **Table 2**. As it can be seen in these results, acetone controls, containing the carrier solvent, did not differ significantly from blank controls. On the contrary, it is observed that the organophosphorus toxicant consistently inhibited the algal population growth in concentrations from 0.50 to 3.00 mg L^{-1}, and the specific growth rate became remarkably lower with the increase of fenthion concentration, which demonstrated that fenthion can inhibit growth of *T. suecica* at the concentration range tested. Obtained values of μ indicated that significant inhibition of the algal densities occurred in treatment levels of fenthion above 1.00 mg L^{-1}. Pesticide concentrations of 1.50, 2.00, 2.50, and 3.00 mg L^{-1} significantly reduced ($p < 0.05$) *T. suecica* densities after 96 h of exposure. Severe reduction in growth occurred at concentration 2.00 mg L^{-1}, while 2.50 and 3.00 mg L^{-1} were found to be lethal.

Treatment concentration		μ^a (d^{-1})
(mg L^{-1})	(μmol L^{-1})	
Control	Control	0.58 ± 0.04
Control + carrier solvent (acetone)	Control + carrier solvent (acetone)	0.54 ± 0.04
0.50	1.80	0.37 ± 0.03
1.00	3.59	0.34 ± 0.01
1.50	5.39	0.33 ± 0.02*
2.00	7.19	0.31 ± 0.03*
2.50	8.98	0.23 ± 0.04*
3.00	10.78	0.01 ± 0.04*

[a]Based on algal densities.

*Significantly different as compared to the controls ($p < 0.05$).

Values are means ± standard deviation of three replicates.

Table 2. Specific growth rate (μ) of *T. suecica* after 96 h of exposure to treatments of fenthion.

Similar results were found by other authors who tested the influence of the organophosphorus insecticide fenitrothion on *Nannochloris oculata* and reported that treatment concentrations higher than 1.00 mg L^{-1} affected algal growth, whereas μ values decreased significantly by concentrations 5.00, 10.00, and 15.00 mg L^{-1} [26].

Using the toxicity data contained in **Table 2**, an estimate of NOEC and LOEC values would be 1.00 and 1.50 mg L^{-1}, respectively, while MATC calculated as the geometric mean between the NOEC and LOEC was estimated to be 1.22 mg L^{-1}. The experimental results of the present study confirmed that fenthion is slightly less toxic toward the target marine microalgae than it was previously reported as the values of NOEC, LOEC, and MATC were reported to be 0.50, 1.00, and 0.70 mg L^{-1}, respectively [16]. The percentage of inhibition data relative to growth in untreated controls (%*I*) was calculated according to Eq. (2).

Figure 3a shows the concentration-response curve of fenthion to *T. suecica,* which obviously corresponded to typical and characteristic sigmoid form (S-shape). Obtained toxicity values of %*I* were linearly related to transformed pesticide concentration values by logarithmic conversion (logC), and the plotted log transformation of the "concentration effect" line is presented in **Figure 3b**. The S-shape is again evident, but the curve approaches a straight line, and a linear portion of the curve is obvious and presented in **Figure 3c**.

The linear regression equation that was derived from this linear part of the curve is described by Eq. (5):

$$\%I = 181.26 \log C + 17.01, \text{ with correlation coefficient } R^2 = 0.9778 \quad (5)$$

where %*I* represents the percentage inhibition ($0 \leq \%I \leq 100$) and C is the pesticide concentration (in mg L^{-1}). High value of correlation coefficient showed that data fitted satisfactorily to the linear model.

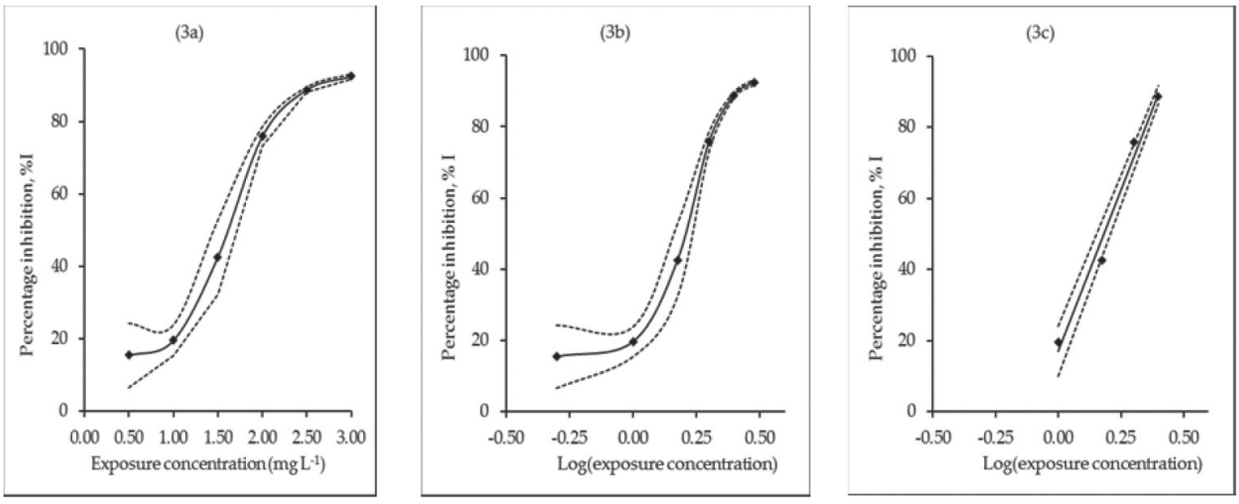

Figure 3. Concentration-response curves of fenthion on growth of *Tetraselmis suecica*. (a) Percentage inhibition versus concentration. (b) Percentage inhibition versus logarithm transformation of concentration. (c) Linear portion of percentage inhibition versus logarithm transformation of concentration. (Dotted lines on each side of the curve represent the 95% confidence limits).

Acute toxicity values of EC_{20} and EC_{50} (in mg L^{-1}) at 96 h were obtained by the above-described relationship (5), and the calculated data are presented in **Table 3**.

EC_{50} values of target compound estimated in the present work are in accordance with toxicity data reported in the literature for the same toxicant toward other green algal species, such as *Scenedesmus subspicatus* (EC_{50}, 1.79 mg L^{-1}) [19] and *Kirchneria subcapitata* (EC_{50}, 1.1 mg L^{-1}) [27]. A large number of ecological toxicity data of fenthion toward several nontarget aquatic organisms are available in published literature. Reported toxicity values indicated that fenthion is moderately toxic to estuarine and marine fish (LC_{50} for *Cyprinodon variegatus*, 1200 μg L^{-1} [27]), moderately highly toxic to freshwater fish on an acute basis (LC_{50} for *Oncorhynchus mykiss*, 0.83 mg L^{-1} and for *Lepomis macrochirus*, 1.7 mg L^{-1} [27]), very highly toxic to estuarine and marine invertebrates (LC_{50} or EC_{50} for *Crassostrea virginica*, 321 μg L^{-1} and for *Americamysis bahia*, 0.22 μg L^{-1} [27]), very highly toxic to freshwater invertebrates on an acute basis (EC_{50} for *Daphnia magna*, 5.2 μg L^{-1} [27]), and finally moderately toxic to nontarget aquatic plants such as marine and freshwater diatoms (EC_{50} for *Skeletonema costatum*, 0.4 mg L^{-1} and for *Navicula pelliculosa*, 1.0 mg L^{-1} [27]).

3.3. Toxicity of fenthion on chlorophyll pigment production of marine algae *T. suecica*

T. suecica Kylin (Butch) is an algal strain that its mass and biochemical composition, mainly in protein, chlorophyll-a, and RNA content, have shown great variabilities, which are related

EC_{20} 96 h		**EC_{50} 96 h**	
(mg L^{-1})	**(mol L^{-1})**	**(mg L^{-1})**	**(mol L^{-1})**
1.04	3.74×10^{-6}	1.52	5.46×10^{-6}

Table 3. Acute toxicity values of fenthion to *T. suecica* after 96 h of exposure.

to changes in nutrient concentrations and that phenomenon has a marked effect on the nutritive value of this microalga as feed in marine culture. According to relevant literature, these observed changes in the chlorophyll-a level either in the stationary or in logarithmic phase of growth were related to nitrogen depletion [15].

Fenthion belongs to a chemical group of pesticides called organophosphates, which share a common mechanism of toxicity; they all affect the nervous system by inhibiting acetylcholinesterase (AChE). The physiological role of AChE is the cleavage of the neurotransmitter acetylcholine at cholinergic synapses and neuromuscular junctions, thereby terminating the neurotransmitter's effects on the postsynaptic membrane. The toxicity of insecticidal organophosphates also called the anticholinesterase insecticides (anti-ChEs) is based on their inhibition of AChE, which results in interference with proper neurotransmission. Therefore, fenthion is not expected to be a direct inhibitor of pigment synthesis nor to induce a direct oxidative stress as a consequence of its biochemical mode of action that would destroy chlorophyll pigments. However, pigment content may change in response to the cascade of events following contamination with the pesticide, regardless of its different mode of action [28, 29]. Results of the effect of fenthion on the pigments were expressed either as pigment content of the culture or as percentage inhibition of pigment increase. These results are shown in **Table 4** and **Figure 4**.

From the collected experimental data, it became obvious that fenthion decreased the contents of photosynthetic pigments (Chl_a, Chl_b, Chl_c, and Chl_{tot}) and statistically significantly different as compared to the controls occurred in photosynthetic activity of *T. suecica* cells that were treated with concentration of fenthion above 1.00 mg L^{-1}. Values of concentration ratio of chlorophyll-a/chlorophyll-b (Chl_a/Chl_b) were calculated, and results are shown in **Table 4**.

Treatment level (mg L^{-1})	Chl_a		Chl_b		Chl_c		Chl_{tot}		Chl_a/Chl_b
	(μg L^{-1})	(pg $cell^{-1}$)	(μg L^{-1})	(pg $cell^{-1}$)	(μg L^{-1})	(pg $cell^{-1}$)	(μg L^{-1})	(pg $cell^{-1}$)	
Control	1125.169	2.632	463.586	1.084	140.194	0.3279	1728.949	4.044	2.43
Control + acetone	1263.114	3.249	540.690	1.391	100.796	0.2593	1904.600	4.899	2.34
0.50	995.208	2.704	378.972	1.030	93.088	0.2530	1467.268	3.987	2.63
1.00	1106.970	3.163	378.506	1.081	95.916	0.2740	1581.392	4.518	2.92
1.50	888.978*	3.556*	296.504	1.186	86.216	0.3449	1271.698*	5.087*	3.00
2.00	587.190*	5.592*	192.943*	1.838*	77.249*	0.7357*	857.383*	8.166*	3.04
2.50	424.708*	8.668*	143.219*	2.923*	37.895*	0.7734*	605.822*	12.364*	2.97
3.00	307.121*	9.307*	105.281*	3.190*	30.632*	0.9282*	443.034*	13.425*	2.92

Mean values of three replicates.

*Statistically significantly different as compared to the controls ($p < 0.05$).

Table 4. Effect on the pigment content of *T. suecica* after 96 h of exposure to treatments of fenthion.

http://dx.doi.org/10.5772/intechopen.72321

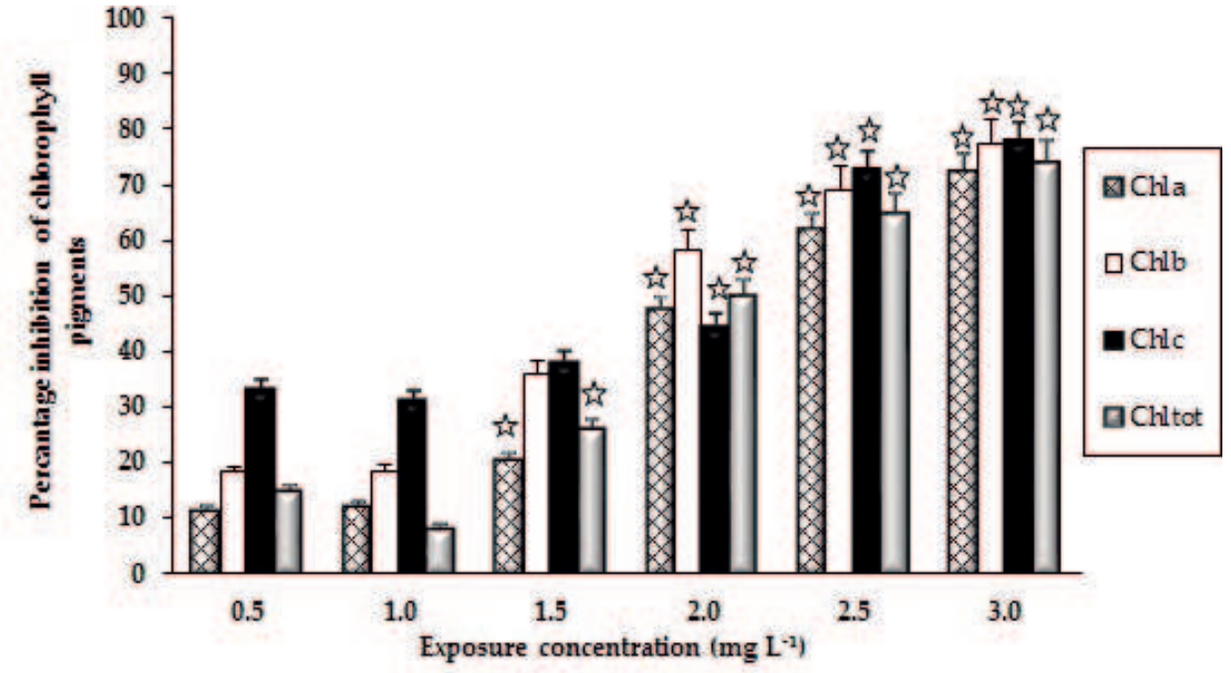

Figure 4. Effect of fenthion on the photosynthetic activity of *Tetraselmis suecica*. [Error bars represent standard deviations of three replicates, ☆ significantly different as compared to the controls ($p < 0.05$)].

It was clear that while cell density decreased with increasing exposure treatments of fenthion, values of Chl_a/Chl_b ratio remained stable or increased, suggesting that the biomass of algae was affected by the organophosphorus insecticide much more strongly than the structure of the chlorophyll body. These data were in agreement with those of Li et al., who reported that cypermethrin induced a drastic decrease in the growth and photosynthesis of *Scenedesmus obliquus* and that production of each chlorophyll pigment separately was more sensitive to cypermethrin than the ratio of Chl_a/Chl_b [25].

Linear correlations between cell density and chlorophyll pigment concentrations of chlorophyll-a, chlorophyll-b, chlorophyll-c, and total chlorophyll were calculated and are described by Eqs. (6)–(9), respectively:

$$Chl_a = 0.00002\,N + 312.41466, \text{ with correlation coefficient } R^2 = 0.9459 \quad (6)$$

$$Chl_b = 0.00001\,N + 84.68639, \text{ with correlation coefficient } R^2 = 0.9213 \quad (7)$$

$$Chl_c = 0.000002\,N + 33.815385, \text{ with correlation coefficient } R^2 = 0.8322 \quad (8)$$

$$\text{and } Chl_{tot} = 0.00003\,N + 430.91643, \text{ with correlation coefficient } R^2 = 0.9530 \quad (9)$$

where Chl_a, Chl_b, and Chl_c are the concentrations of chlorophyll pigments in culture media, Chl_{tot} is the sum of Chl_a, Chl_b, and Chl_c (all in $\mu g\ L^{-1}$), and N is the cell number (in cells).

Compound	Parameters		Predicted EC_{50} 96 h (mg L^{-1})	
	log P_{ow}	log S (at 20°C) (mg L^{-1})	QSAR-log P_{ow} [Eq. (3)]	QSAR-log S [Eq. (4)]
Fenthion	3.73	7.49	4.27	1.35
Fenthion sulfoxide (I)	2.18	240.00	29.47	0.92
Fenthion sulfone (II)	2.34	44.86	24.14	1.09
Fenthion oxon (III)	2.30	810.00	25.38	0.83
Fenthion oxon sulfoxide (IV)	0.87	2597.00	150.94	0.75
Fenthion oxon sulfone (V)	0.91	1773.00	143.59	0.78
Demethyl fenthion (VI)	3.07	93.78	9.72	1.01
Demethyl fenthion sulfoxide (VII)	1.59	1650.00	61.51	0.78
Demethyl fenthion sulfone (VIII)	1.67	590.00	55.67	0.85
Demethyl fenthion oxon (IX)	1.81	2540.00	46.75	0.75
Demethyl fenthion oxon sulfoxide (X)	0.42	19140.00	264.53	0.65
Fenthion phenol (XI)	2.49	1067.00	20.02	0.81
Fenthion phenol sulfoxide (XII)	1.19	8533.00	101.28	0.69
Fenthion phenol sulfone (XIII)	1.04	3163.00	122.11	0.74

Table 5. Calculated EC_{50} values for *T. suecica*.

The above-described linear correlations resulted in high values of correlation coefficients ($R^2 > 0.8322$), a fact which indicated that the use of chlorophyll measurements to estimate biomass concentration is reliable and validated the possibility of using cell chlorophyll content to assess the state of the cells after 96 h of exposure to fenthion, as previously described by other authors for other cases of bioassays [30, 31]. These results confirmed that the commonly accepted hypothesis of chlorophyll pigment content being proportional to growth rate of microalgal species [32] applies for toxicity assessment of fenthion on marine phytoplanktonic species such as *T. suecica*.

Acquired values of chlorophyll content expressed in pg. $cell^{-1}$ are summarized for each pigment in **Table 4**. Unfortunately, there is lack of available published information on photosynthetic activity of this species, and the few data are restricted only to chlorophyll-a concentrations [15]. It is observed that when incubated with fenthion concentrations equal or below 1.50 mg L^{-1}, the content of chlorophyll-a/cell of *T. suecica* reached values between 2.632 and 3.556 pg. $cell^{-1}$. Similar results were obtained by other authors who reported values of chlorophyll-a/cell between 3.1 and 3.8 pg./cell [15]. On the contrary, after exposure to treatment levels higher than 1.50 mg L^{-1}, an increase in these values occurred, and the

http://dx.doi.org/10.5772/intechopen.72321

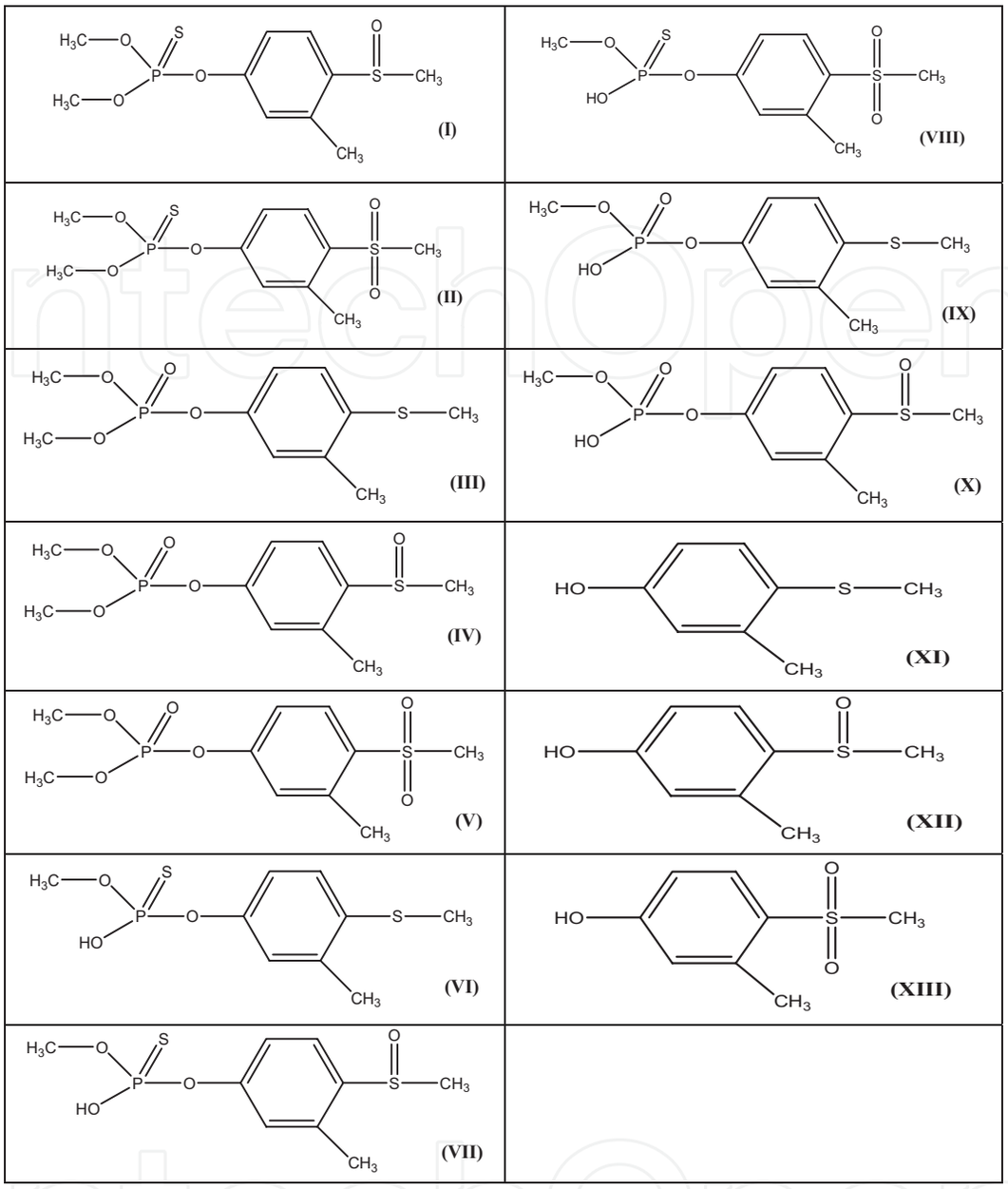

Figure 5. Principal metabolites and degradation products of fenthion. [(I) Fenthion sulfoxide, (II) fenthion sulfone, (III) fenthion oxon, (IV) fenthion oxon sulfoxide, (V) fenthion oxon sulfone, (VI) demethyl fenthion, (VII) demethyl fenthion sulfoxide, (VIII) demethyl fenthion sulfone, (IX) demethyl fenthion oxon, (X) demethyl fenthion oxon sulfoxide, (XI) fenthion phenol, (XII) fenthion phenol sulfoxide, and (XIII) fenthion phenol sulfone].

range was from 5.592 to 9.307 pg. cell^{-1}. This phenomenon suggested that either the determination of chlorophyll-a concerned pigment amounts that were extracted from dead cells as well as from the live ones or that under the stress due to high exposure levels of fenthion, a mechanism of stimulation occurred by the incubated strain and resulted in the increase of chlorophyll-a concentration.

3.4. Toxicity of the metabolites of fenthion on growth of marine alga *T. suecica*

The abiotic and biotic degradation of organophosphorus pesticides has been extensively studied in a large number of studies. Various data concerning the metabolism of several organophosphates in terrestrial and aquatic species, either in vivo or in vitro, are available [4–6]. After the application of Eqs. (3) and (4) for the prediction of the toxicity of 13 principal metabolites and degradation products of fenthion that have been identified in environmental samples, the predicted EC50 values for *T. suecica* are listed in **Table 5**. Additionally, the chemical structures of those compounds, called as metabolites and degradation products, are presented in **Figure 5**.

According to predicted EC_{50} values of Eq. (3), the parent chemical was more toxic than all of its metabolites, while on the contrary, according to Eq. (4), all of the 13 metabolites and degradation products of fenthion were expected to be more toxic than the parent compound. The acquired toxicity based on QSAR containing log P_{OW} data [Eq. (3)] followed the order: fenthion > demethyl fenthion > fenthion phenol > fenthion sulfone > fenthion oxon > fenthion sulfoxide > demethyl fenthion oxon > demethyl fenthion sulfone > demethyl fenthion sulfoxide > fenthion phenol sulfoxide > fenthion phenol sulfone > fenthion oxon sulfone > fenthion oxon sulfoxide > demethyl fenthion oxon sulfoxide. Interestingly, fenthion oxon that is the transformation product of fenthion by oxidative desulfuration was not predicted to be as toxic to *Tetraselmis suecica* up as the parent compound fenthion. EC_{50} value of fenthion oxon was estimated to be 25.38 mg L^{-1}, approximately six times higher than EC_{50} of fenthion, which was 4.27 mg L^{-1}. That fact could be attributed either to physicochemical properties of the compound (such as the highest water solubility and lowest octanol water partition coefficient) or to low persistence of the molecule into marine ecosystems as it undergoes under rapid hydrolysis. On the contrary, the calculated toxicity based on QSAR containing log *S* data [Eq. (4)] followed the order: demethyl fenthion oxon sulfoxide > fenthion phenol sulfoxide > fenthion phenol sulfone > fenthion oxon sulfoxide = demethyl fenthion oxon > fenthion oxon sulfone = demethyl fenthion sulfoxide > fenthion phenol > fenthion oxon > demethyl fenthion sulfone > fenthion sulfoxide > demethyl fenthion > fenthion sulfone > fenthion. This observation is in accordance with EC_{50} values found for the organophosphorus pesticide fenamiphos and its oxidation products fenamiphos sulfoxide (FSO) and fenamiphos sulfone (FSO_2) toward the aquatic alga *Pseudokirchneriella subcapitata* and the terrestrial alga *Chlorococcum* sp., which proved that parent compound was less toxic than its metabolites [33].

4. Conclusions

Based on the results of the current study, it appeared that fenthion can be highly toxic to the marine microalgal strain *T. suecica*. Experimental data revealed that the examined organophosphorus pesticide had marked effects on the growth of the tested algae since treatment concentrations above 1.00 mg L^{-1} affected algal densities and significantly decreased specific growth rate values. The finding that reduction of chlorophyll pigment production was observed due to exposure to fenthion indicated that this parameter could be used as a pollution biomarker. Moreover, two quantitative structure activity relationships, QSARs, based on physicochemical properties of the toxicants were applied for the prediction of toxicity values EC_{50} of the

13 principal metabolites and degradation products of parent organic compound, fenthion. Finally, the comparison between observed and predicted endpoint toxicity data showed that the predictive capability of both employed QSARs could be considered highly satisfactory.

Author details

Maria C. Vagi[1]*, Andreas S. Petsas[1,2], Maria D. Pavlaki[3], Niki M. Smaragdaki[1] and Maria N. Kostopoulou[1]

*Address all correspondence to: mbag@env.aegean.gr

1 Department of Marine Sciences, Laboratory of Environmental Quality and Geo-spatial Applications, School of the Environment, University of the Aegean, Mytilene, Lesvos, Greece

2 Department of Food Sciences and Nutrition, School of the Environment, University of the Aegean, Myrina, Lemnos, Greece

3 Department of Biology and Center for Environmental and Marine Studies (CESAM), University of Aveiro, Aveiro, Portugal

References

[1] U.S. National Library of Medicine, TOXNET, TOXICOLOGY DATA NETWORK. Hazardous Substance Data Bank, (HSDB): Fenthion, CASRN: 55-38-9. Available from: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB [Accessed: Aug 31, 2017]

[2] Tsatsakis A, Tsakiris I, Tzatzarakis M, Agourakis Z, Tutudaki M, Alegakis A. Three-year study of fenthion and dimethoate pesticides in olive oil from organic and conventional cultivation. Food Additives & Contaminants: Part A. 2003;**20**:553-559

[3] UNEP/FAO/WHO/IAEA. Assessment of the state of pollution of the Mediterranean Sea by Organophosphorus compounds. Athens, Greece: Map Technical Reports Series, No. 58; 1991

[4] Kitamura S, Suzuki T, Kadota T, Yoshida M, Ohash K, Ohta S. In vitro metabolism of fenthion and fenthion sulfoxide by liver preparations of bream, goldfish, and rats. Drug Metabolism and Disposition. 2003;**31**(2):179-186

[5] Tsuda T, Kojima M, Harada H, Nakajima A, Aoki S. Accumulation and excretion of fenthion, fenthion sulfoxide and fenthion sulfone by killifish (*Oryzias latipes*). Comparative Biochemistry and Physiology C. 1996;**113**:45-49

[6] Stone BF. Metabolism of fenthion by the southern house mosquito. Journal of Economic Entomology 1969;**62**:977-981

[7] Lacorte S, Jeanty G, Marty JL, Barceló D. Identification of fenthion and temephos and their transformation products in water by high-performance liquid chromatography with

diode array detection and atmospheric pressure chemical ionization mass spectrometric detection. Journal of Chromatography. A. 1997;**777**:99-114

[8] DeLorenzo ME, Scott GI, Ross PE. Toxicity of pesticides to aquatic microorganisms: A review. Environmental Toxicology and Chemistry. 2001;**20**:84-98

[9] Ahlegren G, Lundstedt L, Brett M, Forsberg C. Lipid composition and food quality of some freshwater phytoplankton for cladoceran zooplankters. Journal of Plankton Research. 1990;**12**:809-818

[10] Pehkonen SO, Zhang Q. The degradation of organophosphorus pesticides in natural waters: A critical review. Critical Reviews in Environmental Science and Technology. 2002; **32**(1):17-72

[11] Hanazato T, Kasai F. Effects of the organophosphorus insecticide fenthion on phyto- and zooplankton communities in experimental ponds. Environmental Pollution. 1995; **88**:293-298

[12] Butcher RW. An introductory account of the smaller algae of British coastal waters. Part I: Introduction and Chlorophyceae. Great Britain, London: Minist. Agric. Fish. Food, Fisheries Investigations. 1959;**Series IV**(Part 1):1-74

[13] Kylin H. Die Chlorophyceen der schwedischen Westküste. Lunds Universitets Arsskrift. N.F., Avd. 1949;**245**(4):1-79

[14] van den Hoek C, Mann D, Jahns HM. Algae-an Introduction to Phycology. UK: Cambridge University Press; 1995. 623 pages. ISBN: 0521304199

[15] Fabregas J, Herrero C, Cabezas B, Abalde J. Mass culture and biochemical variability of the marine microalga *Tetraselmis suecica* Kylin (butch) with high nutrient concentrations. Aquaculture. 1985;**49**:231-244

[16] Vagi MC, Kostopoulou MN, Petsas AS, Lalousi ME, Rasouli C, Lekkas TD. Toxicity of organophoshorous pesticides to the marine alga *Tetraselmis suecica*. Global NEST Journal. 2005;**7**(2):222-227. Available from: http://journal.gnest.org/sites/default/files/Journal%20Papers/paper_9_VAGI_375.pdf [Accessed: Jan 23, 2017]

[17] Provasoli-Guillard National Center for Culture of Marine Phytoplankton (PGCCMP). Medium Recipes. Medium f/2. Bigelow Laboratory for Ocean Sciences. West Boothbay Harbor, Maine, USA. 2010. Available from: https://ncma.bigelow.org/[Accessed: Aug 31, 2017]

[18] Organization for Economic Co-Operation and Development, (OECD). OECD Guidelines for Testing of Chemicals, Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test. OECD Publications Office -Environ. Health and Safety Div., OECD Environ. Directorate, Paris, France. 2011. Available from: http://dx.doi.org/10.1787/9789264069923-en [Accessed Aug 31, 2017]

[19] Tomlin CDS. The Pesticide Manual: A World Compendium. 11th ed. Farnham, Surrey, UK: British Crop Protection Council; 1997. 1606 pages. ISBN:1901396118

[20] Nyholm N, Källqvist T. Environmental toxicology review: Methods for growth inhibition toxicity tests with freshwater algae. Environmental Toxicology and Chemistry. 1989; **8**:689-703

[21] Strickland JDH, Parsons TR. A Practical Handbook of Seawater Analysis. Bulletin 167. 2nd ed. Ottawa: Fisheries Research Board of Canada; 1972. 309 pages. Available from: https://epic.awi.de/39262/1/Strickland-Parsons_1972.pdf [Accessed: Dec 9, 2017]

[22] Vagi MC. Hydrolysis and adsorption study of selected organophosphorus pesticides in aquatic and soil systems. Evaluation of their toxicity on marine algae. [PhD thesis] (in Greek). Greece: University of the Aegean, Mylilene; 2007. 355 pages. Available from: https://www.didaktorika.gr/eadd/handle/10442/17779 [Accessed: Aug 31, 2017]

[23] Virtual Computational Chemistry Laboratory (VCCLAB). 2005. Available from: http://www.vcclab.org [Accessed: Aug 31, 2017]

[24] Lewis MA. Algae and vascular plant tests. In: Rand GM, editor. Fundamentals of Aquatic Toxicology-Effects, Environmental Fate, and Risk Assessment. 2nd ed. Boca Raton: CRC Press; 1995. pp. 135-169. ISBN: 156032-091-5

[25] Li X, Ping X, Xiumei S, Zhenbin W, Liqiang X. Toxicity of cypermethrin on growth, pigments and superoxide dismutase of *Scenedesmus obliquus*. Ecotoxicology and Environmental Safety. 2005;**60**:188-192

[26] Ferrando MD, Sancho E, Andreu-Moliner E. Chronic toxicity of fenitrothion to an alga (*Nannochloris oculata*), a rotifer (*Brachionus calyciflorus*) and the cladoceran (*Daphnia magna*). Ecotoxicology and Environmental Safety. 1996;**35**:112-120

[27] United States Environmental Protection Agency (US-EPA). Transmittal of EFED RED for the List A Chemical Fenthion, Case #0290, Chemical #053301. Washington, DC, USA: Office of Prevention, Pesticides, and Toxic Substances; 1996

[28] Petsas AS, Vagi MC. Effects on the photosynthetic activity of algae after exposure to various organic and inorganic pollutants: Review. In: Jacob-Lopes E, editor. Chlorophyll. Rijeka, Croatia: InTech; 2017. pp. 37-77. DOI: 10.5772/67991. Available from: https://www.intechopen.com/books/chlorophyll/effects-on-the-photosynthetic-activity-of-algae-after-exposure-to-variousorganic-and-inorganic-poll [Accessed: Sep 18, 2017]

[29] Couderchet M, Vernet G. Pigments as biomarkers of exposure to the vineyard herbicide flazasulfuron in freshwater algae. Ecotoxicology and Environmental Safety. 2003; **55**(3):271-277

[30] Wilhelm C, Manns L. Changes in pigmentation of phytoplankton species during growth and stationary phases-consequences for the reliability of pigment based methods of biomass determination. Journal of Applied Phycology. 1991;**3**:305-310

[31] Davidson K, Flynn KJ, Cunningham A. Relationships between photopigments, cell carbon, cell nitrogen and growth rate for a marine nanoflagellate. Journal of Experimental Marine Biology and Ecology. 1991;**153**:87-96

[32] Borowitzka LJ, Borowitzka MA. B-carotene (Provitamin A) production with algae. In: Vandamme EJ, editor. Biotechnology of Vitamins, Pigments and Growth Factors. London: Elsevier Applied Science; 1989. pp. 15-26. ISBN: 978-94-009-1111-6

[33] Cáceres T, Megharaj M, Naidu R. Toxicity and transformation of fenamiphos and its metabolites by two micro algae *Pseudokircheriella subcapitata* and *Chlorococcum* sp. Science of the Total Environment. 2008;**398**:53-59