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UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

Dedicated to the memory of Dr. Pearl Weinberger

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ABSTRACT

The fate and persistence of the pesticide, fenitrothion (O,O-dimethyl-O (3-methyl-4-nitrophenyl) phosphorothioate) was monitored in the presence of the alga *Chlamydomonas segnis* Ettl. Under natural sunlight (800 w m⁻²) and Vita Lite[®] high intensity fluorescent lamps (Dura Test, 50 w m⁻², 290-700 nm), there was 10 to 20 times the uptake of ¹⁴C ring-labelled fenitrothion into live and freeze-killed algae as compared to dark conditions. The accumulated label in both the live and freeze-killed algae (with nonfunctioning electron transport systems) was identified as polar metabolites of fenitrothion, their identical percentages suggesting photophysical reactions. As a consequence of increased uptake and degradation of fenitrothion by live algae, the fenitrothion half-life in the media decreased by approximately one half in the presence of algae and light, as compared to lighted media controls.

When accumulation experiments were repeated under anaerobic conditions, polar metabolites were reduced by 50%. Further, as increased pigment chlorosis correlated with increased polar metabolites, it is suggested that photooxidation of fenitrothion by singlet oxygen could have been a degradation route. Fenitrothion photolysis was accelerated by the aquatic alga, *Chlamydomonas segnis*.

RESUME

Une étude a été menée sur le devenir et la rémanence du pesticide fénitrothion (*O,O*-diméthyl-*O* (3-méthyl-4-nitrophényl) phosphorothioate) en présence de l'algue *Chlamydomonas segnis*. L'assimilation du fénitrothion marqué au ^{14}C au noyau aromatique était 10 à 20 fois plus élevée chez les algues vivantes et les algues tuées à l'azote liquide, exposées à la lumière solaire naturelle (800 w m^{-2}) et aux lampes fluorescentes à haute intensité Vita Lite[®] (Dura Test, 50 w m^{-2} , 290-700 nm), que lorsqu'elles étaient à la noirceur.

Le marqueur au ^{14}C dans les algues vivantes et mortes (i.e. les algues n'ayant aucun système photosynthétique fonctionnel de transport d'électrons) a été identifié comme étant des métabolites polaires du fénitrothion. Les pourcentages identiques de ces métabolites retrouvés chez les algues vivantes et mortes suggèrent la présence de réactions photophysiques. Dû à l'assimilation et la dégradation du fénitrothion par *Chlamydomonas segnis*, la demie-vie du fénitrothion retrouvé dans le milieu aqueux s'est vue décroître de moitié en présence d'algues et de lumière.

Les expériences d'accumulation du pesticide par les algues ont été répétées sous conditions anaérobiques; la production de métabolites polaires était réduite de 50%. De plus, sous conditions aérobiques, l'augmentation de pigments dénaturés était en

corrélation avec l'accroissement de métabolites polaires. Il est suggéré que la dégradation du fénitrothion se soit effectuée grâce au processus de photooxydation par la production de singlet d'oxygène. L'algue aquatique *Chlamydomonas segnis* accélère donc la photolyse du fénitrothion.

A - INTRODUCTION

Organic contaminants introduced into the environment can easily find their way into aquatic ecosystems where their fate is determined by a complex set of biological and physiochemical interactions. Light induced reactions, or photolyses, are a major part of these interactions. Most natural waters contain varying amounts of suspended particles of terrigenous and biogenous origins to which partitioning of various aquatic pollutants is likely to alter the contaminants' photoreactivity and photoproducts (Zepp, 1980). Besides direct photolysis, involving light absorption by the chemical itself, there exists indirect or sensitized photolysis, initiated through light absorption by other substances in the system, most likely phytoplankton (Zepp, 1980).

For the past three decades, eastern Canadian forests have been plagued by the defoliant insect, spruce budworm *Choristoneura fumiferana* (Clemens). Since the 1970's, fenitrothion, *O,O* - dimethyl-*O* (3-methyl-4-nitrophenyl) phosphorothioate (IUPAC, 1987), has been used extensively for budworm control and applied as an aerial formulated spray. Aerial application of fenitrothion can often result in its introduction into aquatic systems either directly from spray drift or indirectly as a result of foliage washoff (Symons, 1977). Fenitrothion is known to persist for only short periods of time in aquatic compartments and degradation is

mainly by photolysis and hydrolysis. Its half-life ($t_{1/2}$) is from one to several days depending on environmental factors such as light, pH, temperature and water quality (Weinberger *et al.*, 1982a).

For the most part, the persistence and fate-transport studies of fenitrothion have been carried out in laboratory microcosms devoid of phytoplankton (Makami *et al.*, 1985; & Greenhalgh *et al.*, 1976; Greenhalgh & Marshall, 1980). However, numerous studies have indicated that algae are capable of mediating photobiological transformations of chemicals (Anagnostopoulos *et al.*, 1978; Weinberger *et al.*, 1983; 1982a; 1981a; Zepp & Schlotzhauer, 1983; Zepp & Baughman, 1978).

The purpose of this study was to examine the effects of algae on fenitrothion degradation and persistence in media and determine whether light mediated mechanisms are involved.

1.0 Fenitrothion Use in Canadian Forests

In Canada, aerial applications of pesticides in forests began in Nova Scotia during the late 1920's. At this time, calcium arsenate was used against the spruce budworm. By the mid-1940s, the chlorinated hydrocarbon DDT¹ became the predominant insecticide against forest insects (Nigam, 1975). DDT was the most widely used insecticide in the 1950's and 1960's, its operational or experimental aerial spray applications spanning across all of Canada (Busby *et al.*, 1989). By 1969, however, DDT was banned due to its extreme

¹ DDT: 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane

persistence. For example, Yule (1970) reported that the half-life ($t_{1/2}$) of DDT in temperate forest soils was approximately 10 years.

During the 1960's a new generation of less persistent insecticides appeared. The carbamate aminocarb (Matacil¹)¹ and the organophosphorothioates phosphamidon² and fenitrothion³ (Sumithion³, Accothion³, Novathion³) have been the most used in the spruce budworm control programs in eastern Canada (Armstrong, 1984). Phosphamidon was highly toxic to birds and fenitrothion replaced it, emerging as the prominent forest insecticide until the mid 1980's when several provinces switched to the microbial insecticide *Bacillus thuringiensis* (Busby *et al.*, 1989). Over a ten year period until 1977, it was estimated that 10,000 metric tons of fenitrothion had been deposited on Canadian forests (Symons, 1977). Recently, from 1983 to 1987, 1200 tonnes of formulated and technical fenitrothion were imported into Canada (Statistics Canada, 1987). Presently, only Newfoundland and New Brunswick are applying fenitrothion in large-scale aerial control programs (Busby *et al.*, 1989).

The main criteria used for selecting candidate insecticides for Canadian forest insect control has been high toxicity to the target insect and low toxicity to fish, birds and mammals (Nigam,

¹ aminocarb: 4-dimethylamino-*m*-tolyl methylcarbamate

² phosphamidon: 2-chloro-*N,N*-diethyl-3-hydroxycrotonamide dimethyl phosphate

³ fenitrothion: *O,O* -dimethyl-*O* (3-methyl-4-nitrophenyl) phosphorothioate

1975). Initially, fenitrothion satisfied the basic criteria and was the subject of several reviews by the National Research Council of Canada, the last comprehensive one in 1977 (Roberts *et al.*, 1977). However, in 1989 after an extensive review, Environment Canada recommended a re-evaluation of registered fenitrothion use pattern be undertaken due to undesirable environmental effects (Ernst *et al.*, 1989). Some of the reasons for the recommendations include deleterious effects on pollinators such as honey bees and wild bees, and risks to protected migratory songbirds.

2.0 Contamination of Aquatic Habitats

The spruce budworm control program in New Brunswick is the largest of its kind in the world with the greatest use of fenitrothion in forest protection (Busby *et al.*, 1989). Fenitrothion is sprayed aerially in May or June as an oil based spray or aqueous emulsion in a single treatment of 280 g active ingredient (AI) ha⁻¹ or in two applications of 210 g AI ha⁻¹ with a five day interval between applications (Sundaram *et al.*, 1989). The spraying is not always uniform when large planes are utilized. Upon completion of a spray program, Pierce & Ernst (1988) discovered a variability of application resulting in deposits of fenitrothion which were greater than five times the mean deposit on up to 5% of the spray area. Overspraying on boundaries between spray blocks has also been reported (Symons, 1977, Varty, 1980). Thus, it is conceivable that higher quantities of fenitrothion can be sprayed on the forest

canopies.

Aerial application of fenitrothion can result in direct contamination of aquatic habitats while indirect contamination may result from spray drift or from surface run-off following rainfall (Eidt, 1975; Eidt & Sundaram, 1975; Armstrong, 1984). Protective, no-spray buffer zones beside water bodies were implemented in 1978 to reduce exposure of aquatic ecosystems to fenitrothion (Sexsmith, 1987). However, these restrictions apply only to rivers and large bodies of water and smaller bodies of water could still be sprayed. Even with boundaries in place, drift and surface run-off would continue. For example, low but detectable amounts of fenitrothion have been found in areas 200 m away from a spray block (Sundaram, 1987).

Once in aquatic habitats, fenitrothion may undergo rapid degradation and is not detectable by 40 days post-spray (Roberts, 1975; Malis & Muir, 1984). In distilled and buffered solutions, in vitro degradation has been shown to be by hydrolysis and photolysis (Greenhalgh et al., 1980; Maguire & Hales, 1980; Marshall et al., 1974; Ohkawa et al., 1974). The hydrolysis process is relatively slow, primarily depending on temperature and pH. Greenhalgh et al. (1980) observed the $t_{1/2}$ of fenitrothion was 50 days under laboratory conditions at 23°C and pH 7.5 in the dark. However, under field conditions the $t_{1/2}$ was less than 2 days. They concluded that in the field photolytic and/or microbial processes are the main degradative route of fenitrothion in natural aquatic systems.

Under sunlight conditions, fenitrothion photodecomposes very quickly. Mikami *et al.* (1985) exposed fenitrothion to full sunlight in the fall and found the $t_{\frac{1}{2}}$'s to be 0.6 - 1.0 days in distilled water, 1.5, 1.0, and 0.9 days in buffer solutions of pH 3, 7 and 9 respectively, and 0.9 - 1.1 days in natural water and sea water. Weinberger *et al.* (1982a) found that the photolysis of fenitrothion was generally unaffected by pH of natural waters and that 80% of the pesticide was photolyzed to polar derivatives within 6 hours. Light intensity can also affect the $t_{\frac{1}{2}}$ of fenitrothion. Malis and Muir (1984) discovered the $t_{\frac{1}{2}}$ of fenitrothion in shaded ponds to be 1.6 days while under direct light the $t_{\frac{1}{2}}$ was one day.

Laboratory studies of fenitrothion photodegradation by UV radiation (wavelength 253.7 nm) have revealed the following breakdown products; mainly carboxyfenitrothion¹ as well as fenitrooxon², demethylfenitrothion³, formylfenitrothion⁴, carboxyfenitrooxon⁵, S-methylfenitrothion⁶, 3-methyl-4-nitrophenol (4-nitro-*m*-cresol) and

¹ carboxyfenitrothion: *O,O*-dimethyl *O*-(4-nitro-3-carboxyphenyl) phosphorothioate

² fenitrooxon: *O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphate

³ demethylfenitrothion: *O,O*-dimethyl *O*-hydrogen *O*-(3-methyl-4-nitrophenyl) phosphorothioate

⁴ formylfenitrothion: *O,O*-dimethyl *O*-(4-nitro-3-formylphenyl) phosphorothioate

⁵ carboxyfenitrooxon: *O,O*-dimethyl *O*-(4-nitro-3-carboxyphenyl) phosphate

⁶ S-methylfenitrothion: *O,S*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate

3-carboxy-4-nitrophenyl (4-nitro-*m*-thio cresol) (Greenhalgh & Marshall, 1976; Mikami *et al.*, 1985) (Structures are shown in Figure 15, Appendix I). Analyses of fenitrothion degradation in natural aquatic systems (*in situ*) under sunlight show that similar metabolites are produced. In natural river water, Mikami *et al.* (1985) identified 18 photoproducts over a 40 day period, of which carboxyfenitrothion was the main metabolite formed. Weinberger *et al.* (1982a) monitored field microcosms containing algae maintained under sunlight conditions. Aerially applied fenitrothion was rapidly partitioned into the water compartment of the microcosm and degraded into carboxyfenitrothion, fenitrooxon, demethylfenitrothion, and *S*-methylfenitrothion. They discovered a new metabolite, carboxyamino fenitrothion¹, when algae were irradiated with sunlight.

The adsorption of pesticides and their degradation products on suspended particles, soils and sediments could play a major role in their environmental fate (Baarschers *et al.*, 1983; Eidt *et al.*, 1984). Sediment and soils have been observed to adsorb fenitrothion and 3-methyl-4-nitrophenol in amounts that rise with increasing organic matter content (Baarschers *et al.*, 1983). In field microcosms, Weinberger *et al.* (1981a) found sediment to be a primary sink for fenitrothion (30% of aerially applied fenitrothion partitioned into the sediment 2 days post-spray). However, for the purpose of this study, uptake of fenitrothion by algae and

¹ carboxyamino fenitrothion: *O,O*-dimethyl *O*-(4-amino-3-carboxyphenyl) phosphorothioate

subsequent metabolism will be the focus of further discussion.

3.0 Contamination of Algae

There have been few studies of bioaccumulation of fenitrothion by aquatic plants. In macrophytes, Eidt et al. (1984) found 128 times more fenitrothion per unit dry weight in liverwort, *Jungermannia* sp. In natural lake waters under sunlight, Weinberger et al (1981b) observed a rapid partitioning of fenitrothion and its metabolites into the aquatic macrophytes *Myriophyllum*, *Elodea*, and *Sagittaria*. The aquatic macrophyte *Lemna* accumulated 3 to 6% of added fenitrothion by 2 days in unshaded ponds (Malis & Muir, 1984). In Manitoba, Moody et al. (1978) sampled a stream 10 hours after an aerial application of fenitrothion and found up to 4 $\mu\text{g mL}^{-1}$ fenitrothion in *Lemna minor* verses 44 $\mu\text{g L}^{-1}$ of fenitrothion in the surrounding water.

Algae are small aquatic microphytes and consequently have a high surface-to-volume ratio, thus increasing uptake of lipophilic chemicals (Roberts et al., 1981b). Algae cells also contain relatively large amounts of fats and oils (lipids)(Stewart, 1974). For example, lipids comprise 39% of the cell dry weight of *Chlorella* (Nichols, 1965). The bioaccumulation potential of a lipophilic compound such as fenitrothion could be linked to its n-octanol-water partition coefficient (K_{ow}) and water solubility. Fenitrothion has a high K_{ow} of 3800 and could easily partition into cell lipids of algae.

There is strong evidence of this occurring with organophosphorus pesticides (Boyle, 1984). Weinberger *et al.* (1981a, 1982a) discovered that a mixture of the algae *Chlamydomonas reinhardtii*, *Euglena gracilis* and *Chlorella pyrenoidosa* accumulated fenitrothion and its derivatives several hundredfold after two days and several thousandfold after 6 days under natural light conditions. In a laboratory study under fluorescent lights, Kikuchi *et al.* (1984b) found *Chlorella vulgaris*, *Nitzschia closterium* and *Anabaena flos-aquae* rapidly absorbed fenitrothion from medium with maximum bioaccumulation ratios (in terms of dry weight) of 44, 105 and 53, respectively. Finally, Lal *et al.* (1987) examined bioaccumulation in the blue-green algae *Anabaena* sp. and *Aulosira fertilissima* under fluorescent lights and found levels of fenitrothion varying from 53 to 6651 $\mu\text{g g}^{-1}$ dry weight.

With significant bioaccumulation of fenitrothion in algae, any metabolic changes should be examined. Algae are primary producers in the ecosystem and any deleterious effect of a pesticide could be harmful to the aquatic foodchain. Organophosphorus pesticides are known to inhibit DNA, RNA, protein and carbohydrate synthesis, and reduce ATP levels in various species of algae (Sumida & Ueda, 1974; Bushway, 1978; Clegg & Koevenig, 1974). Recently, Kent & Weinberger (1990) exposed the algae *Chlamydomonas segnis*, *Chlorella pyrenoidosa*, *Scenedesmus obliquus*, *Ankistrodesmus falcatus* and *Selenastrum capricornutum* to fenitrothion levels of 10 $\mu\text{g mL}^{-1}$, significantly increasing cell volumes and weights while reducing growth

rates up to 64 and 96%. Studies by Kikuchi *et al.* (1984a) confirm similar effects of fenitrothion on aquatic algae. They reported inhibition of DNA and RNA synthesis by 60 to 80% when *Chlorella vulgaris* were exposed to 100 $\mu\text{g mL}^{-1}$ fenitrothion. Subsequently, cell replication was reduced while cell weight and volume increased.

4.0 Biodegradation of Fenitrothion by Algae

Although there is bioaccumulation of fenitrothion in algae and subsequent metabolic effects of the pesticide to algae growth, there have been few studies of algae biodegradation of pesticides. Biodegradation is the molecular degradation of an organic substance resulting from the complex action of living organisms (Matsumura *et al.*, 1982a). There is a wealth of information on bacteria and fungi degradation of organic chemicals (Matsumura & Esaac, 1979). Several studies have indicated microbial and fungal biodegradation of fenitrothion. For example, the fungus *Trichoderma viride* is capable of hydrolysing fenitrothion and fenitrooxon in pure culture (Baarschers & Heitland, 1986). Sundaram *et al.* (1984) and Greenhalgh *et al.* (1980) concluded that bacteria biodegraded fenitrothion to aminofenitrothion in their studies.

When the degradative properties of algae alone have been examined, they do not show appreciable degradation capabilities (Matsumura & Esaac, 1979). However, algae are closely associated with sunlight and their functions have to be studied in the

presence of light. Algae are the most important sunlight absorbers in many aquatic environments (Baker & Smith, 1982). Therefore, algae-mediated phototransformation of toxic chemicals may have a significant effect on concentrations in the water column (Zepp & Schlotzhauer, 1983).

Zepp & Schlotzhauer (1983) carried out an extensive screening of 22 organic chemicals and their sunlight induced algal transformations. Their studies indicated that green and blue-green algae at concentrations of 1 - 10 mg L⁻¹ chlorophyll a accelerated photo-reactions of certain polycyclic aromatic hydrocarbons, organophosphorus compounds, and anilines. Parathion¹ and methyl parathion² were photolyzed 390 times more rapidly, and aniline and *m*-toluidine reacted over 12000 times faster when sorbed by algae under sunlight conditions. They claimed the photolysis rate of fenitrothion in sunlight was unchanged in the presence of *Chlorogonium* sp., concluding direct photolysis competed with the indirect algal mediated process. Confirming Zepp & Schlotzhauer's study, Lal *et al.* (1987) examined metabolism of fenitrothion by blue green algae and found no metabolites in the algae. However, the study by Kikuchi *et al.* (1984b) disagreed with the previous authors. They discovered *Anabaena flos-aqua* metabolized fenitrothion most actively to its oxon and demethyl analogs, whereas *Chlorella vulgaris* decomposed the chemical to demethylfenitrothion.

¹ parathion: *O,O*-dimethyl-4-nitrophenyl phosphorothioate

² methyl parathion: *O,O*-dimethyl *O-p*-nitrophenyl phosphorothioate

5.0 Indirect Photolysis

When discussing indirect photolysis, it is important to have a good understanding of the photochemical processes involved. Many indirect processes are dependant upon the collisional transfer of energy from an excited species, called the sensitizer, to the pollutant, and the sensitizer must be a sufficiently long-lived reaction intermediate for there to be a high probability to collide with it (Roberts *et al.*, 1981b). In most cases, the degradative process proceeds through the efficient and longer-lived triplet excited state (excited state with spin unpaired electrons) (Roberts *et al.*, 1981b). The overall sequence of competitive processes is shown in Figure 1. S denotes the singlet state, T denotes the triplet state, 0 is the ground state, 1 is the excited state, ISC is intersystem crossing, k_s is the sensitizer rate constant, and k_q is the quenching rate constant.

The sensitizer begins at ground state where absorbed light leads to electronic excitation to the singlet excited state (1)(Figure 1). The singlet excited state is normally short-lived but can undergo intersystem crossing, involving spin inversion (Knox & Dodge, 1985), to the longer lived triplet state (2). When the sensitizer collides with the pollutant the excited state energy may be passed on to the pollutant (3). At this point either the energy absorbed by the pollutant may cause bonds to break, creating degradation products (4) or the pollutant may lose the energy (5). The quenching formulas, (6) and (7), are important as normal levels

of dissolved oxygen in aquatic waters may quench up to 90% of the energy absorbed by the sensitizer (Roberts *et al.*, 1981b). However, increased levels of the pollutant inside the algae through bioaccumulation, coupled with a decreased efficiency of oxygen quenching in the cell would enhance the indirect photochemical reactions. Singlet oxygen (1O_2) produced during the quenching process could also react with the pollutant to form an oxidative type derivative (8).

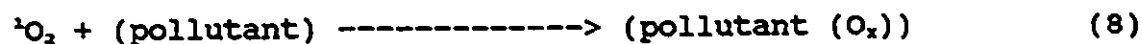
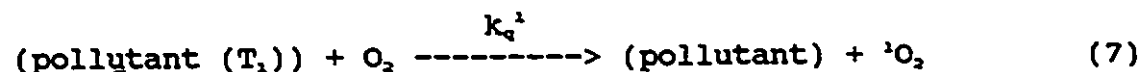
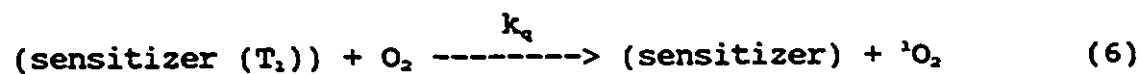
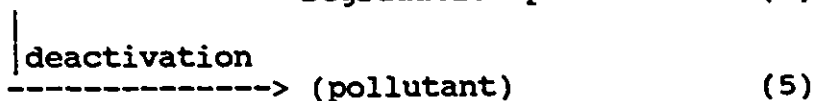
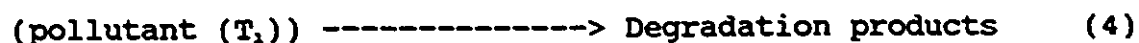
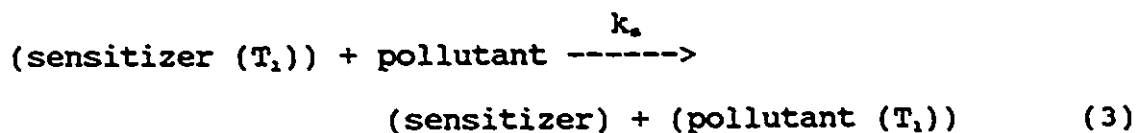
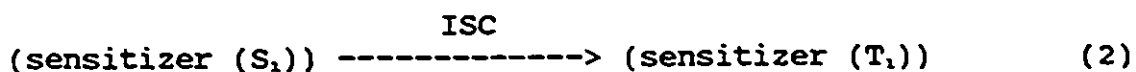
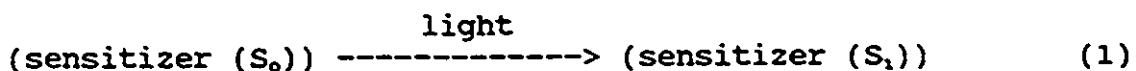


Figure 1. Indirect photolysis sequence of events showing the transfer of energy from the light source to the pollutant from the sensitizer (adapted from Roberts *et al.*, 1981b).

Light harvesting pigments of algae could be sensitizers in an indirect photolysis process. Numerous studies have shown that visible light absorption by the photosynthetic apparatus of algae could result in the photometabolism of polar ionic organic substrates such as carboxylic acids, carbohydrates, and amino acids (Wiessner, 1970). Chlorophyll from spinach chloroplasts acted as a sensitizer for degradation of organophosphorus insecticides (Matsumura, 1982b). Dixon and Wells (1987) used chlorophyll as a sensitizer to photodegrade pirimicarb¹. They discovered that singlet oxygen was implicated in the sensitized photodegradation. While chlorophyll is the primary pigment of the light harvesting complex, there are other accessory pigments such as the carotenoids. These pigments are mainly sensitizers for chlorophyll and could act as indirect sensitizers of fenitrothion as well.

Weinberger's group has shown higher accumulation and metabolism of fenitrothion by algae under natural light conditions (Weinberger *et al.*, 1983; 1982b; 1981a; Moody, 1982). The identification of the new metabolite of fenitrothion, only present when algae are irradiated, supports the theory that a light mediated process is occurring. Since algal pigments are the most affected by light, they could be acting as the chief sensitizers in the indirect photolysis of fenitrothion.

¹ pirimicarb: 2-dimethylamino-5,6-dimethylpyrimidin-4-ol

6.0 Hypothesis and Objectives

The following hypothesis is presented;

Fenitrothion photolysis is accelerated or increased by photo-oxidative processes in aquatic algae.

To test the hypothesis, the following objectives were examined;

- 1: To determine the effect of fenitrothion on algal growth.
 - a. Examine various growth parameters of the algae when exposed to fenitrothion. (Cell number, chlorophyll content, dry weights, photosynthetic rates)
 - b. Measure any increased electron flow in the system by the use of the redox reagent, INT (2-(p-iodophenyl)-3-(p-nitrophenyl)-5-(phenyl tetrazolium chloride)).

- 2: To determine whether axenic algae in sterile medium have an effect on the uptake and degradation of fenitrothion.
 - a. By using C¹⁴ ring labelled fenitrothion, determine whether the label is accumulated by live *Chlamydomonas segnis* under light verses dark conditions.
 - b. With TLC, determine whether polar metabolites are present in the algae and compare to polar metabolites in media.
 - c. Directly measure the actual fenitrothion remaining in

media by extraction and GC methodology and calculate rates of degradation in the media.

3: To determine if the mechanism of enhanced fenitrothion degradation in the presence of algae is a biotic and/or abiotic related process.

a. Repeat 1a and 1b using freeze-killed algae. Determine that the algae are indeed dead by use of a Clark electrode to measure O_2 evolution.

b. Extract any pigments and determine spectrum of any pigments remaining in the algae to determine which pigment, if any, may be responsible for increased fenitrothion degradation.

4: To determine whether the increased photolytic degradation of fenitrothion is an oxidative or nonoxidative reaction.

a. Repeat the experiments under anaerobic conditions and determine whether polar metabolites are present in the algae.

B - MATERIALS AND METHODS

1.0 General Methodology

1.1 Chemicals.

¹⁴C ring labelled fenitrothion (S.A., 5.54 mC mM⁻¹) and technical fenitrothion (95% pure) were donated by Sumitomo Chemical Company, Takatsukasa, Japan. Purity was confirmed by gas chromatography (GC) and thin layer chromatography (TLC). INT dye (2-(p-iodophenyl)-3-(p-nitrophenyl)-5-(phenyl tetrazolium chloride)), NADH (reduced nicotinamide adenine dinucleotide), and NADPH (reduced nicotinamide adenine dinucleotide phosphate) were purchased from Sigma Chemicals. All other chemicals were obtained from BDH Chemicals (Toronto, Canada). All organic solvents used were glass-distilled pesticide grade from BDH Chemicals. The liquid scintillation solution, LSC Cocktail '4698,' was from BDH Chemicals.

1.2 Algae.

1.2.1. Culture Techniques. The unicellular green alga *Chlamydomonas segnis* (Ettl) was obtained axenic from the Culture Collection at the University of Texas. Liquid cultures in 10 L round bottom flasks were grown in a Hotpack growth chamber maintained at 21-23°C under 16 hour light: 8 hour dark periods provided by a bank of two cool-white and two Grow-lux (Sylvania, Ottawa, Canada) fluorescent lamps (5 watts m⁻²). Stock cultures were continually stirred and aerated with air (0.03% CO₂). All experi-

ments were conducted during exponential cell growth, maintained by half-volume dilutions of Kuhl's (1964) nutrient solution every two days (Calculated from growth curve, Appendix II, Figure 16). Prior to an experiment, an aliquot was removed, algal cells counted with an improved Neubauer-Levy haemocytometer and the required concentrations obtained.

1.2.2. Freeze-killed Algae. Freeze-killed algae were prepared by centrifuging an algal aliquot from the required concentration in a Sorvall centrifuge at 5000 rpm for 7 minutes. The supernatant was removed and the pellet was suspended in liquid nitrogen for five minutes. The frozen pellet was resuspended in an equal amount of fresh medium. An aliquot of this was monitored to establish that regrowth did not take place and the algae were indeed dead.

1.3 Statistical Analysis of Data.

All data collected from the experiments were analyzed using Statgraphics 3.0 (Statistical Graphics Corporation) on an IBM personal computer. Various treatments were compared using analysis of variances and Tukey's Multiple Range Tests. Means at individual time periods were compared by Tukey's tests (Tukey, 1949).

All error bars shown in the figures are standard deviation. Missing error bars indicate very low error.

2.0 *Chlamydomonas segnis* Studies.

2.1 Effects of Fenitrothion on Growth.

Experimental treatments consisted of live algae with fenitrothion and controls of live algae only sets. 150 mL aliquots were placed in 500 mL Erlenmeyer flasks to yield final concentrations of 20×10^4 cells mL⁻¹ algae and 10.3 μ g mL⁻¹ fenitrothion (control sets had media only plus algae). The flasks were attached to an Eberbach shaker (Ann Arbor, Mich., USA) and held under cool-white and Grow-lux fluorescent lamps (5 wm^{-2}) at 23°C in a constant temperature room. At 24 hour periods 15 mL aliquots were removed and analyzed.

2.1.1. Algae Cell Number. A total of 8 counts were conducted of each aliquot using the improved Neubauer haemocytometer and averaged.

2.1.2. Algae Cell Weight. 5 mL of each aliquot were filtered through preweighed glass fibre filters (Whatman GF/A). The filters were oven dried at 60°C for 4 hours and weighed.

2.1.3. Algae Pigments. The determination of chlorophyll a and b was calculated following the method of Hansmann (1973). 10 mL of the algal sample was filtered through a Millipore HA (pore size of 0.45 μ m). The filter was placed in 5-10 mL of 90% acetone and vigorously shaken until the filter was completely dissolved. A blank containing only a filter and acetone was prepared and all samples were placed in a darkened refrigerator for 24 hours. After the extraction period, the samples were warmed to room temperature and made up to 10 mL with 90% acetone. Finally, the samples were

centrifuged for 5-10 minutes at 3000-5000 rpm. Using the filter and acetone as a blank, the samples were placed in a Spectronic 21 Bausch and Lomb spectrophotometer and absorptions (D) were measured at 630, 645, and 665 nm. Chlorophyll a and b were calculated by the following equations;

$$\text{Chlorophyll a} = 11.6 \times D_{645} - 1.21 \times D_{665} - 0.14 \times D_{630}$$

$$\text{Chlorophyll b} = 20.7 \times D_{645} - 4.34 \times D_{665} - 4.42 \times D_{630}$$

2.1.4. Photosynthesis. Photosynthetic rates were calculated by measuring O_2 with a Clarke electrode (Delieu & Walker, 1972). The Clarke electrode was connected to a YSI Model 53 oxygen monitor. Saturating light intensity of 320 w m^{-2} (measured inside each reaction chamber with a YSI Kettering Model 65A Radiometer) was provided by a 150 w Sunbeam parchlor lamp. Saturating light intensity was calculated from Figure 17 (Appendix III), which measured photosynthesis over a series of differing light intensities. $8 \mu\text{l mL}^{-1}$ of a 1 M NaHCO_3 (8 mM) solution was used as a CO_2 source for all the experiments.

Prior to the O_2 readings, the Clarke electrode was calibrated to 100% with air saturated media only. Five mL of algae were placed inside the reaction chamber, maintained at 23°C by a water jacket. Air was bubbled through the algal medium and the NaHCO_3 added. A magnetic stirrer was placed inside the reaction chamber, the probe was inserted and the lights were turned on. One minute after the procedure was started, oxygen evolution rates were measured for five minutes and oxygen levels calculated.

2.2 Algae INT Assay

The methodology followed was based on Kenner and Ahmed (1975) and was as follows.

2.2.1. Assay Solutions. The substrate solution consisted of 0.133 M disodium succinate, 0.835 mM NADH, 0.24 mM NADPH, and 0.2% (v/v) Triton X-100, all dissolved in 50 mM sodium phosphate buffer at pH 7. This solution was prepared just before use and kept on ice until INT was added. The INT solution was made up of 2.5 mM INT (Grade A) in distilled water and stored on ice. All solutions were prepared just before use. 0.2 N HCl was used for the termination solution.

2.2.2. Assay Procedure. For treatment purposes, a 60 $\mu\text{g mL}^{-1}$ solution of fenitrothion in water was prepared prior to each experiment and kept in darkness. Each experiment consisted of three replicates of 12 treatments:

1. live cells in sunlight plus fenitrothion
2. live cells in sunlight, media only
3. freeze-killed cells in sunlight plus fenitrothion
4. freeze-killed cells in sunlight, media only
5. blank (no cells) in sunlight plus fenitrothion
6. blank (no cells) in sunlight, media only
- 7 - 12 identical to 1 - 6 except the tubes were wrapped in foil (darkness).

The treatment solutions were made up in 50 mL screw cap culture tubes (Pyrex No. 9825) in the following order; 3 mL substrate, 1 mL fenitrothion (or media), 1 mL algae (live or freeze-

killed, media in the blanks), and 1 mL of INT, resulting in final concentrations of 66.7 mM succinate, 0.418 mM NADH, 0.12 mM NADPH, 0.1% (v/v) Triton X-100, 25.0 mM phosphate buffer, and 0.417 mM INT per 6 mL treatment sample. In the appropriate treatments, the final concentration of fenitrothion was $10 \mu\text{g mL}^{-1}$ and the alga cell number was $20 \times 10^4 \text{ cells mL}^{-1}$. Each sample was vigorously mixed, quickly brought up to 23°C in a water bath and placed at a 20° incline on a Eberbach shaker (Ann Arbor, Mich., USA) under natural sunlight (800 Wm^{-2}). After the required time period, the termination solution was added, the solutions mixed, and absorptions recorded at 490 nm on a Spectronic 21 Bausch and Lomb spectrophotometer.

3.0 Fenitrothion Interaction Studies

3.1 Accumulation in Algae.

3.1.1. Total ^{14}C Ring Label Accumulation Study. Each experiment consisted of a minimum of three replicates of four treatment sets. The sets included;

1. live cells in light plus fenitrothion
2. live cells in darkness plus fenitrothion
3. freeze-killed cells in light plus fenitrothion
4. freeze-killed cells in darkness plus fenitrothion.

The experimental sets were repeated at least twice at two different time periods. In all sets, five ml of $90 \times 10^4 \text{ cells mL}^{-1}$ concentration of live or freeze-killed cells were added to 5 ml of a 'hot': 'cold' fenitrothion mixture ($0.6 \mu\text{g mL}^{-1}$ 'hot' ^{14}C ring

labelled fenitrothion :20 $\mu\text{g ml}^{-1}$ 'cold' fenitrothion) in 50 ml culture tubes to yield final concentrations of 45×10^4 cells ml^{-1} and 10 $\mu\text{g ml}^{-1}$: 0.3 $\mu\text{g ml}^{-1}$ 'cold': 'hot' fenitrothion. Dark treatment tubes were wrapped in aluminum foil. The series of 4 treatment sets were placed together on racks at a 20° incline on a Eberbach shaker, either under natural sunlight (800 w m^{-2}), or Vita Lite[®] high intensity fluorescent lamps (Duro-Test, Toronto, Ont., Canada, 50 w m^{-2} , 290-700 nm).

At the end of each experimental period, 8 ml of medium were taken from each tube and centrifuged for 8 minutes at 6000 rpm. One ml of the supernatant was removed and counted in a liquid scintillation counter (Packard LSC, model 2000 CA, Downers Grove, Ill., USA). The supernatant and pellet remaining in each tube were vigorously mixed and filtered under vacuum through a glass fibre filter (Whatman GF/A). Each centrifuge tube was rinsed three times with 5 ml of distilled water and poured on the same filter. The filter was rinsed 3 times with 5 ml of distilled water with each rinse remaining on the filter for one minute. The filter was dried at room temperature, weighed, immersed in scintillation cocktail and analyzed by LSC. Sample counts (cpm) were automatically corrected for background and quenching, and counting efficiency (dpm) was determined by the external standard method.

3.1.2. ¹⁴C Ring Label TLC Study.

(i) Light and Dark Regimes. Treatment sets consisting of live and freeze-killed algae were exposed to Vita Lite[®] lamps and darkness for 25 hours (time of greatest accumulation in ¹⁴C ring

labelled fenitrothion experiments (Caunter & Weinberger, 1988)). Control sets consisted of ¹⁴C ring labelled fenitrothion and media only. The initial procedure of algal separation followed the accumulation procedure. The isolated algal pellet was extracted with 2 ml of acetone. The acetone slurry was reduced to 50 µl and spotted on a 2 cm wide strip of Silica Gel Eastman Chromagram Sheet (non-fluorescent)(Eastman Kodak Company, USA). Each sheet was developed in a 3:1 cyclohexane:ethyl acetate mixture and allowed to dry. The strip was cut into 0.5 cm pieces which were immersed in liquid scintillation solution and counted in a liquid scintillation counter (Packard LSC, model 2000 CA, Downers Grove, Ill., USA). For comparison purposes, counts below a Rf of 0.5 were considered to be polar metabolites and above 0.5 were either nonpolar metabolites and/or fenitrothion.

Analysis of polar and nonpolar metabolites in media was as follows. Fenitrothion and chloroform dissolved metabolites were extracted following the procedure listed under fenitrothion extraction (Sec. 3.2.1). However, the reduced solution of chloroform was spotted on a TLC plate and developed. Four mL of the water fraction were removed and evaporated to near dryness. Acetone was used to spot this fraction on a separate TLC plate and the plate analyzed.

(ii) **Aerobic and Anaerobic Regimes.** Light (Vita Lite[®]) treatments were extended under aerobic conditions (air) and anaerobic conditions (N₂ bubbled through system) to determine whether oxidative metabolites could be present. Algae extraction and TLC

plating procedures were identical.

3.1.3. Pigment Analysis. Fenitrothion treated sets consisting of live and freeze-killed algae were exposed to Vita Lite[®] lamps and darkness for 25 hours. Five ml of algal suspension were removed and filtered through a Millepore filter (HA. 0.45 μm). Each filter was subsequently dissolved in 5 ml of 90% acetone and the extract analyzed in a Varion-Cary UV-Visible Spectrophotometer (Varion Techtron Pty. Ltd., Australia)

3.2 Fenitrothion Persistence in Media

Experimental procedure was the same as the 3.1.1 accumulation experiments with the following exceptions. Control sets of fenitrothion and media only were run concurrently as the fenitrothion and algae in media sets. Only cold fenitrothion was used at the same final concentration of 10.3 $\mu\text{g mL}^{-1}$. Experiments were conducted under Vita Lites[®] only in the same environmental conditions (50 Wm^{-2} , 23°C). At the end of each experimental time period the media was centrifuged and 6 mL of the supernatant was quickly frozen at -20°C.

3.2.1. Fenitrothion Extraction. The frozen filtered media were quickly brought up to room temperature, four mL removed and immediately extracted three times with 5 ml of chloroform. The chloroform solution was evaporated down to near dryness on a Brinkman Roto-evaporator (Toronto, Ont., Canada) and brought up to 4 mL in acetone for GC analysis.

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3.2.1. Fenitrothion Extraction. The frozen filtered media were quickly brought up to room temperature, four mL removed and immediately extracted three times with 5 ml of chloroform. The chloroform solution was evaporated down to near dryness on a Brinkman Roto-evaporator (Toronto, Ont., Canada) and brought up to 4 mL in acetone for GC analysis.

3.2.2. Gas Liquid Chromatography (GC) Analysis. Samples were analyzed with a Hewlett-Packard model 5880 gas liquid chromatograph (GC) equipped with a flame phosphorous detector. The glass column (1.0 m x 2 mm (id.)) contained 3% SE 30 Ultraphase on Chromosorb W-80-100 mesh. Column temperature was 195°C, gas flow was 40 mL min⁻¹ of nitrogen and detector flow was 500 ml min⁻¹ of air and 35 mL min⁻¹ of hydrogen. Peak areas of sample injections were compared with injections of fenitrothion standards and µg mL⁻¹ of fenitrothion remaining in the media were calculated.

C - RESULTS

1.0 Effects of Fenitrothion on *Chlamydomonas segnis*

1.1 Growth Study

1.1.1. Algae Cell Number. The effects of $10.3 \mu\text{g mL}^{-1}$ fenitrothion on *C. segnis* are shown in Figure 2 (Table 1, Appendix IV). After 2 days, there was an overall significant decrease ($p < 0.05$ in a Tukey's Multiple Range Test) in algae cell numbers in fenitrothion treated media as compared with controls. Rate of growth during exponential phase decreased from 0.933 day^{-1} in media controls to 0.763 day^{-1} in media with fenitrothion. Carrying capacity of the media dropped from $3.32 \times 10^6 \text{ cells mL}^{-1}$ to $2.36 \times 10^6 \text{ cells mL}^{-1}$ in media with fenitrothion. At carrying capacity of the media, the cells were noticeably larger in fenitrothion treated media.

1.1.2. Algae Dry Weight. Fenitrothion treated *C. segnis* cells showed a significant increase in dry weight during the first four days of growth compared to controls (Figure 3, Table 2 (Appendix IV)). At day one treated cells showed the largest increase in dry weight over media only cells (85%). By days 3 and 4 the dry weights of the treated cells were approximately 30% greater than the nontreated cells. A MANOVA test indicated a significant ($p < 0.05$) increase in dry weight in fenitrothion treated algae in the whole treatment period.

1.1.3. Algae Pigments. Chlorophyll a and b per cell in both treated and nontreated *C. segnis* are listed in Tables 3a and 3b (Appendix IV) and Figures 4a and 4b. Until day 3 there was no significant difference in either Chlorophyll a and b in both treated and nontreated sets (Figures 4a and 4b). From day 4 until 7, chlorophyll a and b contents per cell were significantly higher in the fenitrothion treated algae compared with media only controls. As the algae populations reached the end of exponential phase and approached carrying capacity, chlorophyll a and b per cell started to increase in both the fenitrothion treated and control sets of algae. A MANOVA treatment of the data indicated a significant increase ($p < 0.05$) in chlorophyll a and b in algae treated with fenitrothion versus controls.

1.1.4. Photosynthesis. Photosynthetic rates ($\text{pg O}_2 \text{ min}^{-1} \text{ cell}^{-1}$) as measured by Clark electrode are shown in Figure 5a (Table 4, Appendix IV). A MANOVA test indicated no significant difference between treated and nontreated algal sets at the 0.05 level. However, there were significant increases in photosynthetic rates per cell of fenitrothion treated *C. segnis* over nontreated control sets between days 4 to 6. These findings correspond with increases in chlorophyll a and b in treated algae from day 4 to 7.

Photosynthetic rates as measured by $\text{pg O}_2 \text{ min}^{-1} \text{ pg chlorophyll a}^{-1}$ show a different phenomenon (Figure 5b). There were significant decreases in photosynthetic rates in fenitrothion treated sets for the first three days. By day four, photosynthetic rates per pg chlorophyll a were the same as controls.

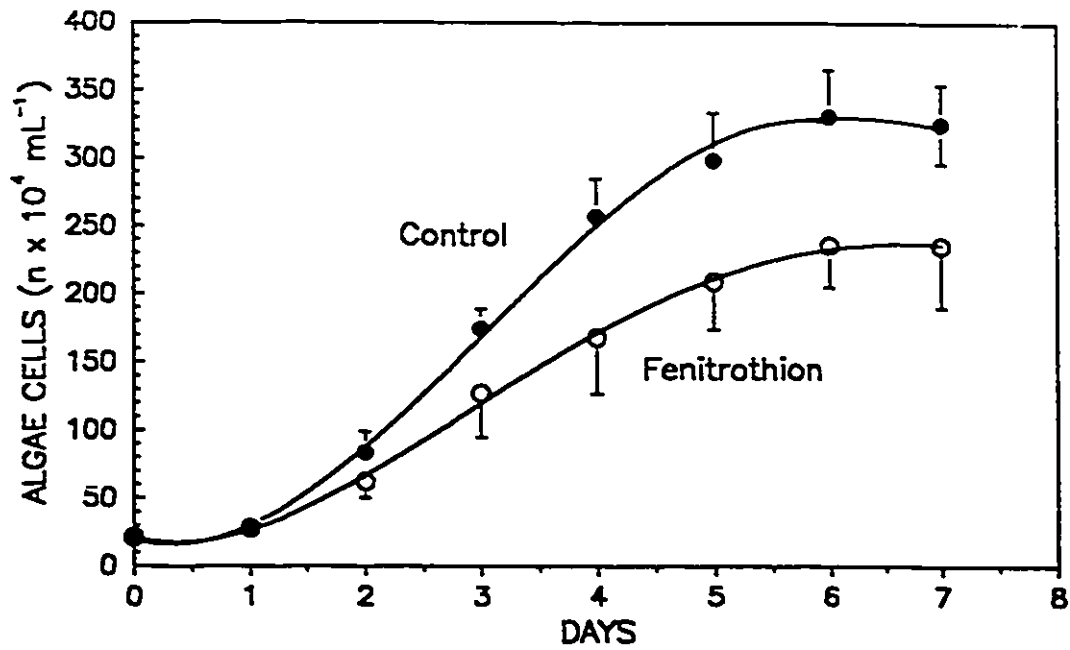


Figure 2. Growth curve of *C. segnis* treated with $10 \mu\text{g ml}^{-1}$ fenitrothion and control (Kuhl's media only) under fluorescent lights (5 w m^{-2} , 24 hours light, 23°C).

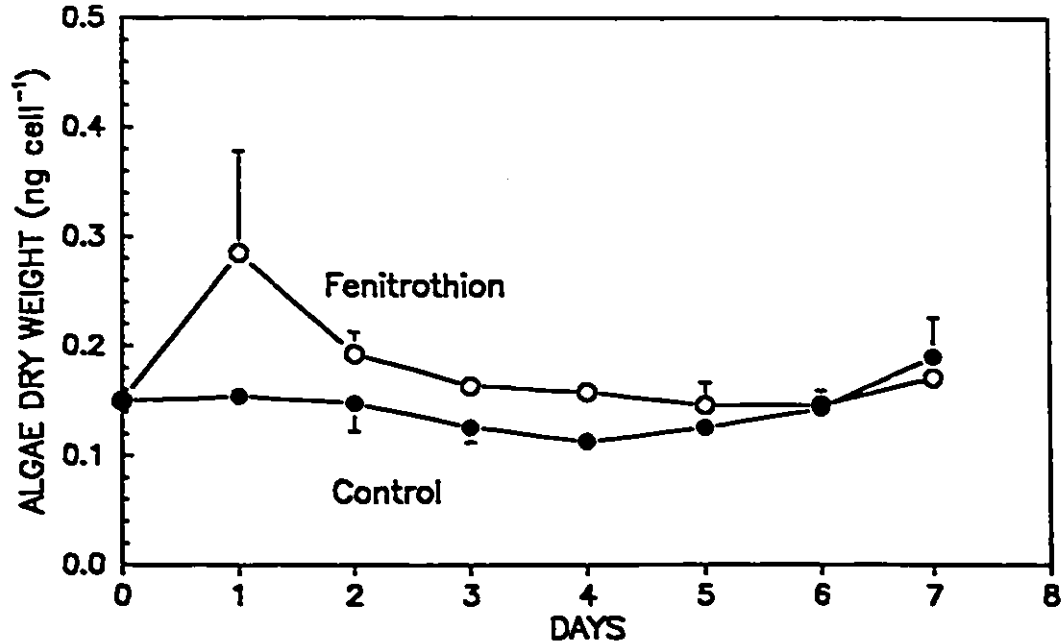


Figure 3. Dry weights of *C. segnis* treated with $10 \mu\text{g ml}^{-1}$ fenitrothion and controls under fluorescent lights (5 w m^{-2} , 24 hours light, 23°C).

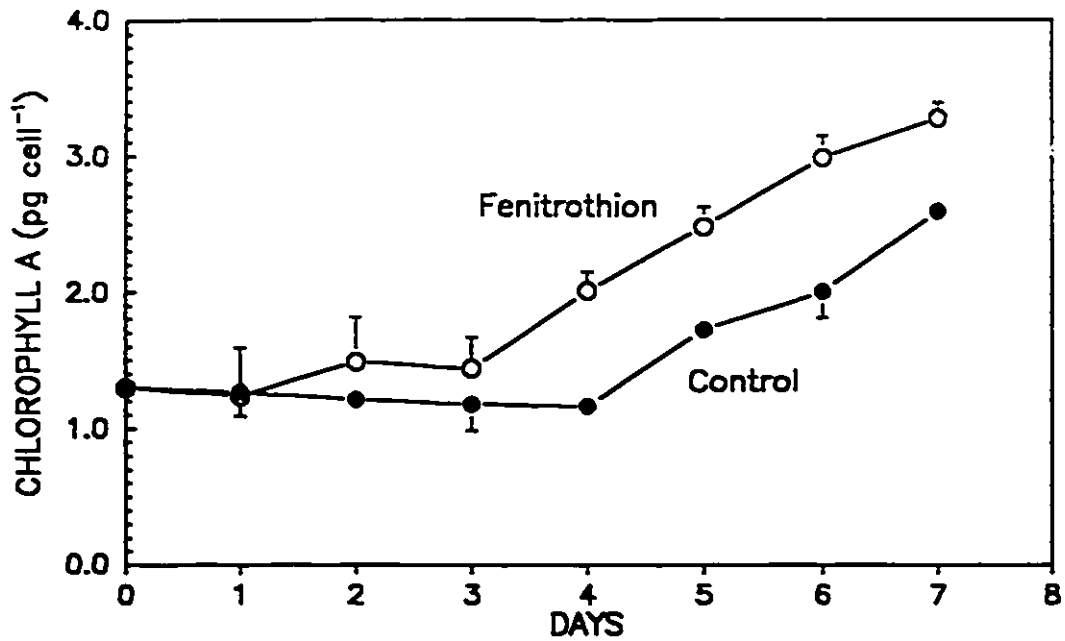


Figure 4a. Chlorophyll a content of *C. segnis* treated with $10 \mu\text{g ml}^{-1}$ fenitrothion and controls under fluorescent lights (5 w m^{-2} , 24 hours light, 23°C).

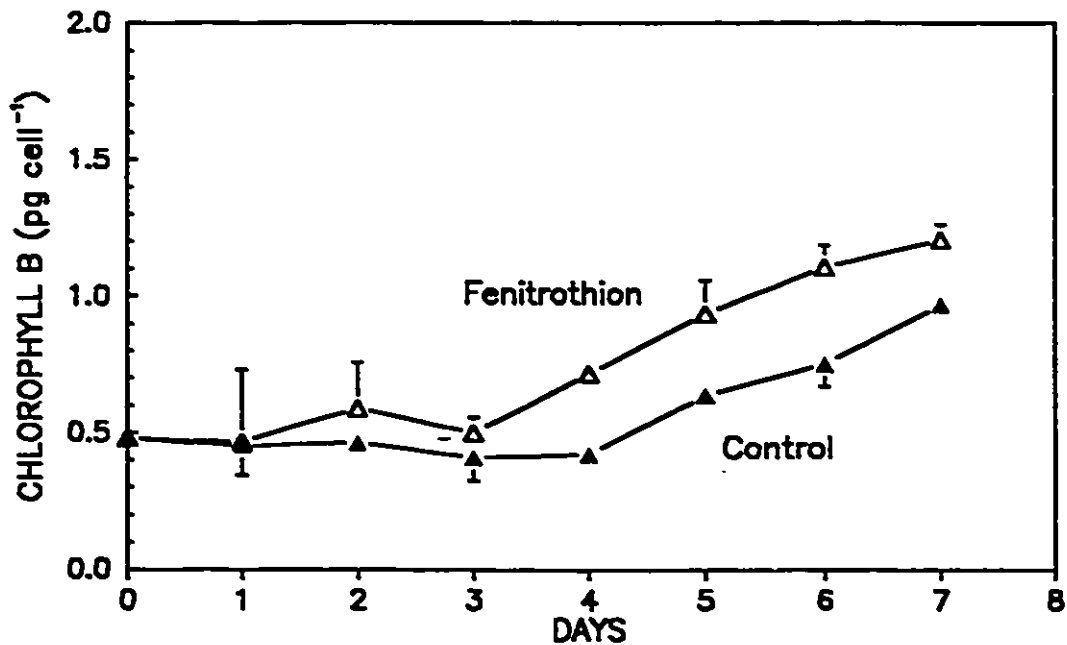


Figure 4b. Chlorophyll b content of *C. segnis* treated with $10 \mu\text{g ml}^{-1}$ fenitrothion and controls under fluorescent lights (5 w m^{-2} , 24 hours light, 23°C).

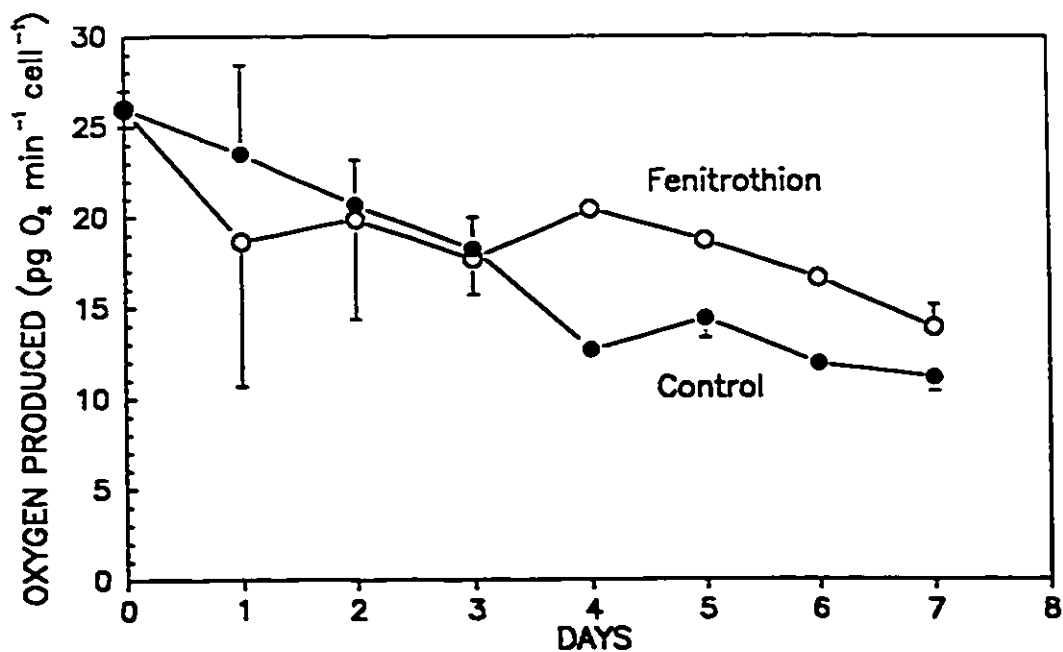


Figure 5a. Photosynthetic rates (measured by oxygen production) per cell of *C. segnis* treated with 10 $\mu\text{g ml}^{-1}$ fenitrothion and controls under fluorescent lights (5 w m^{-2} , 24 hours light, 23°C).

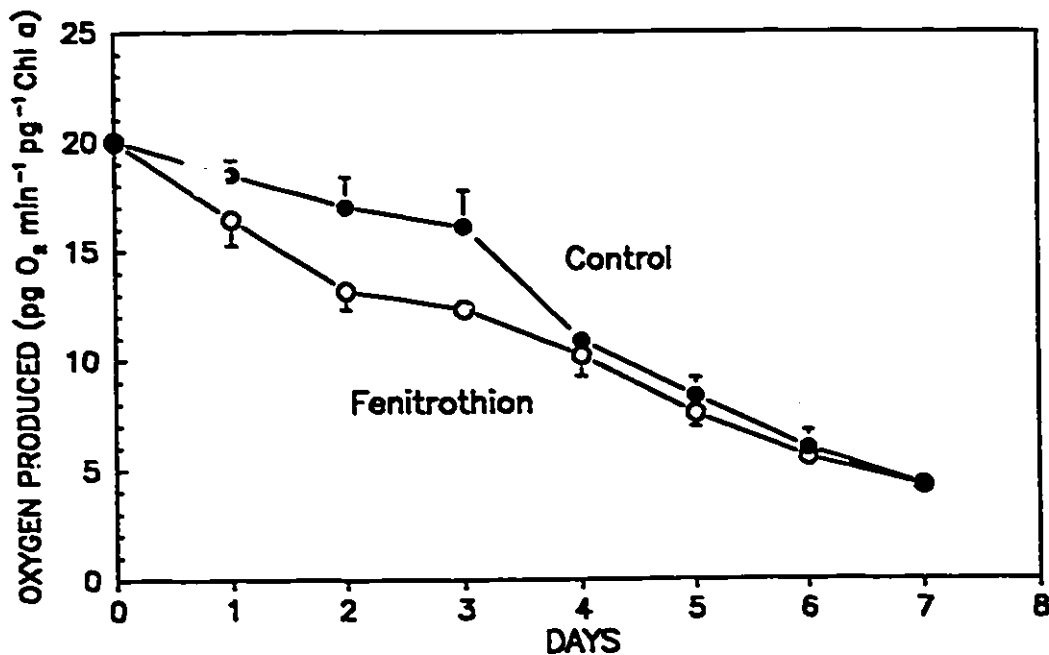


Figure 5b. Photosynthetic rates (measured by oxygen production) per chlorophyll a of *C. segnis* treated with 10 $\mu\text{g ml}^{-1}$ fenitrothion and controls under fluorescent lights (5 w m^{-2} , 24 hours light, 23°C).

1.5 Algae INT Assay

1.5.1. Live Algae. Experimental trials measuring INT reduction of fenitrothion treated and control algal sets were conducted under sunlight during the summer. INT reduction was significantly higher ($p < 0.05$) in the fenitrothion treated cells (Figure 6a). Only the first three hours were analyzed statistically because complete chlorosis of the algae was observed after this time. Live algal cells kept in the dark indicated the opposite reaction. Algae exposed to fenitrothion showed a significant decrease ($p < 0.05$) in INT reduction compared with controls (Figure 6b).

1.5.2. Freeze-Killed Algae. Freeze-killed algal sets kept under either sunlight or darkness conditions showed no significant changes ($p > 0.05$) in INT reduction rates between fenitrothion treatments and media only controls (Figures 7a and 7b).

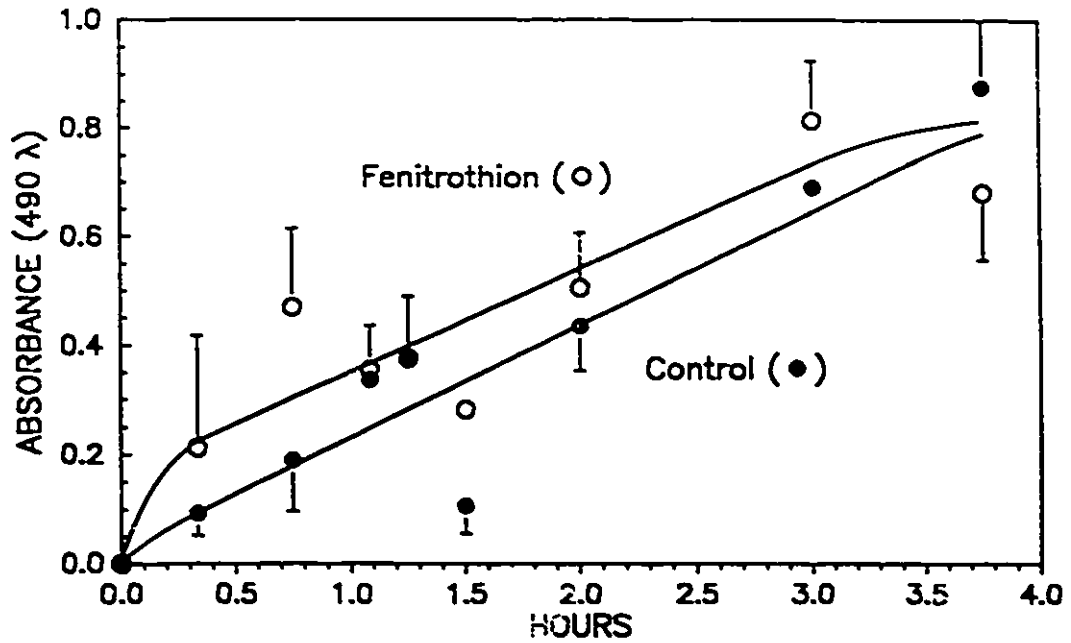


Figure 6a. INT reduction in live *C. segnis* treated with $10.3 \mu\text{g ml}^{-1}$ fenitrothion and media only controls under sunlight (800 w m^{-2} , 23°C).

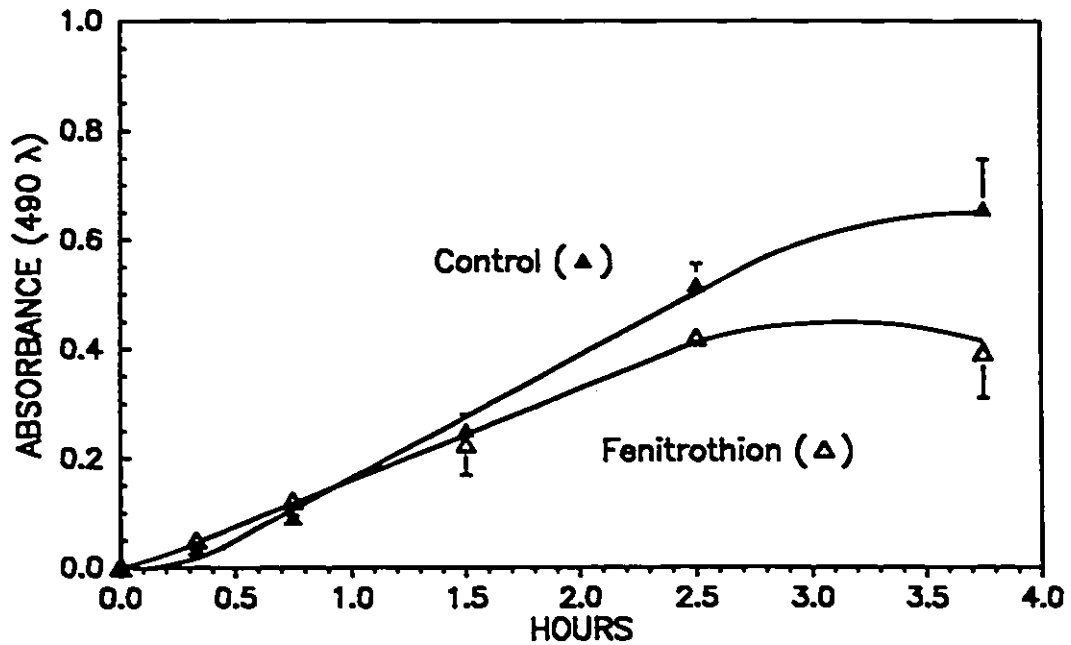


Figure 6b. INT reduction in live *C. segnis* treated with $10.3 \mu\text{g ml}^{-1}$ fenitrothion controls under darkness (23°C).

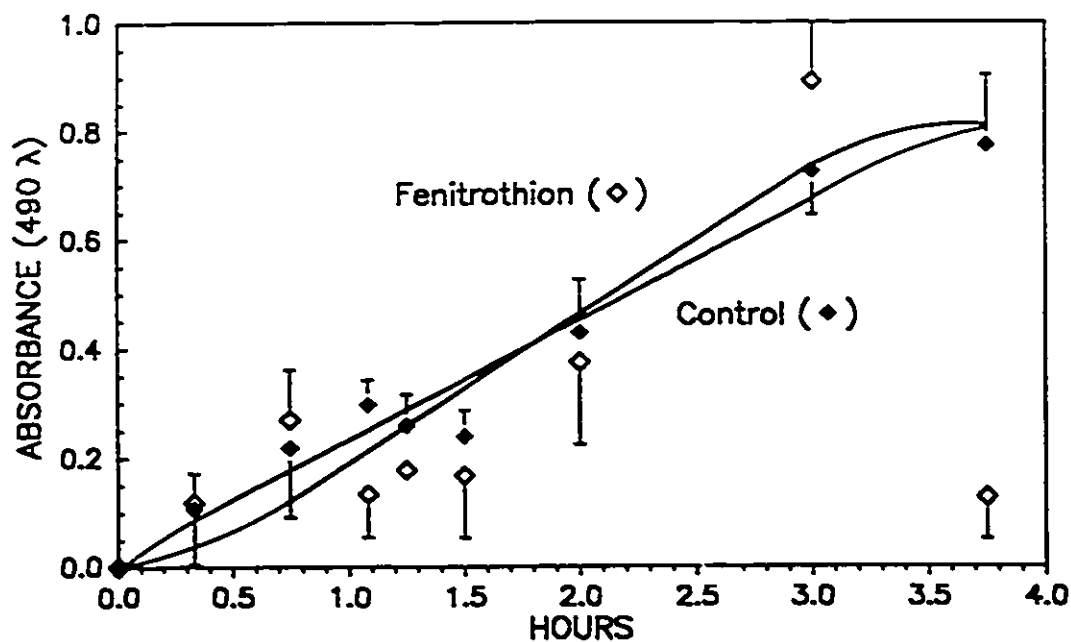


Figure 7a. INT reduction in freeze-killed *C. segnis* treated with $10.3 \mu\text{g ml}^{-1}$ fenitrothion and controls under sunlight (800 w m^{-2} , 23°C).

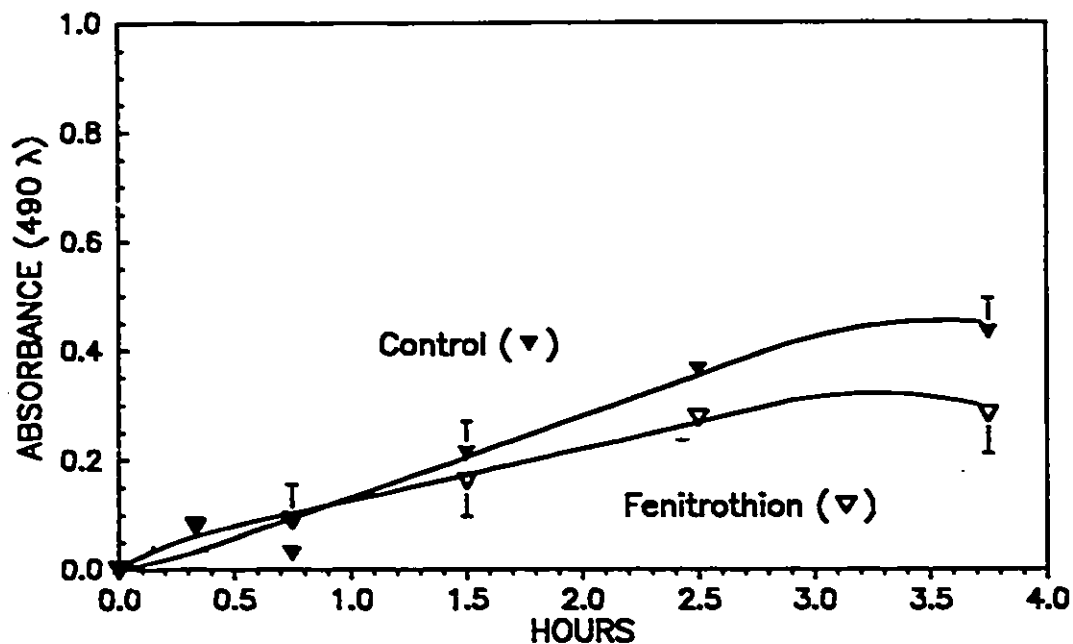


Figure 7b. INT reduction in freeze-killed *C. segnis* treated with $10.3 \mu\text{g ml}^{-1}$ fenitrothion and controls under darkness (23°C).

2.0 Fenitrothion Interaction Studies

2.1 Uptake of Fenitrothion in Algae

2.1.1. Total ^{14}C Ring Label Uptake Study

(i) Live Algae. Experiments monitoring the uptake of ^{14}C ring labelled pesticide were originally conducted under natural sunlight (800 w m^{-2}). The uptake in live cells increased significantly ($p < 0.05$) in the light (Figure 8a, Table 5 (Appendix IV)). Accumulation peaked under sunlight after 3.75 hours, with 13.6 times the amount of ring labelled compound sorbed in the light ($9.4 \mu\text{g } ^{14}\text{C}$ ring label mg^{-1} algae) compared with sets kept in the dark ($0.7 \mu\text{g } ^{14}\text{C}$ ring label mg^{-1} algae). The initial rate of uptake until 3.75 hours was $2.61 \mu\text{g mL}^{-1} \text{ hr}^{-1}$.

In parallel experiments, Vita Lite[®] fluorescent lights (50 w m^{-2}) were used instead of natural sunlight, to maintain constant and reproducible light and temperature regimes. Again, the same phenomenon was observed (Figure 8b, Table 6 (Appendix IV)). Significantly more ^{14}C ring labelled compound was sorbed in algae kept in the light compared with sets in the dark ($p < 0.05$). In live algae, uptake peaked at 25 hours with 20 times more in the light than in the dark ($16.6 \mu\text{g } ^{14}\text{C}$ ring label mg^{-1} algae in light versus $0.8 \mu\text{g } ^{14}\text{C}$ ring label mg^{-1} algae in dark). The rate of uptake was $0.65 \mu\text{g mL}^{-1} \text{ hr}^{-1}$. While uptake was much faster in natural sunlight (the intensity was 16 times greater), the total amount sorbed in natural sunlight was less than that found in the sets kept under Vita Lites[®].

(ii) Freeze-killed Algae. Uptake of ring labelled compound was significantly increased ($p < 0.05$) under sunlight in freeze-killed cells (Figure 9a, Table 7 (Appendix IV)). In freeze-killed cells uptake peaked in the light after 3.75 hours, with 10 times more pesticide sorbed per mg algae ($15.7 \mu\text{g } ^{14}\text{C}$ ring label mg^{-1} algae in light versus $1.6 \mu\text{g } ^{14}\text{C}$ ring label mg^{-1} algae in dark). Uptake of the ring label was at a rate of $3.95 \mu\text{g mL}^{-1} \text{hr}^{-1}$, greater than the live algae.

Under Vita Lites[®], sorption of ring label by freeze-killed algae peaked by 15 hours and decreased after 25 hours. There was 12.6 times more uptake into the algae in the light than in the dark ($28.8 \mu\text{g } ^{14}\text{C}$ ring label mg^{-1} algae in the light versus $2.3 \mu\text{g } ^{14}\text{C}$ ring label mg^{-1} algae in the dark). The rate of uptake was $1.28 \mu\text{g mL}^{-1} \text{hr}^{-1}$ until the peak accumulation. The freeze-killed cells had an apparent higher gross uptake of ring labelled compound on a dry weight basis (Figure 9b, Table 8 (Appendix IV)). This was due to lysing of algal cells observed after the freezing process. After 28 hours, disintegration of most of the freeze-killed cells had occurred. Weighing errors of remaining particulates resulted and the subsequent analysis of uptake of fenitrothion was too variable to provide meaningful data.

2.1.2. ^{14}C Ring Label TLC Metabolite Study. The algal fractions were extracted and the extract completely applied on the TLC sheets. Polar and nonpolar ring label residues after the TLC development were easily identified. In contrast, determination of polar and nonpolar residues in the media fraction was more complex.

As outlined in the methodology, fenitrothion and chloroform dissolved metabolites were extracted from the media fraction and spotted on the TLC plates. The water fraction of the media should only contain polar metabolites (Boulton, 1980). A verification was run where the water was evaporated and transferred into acetone. The acetone extract was analyzed on a TLC plate and all ring label remained at or near the origin, indicating polar metabolites. The polar fractions from both the chloroform extraction and the remaining water fraction were combined and compared to the nonpolar metabolites.

(i) **Light and Dark Regimes.** Polar and nonpolar percentages of the total dpm of algae and media extractions were calculated and are shown in Figure 10. Under Vita Lites² in both live and freeze-killed algae, the algae extractions showed 71% of the radioactivity counted remained at the origin, indicating polar metabolites were the major form of ¹⁴C ring labelled compound present in the algae. In total, 78.7% and 77.6% of the ring label was polar (Rf below 0.5) in live and freeze-killed algae. Dark treated live and freeze-killed cells had substantially lower quantities of ¹⁴C ring label of which 77% was found to be actual fenitrothion. Of the ring label present in the dark treated sets, 16.7% and 21.4% were polar metabolites in the live and freeze-killed algae, respectively.

When media extractions of ¹⁴C ring labelled compound were spotted on plates and counted, the major proportion was identified as non-polar fenitrothion in both light and dark controls, with

some polar metabolites detected in the light treatments (Only 14.0% and 17.9% were polar in the live and freeze-killed sets). Dark treatments were similar to a control of standard ^{14}C ring labelled fenitrothion.

(ii) **Aerobic and Anaerobic Regimes.** Percent polar and nonpolar products from algae and media extractions are shown in Figure 11. After 24 hours in live algae under aerobic conditions, 77.1% of the ^{14}C ring label present was polar. In anaerobic conditions, only 33.2% of the ^{14}C ring label was polar, indicating a significant decrease ($p < 0.05$) of oxidative metabolites. In freeze-killed algae, in aerobic conditions 65.2% of the ring label was polar, while in anaerobic conditions only 42.8% of the ring label was polar.

Media extractions showed a major component of ^{14}C ring label to be nonpolar. Aerobic treatments indicated increased degradation of fenitrothion, with approximately 20% of the ^{14}C ring label found to be polar.

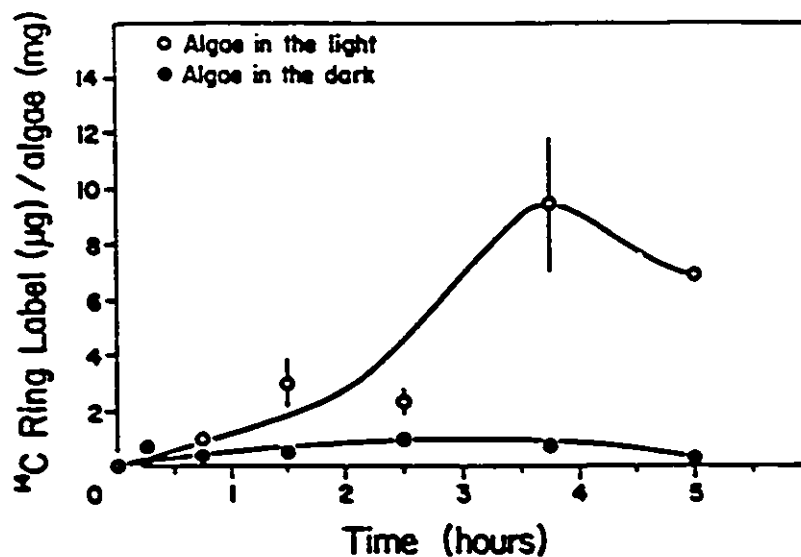


Figure 8a. Uptake of ^{14}C ring label in live *C. segnis* treated with $10.3 \mu\text{g ml}^{-1}$ ^{14}C ring labelled fenitrothion under sunlight (800 w m^{-2} , 23°C) and darkness.

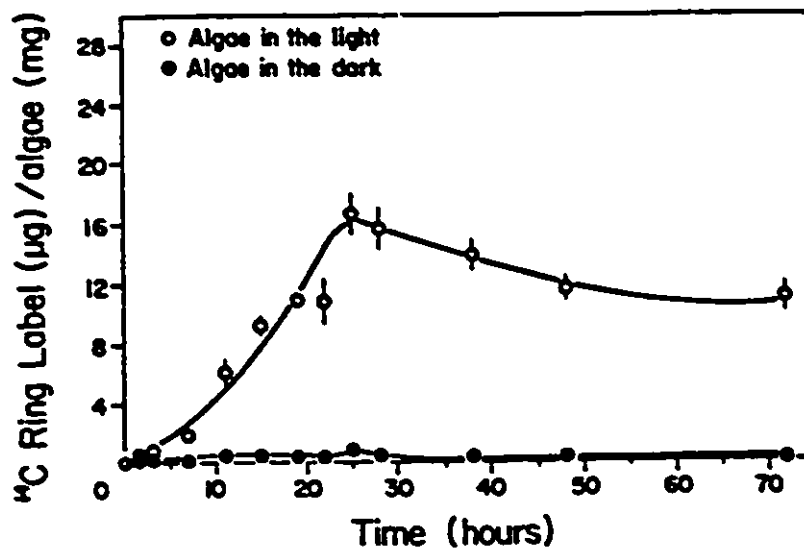


Figure 8b. Uptake of ^{14}C ring label in live *C. segnis* treated with $10.3 \mu\text{g ml}^{-1}$ ^{14}C ring labelled fenitrothion under Vita Lites² (50 w m^{-2} , 23°C) and darkness.

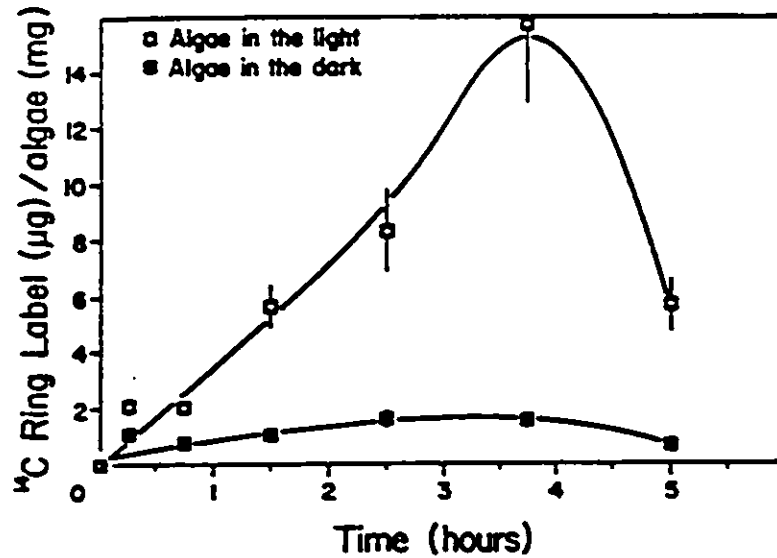


Figure 9a. Uptake of ^{14}C ring label in freeze-killed *C. segnis* treated with $10.3 \mu\text{g ml}^{-1}$ ^{14}C ring labelled fenitrothion under sunlight (800 w m^{-2} , 23°C) and darkness.

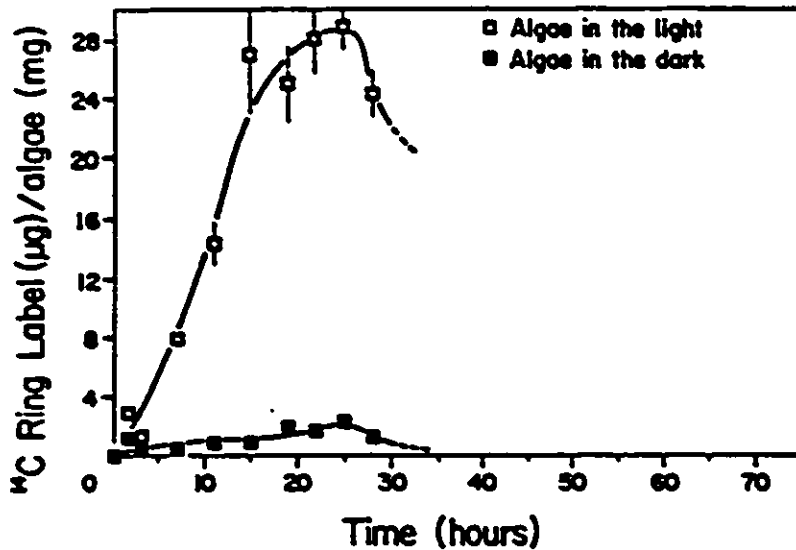


Figure 9b. Uptake of ^{14}C ring label in freeze-killed *C. segnis* treated with $10.3 \mu\text{g ml}^{-1}$ ^{14}C ring labelled fenitrothion under Vita Lites² (50 w m^{-2} , 23°C) and darkness.

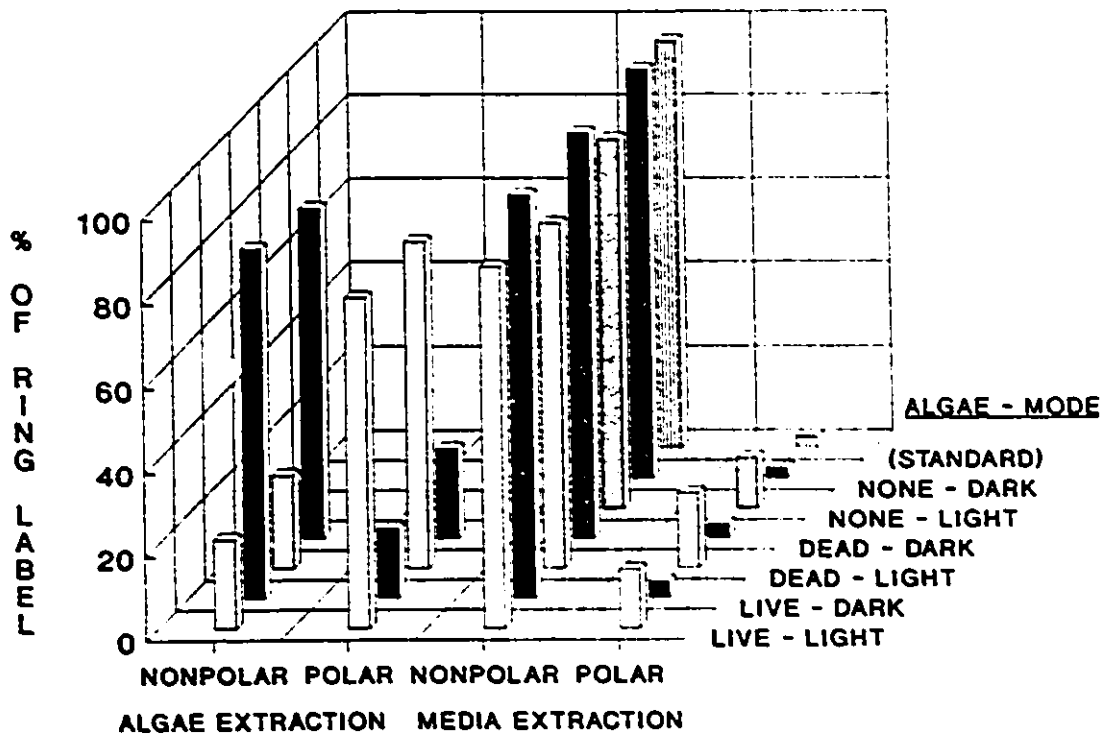


Figure 10. Percent polar and nonpolar ^{14}C ring label in live and freeze-killed algae and media extractions under Vita Lites[®] and dark conditions for 24 hours (50 w m^{-2} , 23°C).

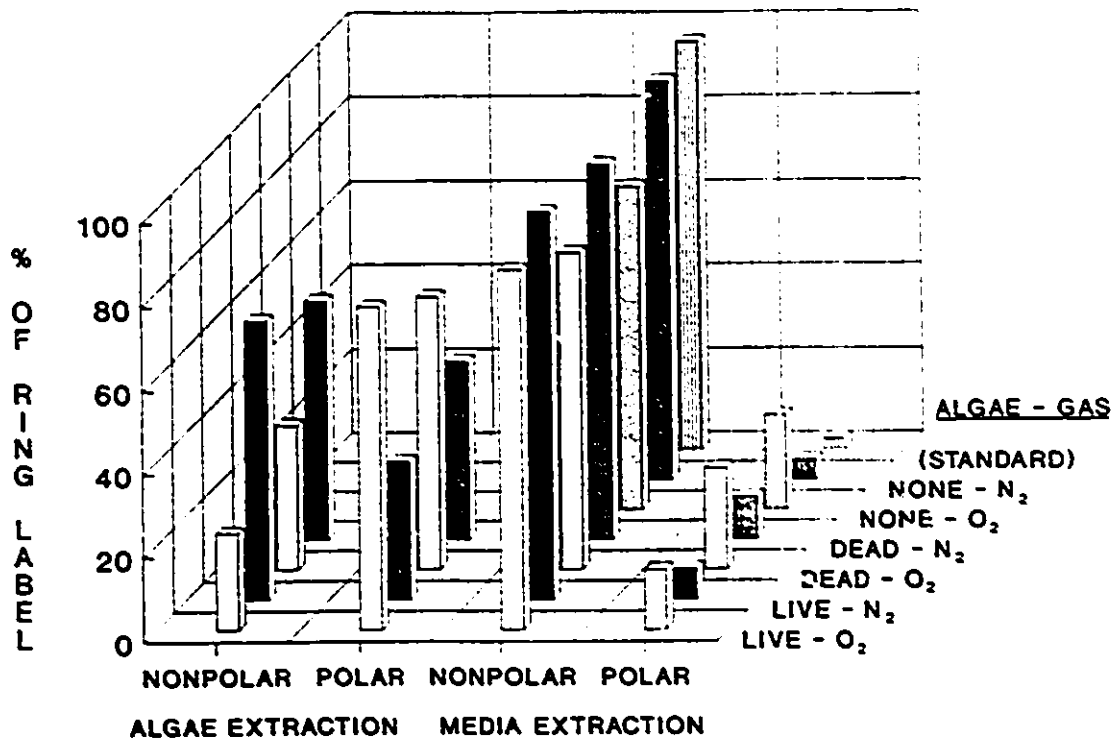


Figure 11. Percent polar and nonpolar ¹⁴C ring label in live and freeze-killed algae and media extractions under Vita Lites[®] in aerobic and anaerobic conditions for 24 hours (50 w m⁻², 23°C).

3.1.3. Measurement of Algal Pigments. In natural sunlight the complete chlorosis in the living and dead cells was obtained following 3.75 and 1.5 hours, respectively. Dead cells under Vita Lite[®]'s were obviously chlorotic after 15 hours of exposure, while live cells held under comparable conditions showed no visible chlorosis. The most light induced uptake and metabolism occurred under Vita Lites[®] in live algae where no chlorosis took place, signifying the importance of healthy metabolism.

The pigment analysis using the Varion-Cary Spectrophotometer at 25 hours of Vita Lite[®] exposure revealed all pigments in the freeze-killed chlorotic algae to be destroyed. Live algae under lights showed a 15% decrease in all pigment concentration, indicating some denaturation by light. After 3 hours exposure to Vita Lites[®], pigment analysis of freeze-killed algae showed substantial reductions in all pigments.

Pigment analysis was possible in the TLC studies as well. The various pigments separated on the TLC plates and were easily identified. TLC separations of pigments are shown in Figure 12. The observed pigments could have been the following; G₁ (dark green) was chlorophyll b, G₂ (light green) was chlorophyll a, Y (yellow) was carotenes, Gr (gray) was phaeophytin, and Y-O (yellow-orange) could have been either carotenes or xanthophyll (Meidner, 1984). The light-dark TLC extractions indicated all pigments to be present in dark controls and live algae under lights. Freeze-killed algae contained no pigments. The oxygen-nitrogen TLC extractions showed all pigments were present in nitrogen treated

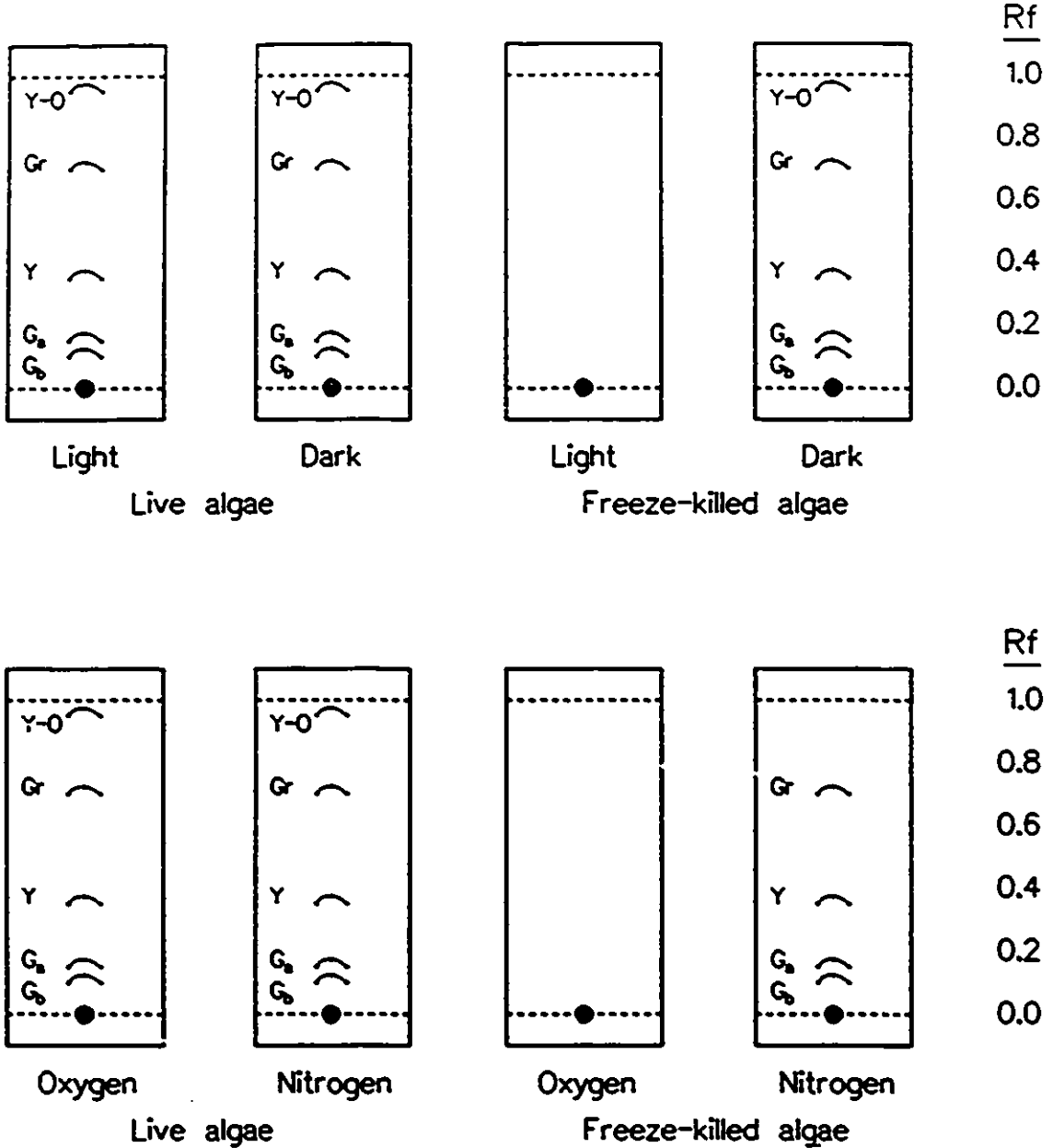


Figure 12. TLC separation of pigments (3:1 cyclohexane:ethyl acetate) on silica gel plastic plates. Pigment colours are listed by G₁ and G₂ for green, Y for yellow, Gr for grey, and Y-O for yellow-orange.

environments and in live algae under aerobic conditions. Only freeze-killed algae in aerobic conditions had no pigments.

2.2 Persistence of Fenitrothion in Media

2.2.1. Media Containing Live Algae. When live algae were present in the media, depletion of fenitrothion significantly increased ($p < 0.05$ by a MANOVA test) compared with controls (media only). Under Vita Lite[®] lamps, fenitrothion levels in the media with live algae decreased in a first order reaction with a rate constant of $4.3 \text{ E-}02 \text{ hr}^{-1}$ (Figure 13a, Table 9 (Appendix IV)). Fenitrothion levels in media alone showed a first order disappearance with a rate constant of $2.2 \text{ E-}02 \text{ hr}^{-1}$. The $t_{1/2}$ of the fenitrothion in the media significantly decreased from 30.7 hours to 16.1 hours in the systems that were irradiated.

Under dark conditions, fenitrothion levels decreased in the media slowly. Fenitrothion depletion was slightly increased in media with live algae with a rate constant of $0.76 \text{ E-}02 \text{ hr}^{-1}$ as compared to the controls (media only) (rate constant of $0.49 \text{ E-}02 \text{ hr}^{-1}$) (Figure 13b, Table 10 (Appendix IV)). The $t_{1/2}$'s of fenitrothion in media and algae decreased to 91.5 hours from 140.1 hours in media only.

2.2.2. Freeze-killed Algae. When freeze-killed algae were combined with fenitrothion in the media, the same phenomena was observed, although at a reduced level. Under Vita Lites[®], fenitrothion decreased at a rate constant of $2.4 \text{ E-}02 \text{ hr}^{-1}$ resulting in a $t_{1/2}$ of 29.1 hours (Figure 14a, Table 11 (Appendix IV)) (The control

with the media only showed the degradation of fenitrothion with a rate constant of 1.5 E-2 hr^{-1} and a $t_{1/2}$ of 45.8 hours). A MANOVA test of the data indicated a significant ($p < 0.05$) decrease in fenitrothion levels in the presence of algae.

The fenitrothion levels with freeze-killed algae under dark conditions decreased with a rate constant of $0.39 \text{ E-02 hr}^{-1}$ and the $t_{1/2}$ was greater than 150 hours (Figure 14b, Table 12 (Appendix IV)). In the media only, the fenitrothion decreased with a rate constant of 0.44 E-2 hr^{-1} and had a $t_{1/2}$ of over 150 hours. There was no significant difference ($p > 0.05$) in fenitrothion depuration between media with freeze-killed algae and media controls in darkened conditions.

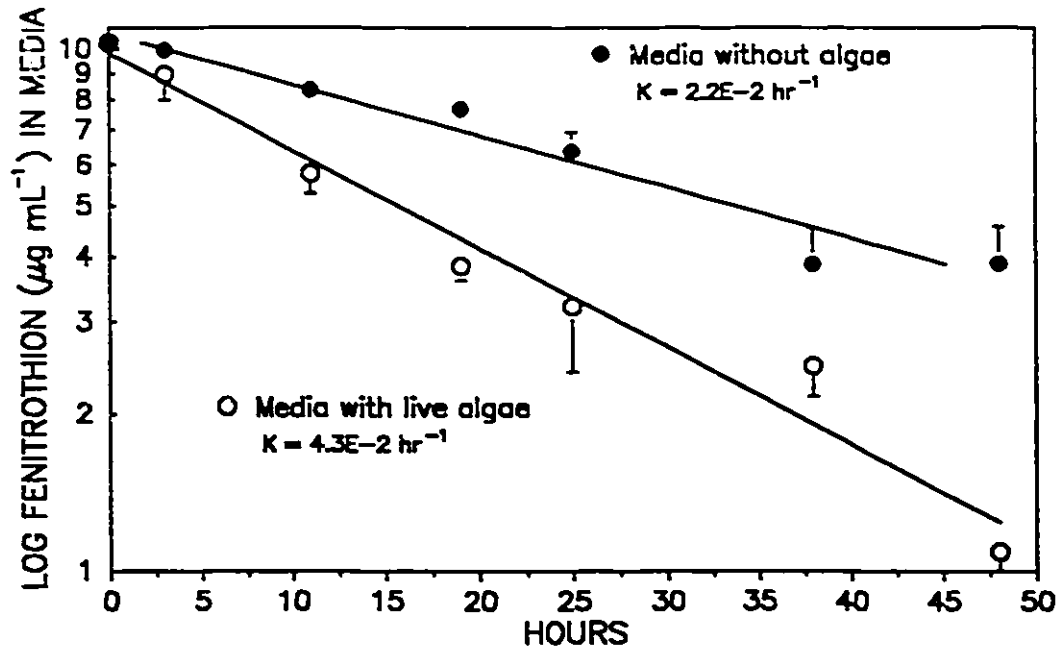


Figure 13a. Depletion of fenitrothion in media containing live *C. segnis* treated initially with $10.3 \mu\text{g ml}^{-1}$ fenitrothion and media only controls under Vita Lites[®] (50 w m^{-2} , 23°C).

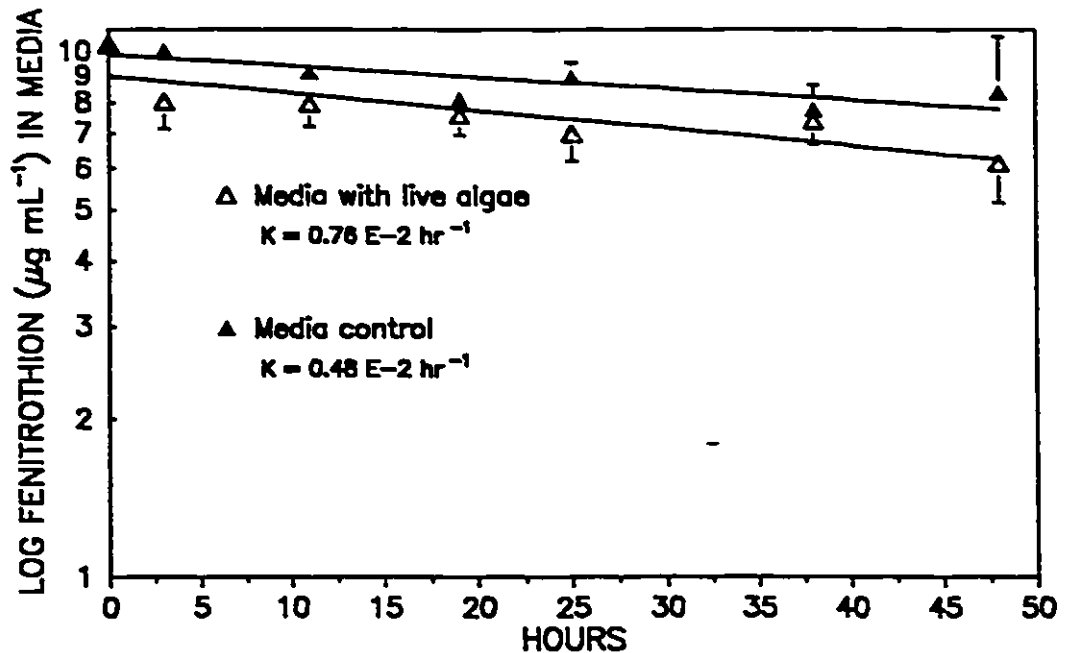


Figure 13b. Depletion of fenitrothion in media containing live *C. segnis* treated initially with $10.3 \mu\text{g ml}^{-1}$ fenitrothion and media only controls under dark conditions (23°C).

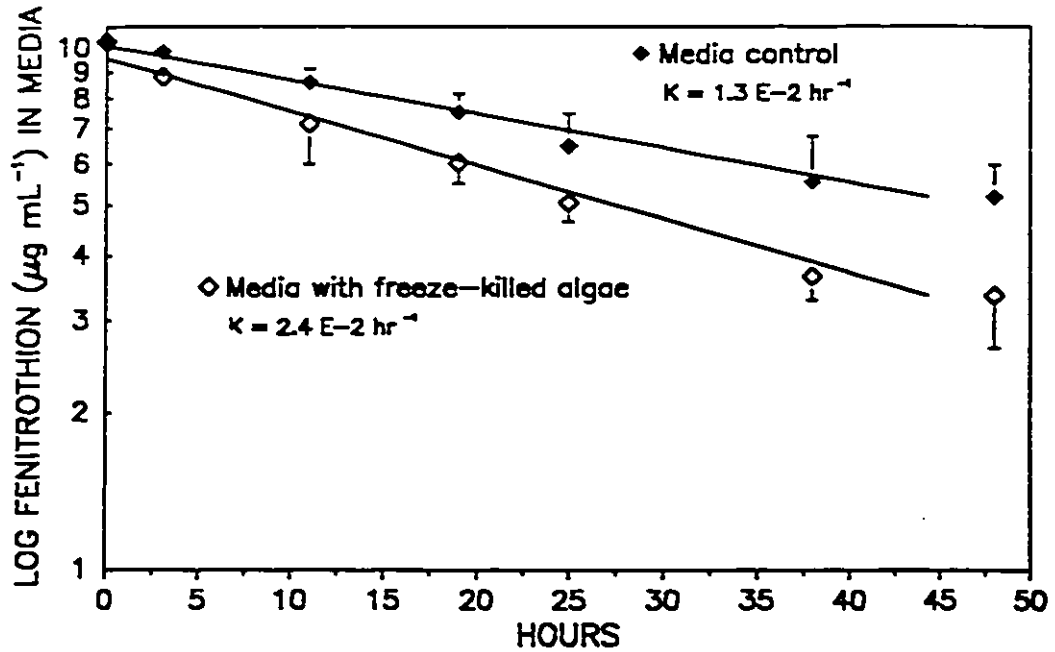


Figure 14a. Depletion of fenitrothion in media containing freeze-killed *C. segnis* treated initially with $10.3 \mu\text{g mL}^{-1}$ fenitrothion and media only controls under Vita Lites[®] (50 w m^{-2} , 23°C).

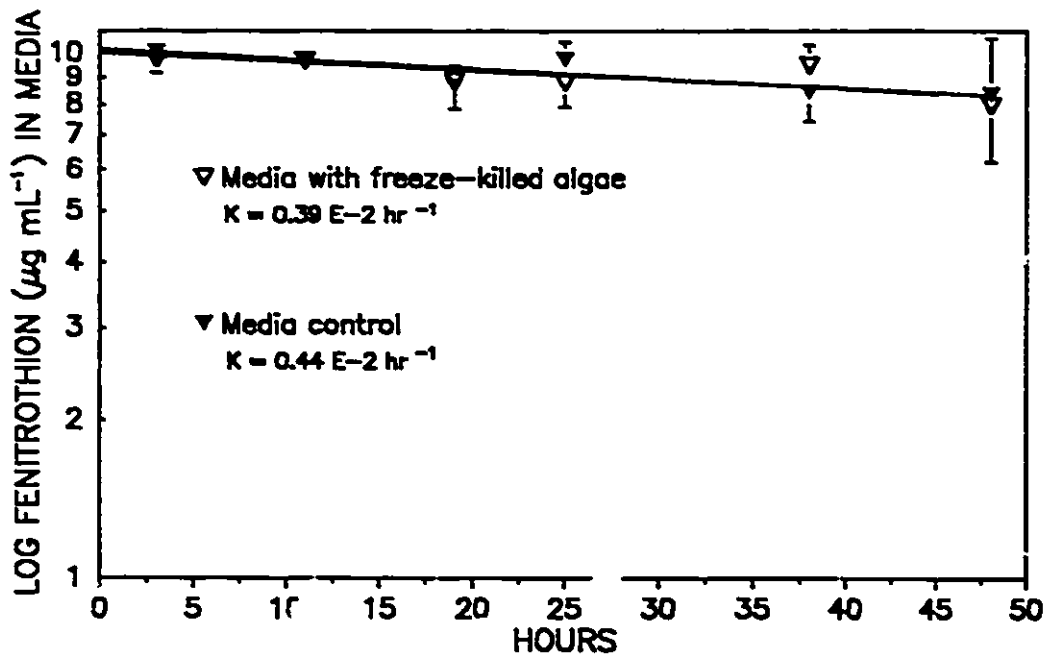


Figure 14b. Depletion of fenitrothion in media containing freeze-killed *C. segnis* treated initially with $10.3 \mu\text{g mL}^{-1}$ fenitrothion and media only controls under dark conditions (23°C).

D - DISCUSSION

This study has demonstrated that illuminated *Chlamydomonas segnis* played a significant part in increased fenitrothion degradation and depletion in an aquatic medium. Enhanced photolysis of fenitrothion in algae appears to be a photooxidative process, thus supporting the proposed hypothesis.

1.0 *Chlamydomonas segnis* Studies

1.1 Fenitrothion Concentration

A major criterion for measuring pesticide toxicity on algae is a relevant concentration of fenitrothion to use for experimental purposes. A pesticide concentration was needed that would stress the organism but not cripple physiological processes to the extreme of an algicidal response (Death of the algae following chronic exposure (Payne & Hall, 1979)). Preliminary experiments indicated that 10 $\mu\text{g mL}^{-1}$ of fenitrothion caused growth inhibition in *Chlamydomonas segnis*. Fenitrothion at 100 $\mu\text{g mL}^{-1}$ caused an algistatic response (no net change in cell number after chronic exposure but does not cause death (Payne & Hall, 1979)) and 1 $\mu\text{g mL}^{-1}$ fenitrothion showed little effect on algae growth (Kent, 1985). As the data shows, with an exposure of 10 $\mu\text{g mL}^{-1}$ fenitrothion, the major effect on algae was reduced cell division, resulting in lower cell numbers. Physiological responses such as photosynthesis and chlorophyll synthesis were not affected but the algae were

obviously under stress.

In the natural aquatic environment, fenitrothion applied aerially could result in maximum concentrations of $4.13 \mu\text{g mL}^{-1}$ in the top 1 cm of a water body situated within the spray region (Moody, 1982). This value could double where spray overlap occurs, especially over rivers and lakes used as boundary markers (Symons, 1977, Varty, 1980). Thus for experimental purposes, a concentration of $10 \mu\text{g mL}^{-1}$ of fenitrothion is relevant as compared to possible maximal field concentrations. This concentration also facilitated experimental design and extraction procedures.

1.2 Growth Study

Although the number of *C. segnis* cells were reduced in fenitrothion treated sets, the cells were observed to be larger in volume, as compared to controls. These observations coincided with the other growth parameters which were examined. In fenitrothion treated experiments, algae dry weight per cell and chlorophyll a and b per cell were higher than for the controls (Figures 3, 4a and 4b). Subsequently, photosynthesis per cell also increased in the fenitrothion treatments (Figure 5a).

These results are similar to those of Kent and Weinberger (1990), where $10 \mu\text{g mL}^{-1}$ of fenitrothion increased cell volumes and weights of *Chlamydomonas segnis*. Kikuchi *et al* (1984a) also discovered that fenitrothion concentrations of 56 and $100 \mu\text{g mL}^{-1}$ affected various growth parameters of *Chlorella vulgaris*, a unicellular alga. At $100 \mu\text{g mL}^{-1}$ fenitrothion, average cell volume,

dry weight of cell and photosynthetic $^{14}\text{CO}_2$ fixation per cell were increased over those of the control. Cell replication was inhibited by fenitrothion but chlorophyll and dry weight of each cell increased.

Decreased algal replication observed in this study could be caused by inhibition of RNA and DNA synthesis by fenitrothion. After 24 hours of $100 \mu\text{g mL}^{-1}$ fenitrothion exposure, Kikuchi *et al* (1984a) found a 60 percent reduction of RNA and DNA and substantially lower amounts of leucine, uracil, and thymine in *Chlorella vulgaris*. As a result, cell volume increased slowly and cell division was retarded. Kent and Weinberger (1990) found growth rates of algae were reduced by 64% when exposed to fenitrothion levels of $10 \mu\text{g mL}^{-1}$. The growth rate in this study decreased 18.2%, from 0.933 day^{-1} in media controls to 0.763 day^{-1} in media with $10 \mu\text{g mL}^{-1}$ of fenitrothion.

Other organophosphorus compounds have affected biosynthesis and metabolic processes in algae. When *Chlorella ellipsoidea* cells were treated with $10 \mu\text{g mL}^{-1}$ of the herbicide S-2846¹, RNA and carbohydrate synthesis were inhibited (Sumida & Ueda, 1974). $10 \mu\text{g mL}^{-1}$ of the organophosphorus insecticides malathion² and guthion³ have been shown to inhibit DNA, RNA and protein synthesis in

¹ S-2846: O-ethyl O-(3-methyl-6-nitrophenyl)-N-sec-butyl phosphoramidate

² malathion: O,O-dimethyl S-1,2-di(ethoxycarbonyl) ethyl phosphorodithioate

³ guthion: O,O-dimethyl S-[(4-oxo-1,2,3-benzotriazin-3,(4H)-yl)methyl] phosphorodithioate

Dictyostelium dicoideum (Bushway, 1978). Clegg and Koevenig (1974) showed that $100 \mu\text{g mL}^{-1}$ of diazinon¹ reduced ATP levels in three species of freshwater algae, subsequently reducing metabolism and protein synthesis. Christie (1969) reported the total population size of a mixed algal community was temporarily repressed by $100 \mu\text{g mL}^{-1}$ of malathion.

During the course of the growth study, there were changes in the observed parameters that most likely were independent of fenitrothion exposure. For example, the dry weights per cell of the control sets started to increase as the population reached carrying capacity or stationary phase. Fenitrothion treated sets showed a smaller increase by day 7 (Figure 3). These observations are a result of the ratio between cell numbers and dry weight of the cells. Cell numbers of an algal population tend to increase quickly and plateau near the carrying capacity of the population while dry weights per cell show a slower rate of increase (Maestrini *et al.*, 1984). Therefore the ratio between dry weight and cell number will increase due to a cell size expansion near the carrying capacity of the population.

Chlorophyll concentrations per cell showed dramatic increases in both control and fenitrothion treated algae after day 4 of the growth experiments (Figures 3a and 3b). Again this phenomenon is caused by the ratio between cell number and chlorophyll concentrations of the cells. Chlorophyll concentrations increase at a

¹ diazinon: *O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate

slower rate compared to cell numbers in a population (Maestrini et al., 1984), probably because of the complexity of chlorophyll biosynthesis. As the initial lag phase of chlorophyll biosynthesis ended, the actual increase of cell numbers began to decline. Therefore chlorophyll content per cell showed an increase. The stressed population of fenitrothion treated cells reached the stationary phase before the controls. Subsequently, chlorophyll concentration per cell of the fenitrothion treatments started to increase before the controls (day 4 versus day 5).

On a photosynthetic rate per cell basis, fenitrothion treated cells showed increases of photosynthesis by day four (Figure 5a). These increases are most likely a result of the chlorophyll concentrations of the cells. By day four, the chlorophyll concentrations of fenitrothion treatments were higher, thus resulting in increased photosynthesis over controls. Similar results were recorded by Kikuchi et al. (1984a), where high concentrations of fenitrothion caused increased photosynthetic $^{14}\text{CO}_2$ fixation per cell in *Chlorella vulgaris* corresponding with higher chlorophyll a levels.

During the 7 day growth experiment, chlorophyll per cell concentrations increased yet photosynthesis rates per cell showed an overall reduction. While it would be expected that an increase in chlorophyll concentration would lead to an increase in photosynthesis per cell, the decrease in photosynthesis is probably due to shading effects. The light source used for the Clark electrode was unidirectional. As the cells were being stirred in the reaction

chamber they would undergo some shading by other cells, especially as cell concentrations increased. A preliminary experiment was initially run to test photosynthetic rates versus cell concentrations. As shown in Figure 17 in Appendix III, there was a slight decrease in photosynthetic rates as cell concentration increased to a maximum of 6.0×10^5 cells mL^{-1} . In the growth experiment cell concentrations increased to over 20.0×10^5 cells mL^{-1} . Thus it would follow that shading effects would increase and photosynthesis decrease. Another limiting factor could have been nutrient levels.

If the photosynthesis rate was calculated on a per chlorophyll a basis, fenitrothion caused an initial inhibition of photosynthesis (Figure 5b). Other organophosphorus pesticides are known to affect photosynthesis. Sances *et al.* (1981) reported that parathion and methyl-parathion reduced photosynthesis in lettuce possibly by inhibiting certain processes in the chloroplasts. Parathion also inhibited photosynthesis in *Chlorella pyrenoidosa* (Cole & Plapp, 1970). Studies by Moody *et al.* (1983) and Moody (1982) determined 10-20 $\mu\text{g ml}^{-1}$ treatments of fenitrothion could totally inhibit photosynthesis in *Chlamydomonas reinhardtii* as determined by fluorometric measurements. Any inhibition of electron transport before the PS-II donor side would cause a sharp decrease in the fluorescence levels (Murthy, 1983) while a blockage of the ETS between PS centres could be indicated by increases of fluorescence. However, mechanisms of organophosphorus pesticide induced inhibition of photosynthesis are difficult to access with the current paucity of information.

1.3 INT Assay

The INT reduction in dark treated experimental sets was significantly decreased in the presence of fenitrothion only in live algae sets. In biotic systems, the INT's site of action in the respiratory electron transport system is at the cytochrome b complex or at the flavoprotein level in the presence of Triton X-100 (Nachlas et al., 1960; Lester & Smith, 1961). Because respiration is closely associated with active cellular metabolism, the deposition of formazan from INT is a good indicator of dehydrogenase activity in living cells (Zimmerman et al., 1978). Such enzyme activity could be classified as respiratory potential or electron transport system (ETS) activity (Jones & Simon, 1979). Any toxic effect of a toxicant on a respiring organism resulting in a decrease or increase of ETS activity would be indicated with INT (Trevors et al., 1983). Fenitrothion under darkened conditions decreased ETS in the *Chlamydomonas segnis* as measured by INT. Whether the fenitrothion directly or indirectly interfered with ETS is difficult to determine.

The small observed INT reduction in freeze-killed cells was probably the result of the large amounts of succinate, NADH, and NADPH added to the reaction tubes. Although not significant at the 0.05 level, there was some decreased INT reduction at 3 hours by the fenitrothion (Figure 7b). The ETS of the freeze-killed algae should be non-functioning, therefore it is likely that the fenitrothion might have competed with the INT for the surplus of

electrons provided by the succinate.

Experiments monitoring INT reduction in live *C. segnis* under light conditions showed the opposite result to the dark. Actual INT reduction was increased in the presence of fenitrothion (Figure 6a). In a cytochemical study of tetrazolium reduction by *Vicia faba* chloroplasts, Imaizumi & Hiraoka (1982) isolated the site of INT photoreduction to be in the PS-II of the chloroplasts. Their results were based on the fact that the additions of DCMU¹, DCPA², MCC³, CIPC⁴, and CAT⁵ at low concentrations inhibited formazan production by chloroplasts under illumination remarkably. Other studies showing DCMU inhibition on the photoreduction of INT have been documented by Howell and Moudrianakis (1967) and Shumway and Park (1969).

If INT is reduced in the PS-II of chloroplasts, it follows that an increased INT reduction in cells exposed to fenitrothion would indicate fenitrothion could be initially blocking the ETS between PS-II and PS-I. Excited electrons from the PS-II complex could be reducing the INT. This supports the earlier growth experiment showing fenitrothion induced photosynthetic inhibition. However, it is difficult to correlate the initial photosynthetic inhibition to the initial increased INT reduction because the two

¹ DCMU: 3-(3,4-dichlorophenyl)-1,1-dimethylurea

² DCPA: 3',4'-dichloropropionanilide

³ MCC: methyl 3,4-dichlorocarbanilate

⁴ CIPC: isopropyl *m*-chlorocarbanilate

⁵ CAT: 2-chloro-4,6-bis(ethylamino)-s-triazine

studies were performed under completely different environmental conditions (INT studies under 800 w m² sunlight and photosynthesis studies under 5 w m² fluorescent lights). Under sunlight, photosynthesis halted after 30 minutes exposure and pigments were bleached by 1.5 hours. Further experiments would be needed to identify the INT site of action and fenitrothion effects on photosynthesis.

2.0 Fenitrothion Interaction Studies

2.1 Light Regimes.

Light regimes are a very important factor when examining any light related parameters such as accumulation and photolysis rates of chemicals, especially photolytically degradable pesticides such as fenitrothion. Care should be taken when examining photolysis rates using natural sunlight versus indoor lighting (Miller & Zepp, 1983).

Early photolytic studies of fenitrothion tended to use ultraviolet light (UV) lamps or mercury lamps with high levels of UV light (Greenhalgh & Marshall, 1976, Ohkawa *et al.*, 1974). Fenitrothion is a strong absorber of UV (Greenhalgh & Marshall, 1976). As shown in Figure 18 of Appendix V, UV absorption of fenitrothion in methanol peaks at 210 and 270 nm, gradually trailing off to no absorbance by 360 nm (Weinberger *et al.*, 1981b). Under UV light regimes, photolysis rates of fenitrothion were reported to have been lower and there were some minor differences in metabolites when compared to studies with natural sunlight

(Ohkawa et al., 1974).

Laboratory studies of fenitrothion and algae have been carried out under regular cool white fluorescent lamps (Kikuchi et al., 1984b; Weinberger et al., 1981b; Weinberger et al., 1982b). They reported some increases in fenitrothion accumulation in *C. segnis* and *Chlamydomonas reinhardtii* and resulting reductions in fenitrothion half lives ($t_{\frac{1}{2}}$) under the lamps. In contrast, a preliminary experiment studying fenitrothion uptake by algae during the growth experiments of this research (Figure 20, Appendix VI) showed no significant levels of fenitrothion in *C. segnis*. This growth study was conducted under cool white fluorescent lamps with a very low intensity of 5 w m^{-2} .

Experiments conducted under natural sunlight and Vita Lite[®] high intensity full spectrum lamps show distinguishing characteristics. Fenitrothion uptake in *C. segnis* was greatly enhanced by natural sunlight and Vita Lite[®] lamps (Figures 8a to 9b). $T_{\frac{1}{2}}$'s were decreased dramatically under Vita Lite[®] lamps in the presence of algae. Studies by Weinberger et al. (1982b) and Mikami et al. (1985) have shown similar results under natural sunlight. Sunlight intensity and quality can vary according to latitude, season and time of day (Zepp & Schlotzhauer, 1983; Roberts et al., 1981b). Some attempt was made during the course of the study to monitor the intensity of the sunlight and an average of 800 w m^{-2} was calculated. However, the experimental period spanned a period of three months and experiments were conducted at various times of the day. While light intensities of sunlight (800 w m^{-2}) are 16 times higher

than Vita Lite[®] lamps (50 w m⁻²), Vita Lite[®] lamps provided a consistent intensity of light as compared to natural sunlight. Consequently, the uptake data from Vita Lite[®] lamps had less deviation as compared to the data from natural sunlight.

Varying light intensities of light sources used for these photolytic studies could explain many of the differences of the uptake and persistence experiments. Of equal importance, however, would be the light spectrums of the light sources. Figure 19 (Appendix V) shows the wavelengths of cool white fluorescent lights, sunlight and Vita Lite[®] fluorescent lamps, all compared at relative irradiance (Hughes & Neer, 1981). Cool white fluorescent lamps have very low amounts of near-UV light. Apart from a small peak of near-UV light of 310 nm, only at 360 nm is there any significant irradiance. The relative spectrum of Vita Lite[®] lamps is very similar to natural sunlight. The curves are almost identical, starting at 310 nm and continuing until 760 nm (Figure 19, Appendix V). Vita Lites[®] have substantially more UV in the 310 nm to 360 nm range as compared to cool white fluorescent lights. This particular range of UV light also corresponds to the latter end of fenitrothion absorption (Figure 18, Appendix V). This important spectrum window will be discussed later.

Light wavelengths below 300 nm could affect photolysis rates of fenitrothion under natural light. However, the experiments used Pyrex glass tubes or flasks. Weinberger *et al.* (1981a) showed the Pyrex glass vessels absorbs UV below 300 nm. Extensive fenitrothion photolysis was observed above 300 nm and below 360 nm.

2.2 Uptake of Fenitrothion in Algae

There were differences in the data between natural sunlight and Vita Lite[®] lamps (Figures 8a to 9b). Ring labelled pesticide was taken up at a faster rate under sunlight but algae under Vita Lite[®] lamps had a greater accumulation. Live and freeze-killed algae under Vita Lite[®] lamps accumulated 1.6 times and 2 times more of the label than algae under sunlight. The faster rate of uptake was most likely a result of the higher light intensity (800 w m^{-2} versus 50 w m^{-2}). However, while the light intensity was 16 times greater, rates of uptake were only 4 and 3.1 times greater in live and freeze-killed algae under sunlight versus the Vita Lite[®] lamps. If the process involved was strictly a photophysical phenomenon linked directly to light intensity, higher rates of uptake and greater accumulation would be expected.

The rapid uptake and accumulation of ¹⁴C ring label fenitrothion by the unicellular alga *C. segnis* in the present study were consistent with previous studies. Moody (1982) and Weinberger *et al.* (1982b) showed a 2 to 5 fold increase of accumulated fenitrothion in both *Chlamydomonas reinhardtii* and *Chlorella pyrenoidosa* under sunlight conditions. Kikuchi *et al.* (1984b) discovered significant bioaccumulation of fenitrothion in algal species such as *Chlorella vulgaris* and *Nitzschia closterium* under artificial lighting. Zepp and Schlotzhauer (1983) indicated increased pesticide sorption and subsequent metabolism by various species of green and blue green algae under sunlight conditions. However, in contrast to the findings of the present study, they found no

effects of *Chlamydomonas* species on the degradation of fenitrothion.

Before possible mechanisms of this phenomenon can be explored, it has to be clarified whether the observed accumulation of the labelled compound is a result of absorption or adsorption. Adsorption is the process in which a compound adheres to the surface but does not pass through (Brown & LeMay, 1977). Absorption of a compound through a membrane results in its bioaccumulation, the ratio of the concentration of a chemical inside an organism to the concentration in the water (Boyle, 1984). In the accumulation experiment, the algae cells were washed at least three times with water. If there had been any adsorption of fenitrothion, the rinsing should have removed any compound loosely adsorbed to the surface of the cell. However, washing probably would not remove any fenitrothion tightly bound to the cell wall. In support of these assumptions, studies using phenol type herbicides showed no significant adsorption to isolated cell walls of *Ankistrodesmus braunii* (Neumann et al., 1987).

In recent years it has been suggested that steady state bioaccumulation factors of organic chemicals in aquatic plants is dependant upon their lipid contents (Roberts et al., 1981b.). There could be passive uptake of fenitrothion ($K_{ow} = 3800$) into algae which have large quantities of lipids (Weinberger et al., 1982b; Moody, 1982). The permeability of liposomes has been shown to be increased by organophosphorus pesticides (Artunes-Madeira & Madeira, 1979). *Chlamydomonas* species have a large vacuole sur-

rounded by its large chloroplast. Any fenitrothion could easily be sequestered into the lipid fractions of the chloroplast (50-55% lipid (Noggle & Fritz, 1983)).

Upon examination of the accumulation experiments it is obvious that the process of label uptake is light dependant. It has been shown that lipid membranes can be altered by light (Nichols, 1965). The cell membrane potential appears to change upon illumination, the light response being quite complex, with more than one transport system (both active and passive) influencing the direction in which the potential changes (Lucas, 1983). There have been conflicting reports of either light-induced hyperpolarization or depolarization of membrane potentials (Lucas, 1983). Recent studies support membrane hyperpolarization of the macro alga *Chara corallina* under illumination, especially in the presence of HCO_3^- ions (Lucas, 1982). There is strong evidence that the light-induced changes in membrane potentials are correlated to photosynthesis in aquatic plant species (Andrianov *et al.*, 1970; Volkov, 1973). Lucas (1983) suggests that the phenomena of membrane changes is a mechanism by which aquatic plants and algae can assimilate larger quantities of exogenous HCO_3^- to be utilized for photosynthesis. As a consequence of the increased polarization of the membranes, lipophilic compounds such as fenitrothion could be transported with less resistance through the membranes. Further studies are needed to examine this possibility.

There were observed differences in relative amounts of labelled compound accumulated between live and freeze-killed

Chlamydomonas segnis. In a light to dark comparison, label uptake was greater in live cells with a higher ratio. However, on a viability basis, freeze-killed algae accumulated 1.7 times more label than live algae. The cells were frozen in liquid nitrogen, thus undergoing a fast cooling rate. At fast cooling rates it has been proposed that lysing or injury to the plasma membrane occurs, caused by intracellular ice formation (Steponkus, 1984). Rupture of the cell because of the expansion of ice is considered unlikely (Steponkus, 1984).

Wolfe and Steponkus (1983) have suggested that expansion-induced lysis during a freeze-thaw cycle could be a result of severe decreases and increases of tension in the plane of the membrane. Rapid freezing results in a hypertonic condition producing volumetric contractions of the protoplasts. Upon return to isotonic conditions during thawing, rapid entry of water causes incorporation of membrane material into the membrane (Wolfe and Steponkus, 1983). The findings of the accumulation experiment suggest the freezing process did increase the 'porous' nature of the cell membranes. However, the freezing of the cells did not effect the light induced uptake and accumulation of the ¹⁴C ring label compound.

2.3 TLC Study of Metabolites in Algae

The preceding accumulation experiment examined the uptake of ¹⁴C ring labelled compound. Whether or not the ring label compound

detected was still the original ring labelled fenitrothion or ring labelled metabolites was determined with the TLC study. For the purposes of this study, it was not necessary to positively identify metabolites. Most fenitrothion metabolites are more polar than fenitrothion (Boulton, 1980) and thin layer chromatography (TLC) examination of labelled compound was the most straightforward way to determine whether metabolism of the fenitrothion took place.

Once the TLC sheets were developed, it was resolved that an Rf value of 0.5 was the boundary between "polar" metabolites and "nonpolar" fenitrothion. The original ¹⁴C ring label fenitrothion was spotted on the same plate and had a Rf of 0.8. This value was higher than the Rf value of 0.57 listed in the literature (Greenhalgh & Marshall, 1976; Boulton, 1980), both using a 3:1 mixture of cyclohexane : ethyl acetate. The only difference between the former studies and this study was the kind of developing plate used. The other studies used silica gel glass plates while in this study plastic silica gel sheets were utilised. The silica gel coating is slightly thinner on the plastic sheets, thus offering less resistance to the solvent. A check using silica gel glass plates was performed and an identical Rf value of 0.57 was obtained for the fenitrothion control. In the literature, most of the fenitrothion metabolites had Rf values less than 0.35 when the cyclohexane : ethyl acetate developing system was used with the glass plates (Greenhalgh & Marshall, 1976; Boulton, 1980). Assuming the metabolites would move on the plastic sheets in a similar manner as the parent fenitrothion control, the correspon-

ding Rf value would be 0.5 on the plastic sheets. Upon examination of the data, an Rf of 0.5 was clearly a dividing point between the ^{14}C label products and original fenitrothion.

The light and dark TLC study definitely showed a light mediated degradation of fenitrothion inside the algae (Figure 10). The dark treatments showed little degradation. Of the small amounts of label compound found inside algae in the dark treatments, the relative amounts of polar metabolites present were identical to the polar metabolites in the original standard (between 2.8 to 4.0 %). Under light conditions, the algae extractions were completely opposite to the media extractions in both live and freeze-killed algae. Labelled polar metabolites did not diffuse into the algae from the media as there were no differences in % polar metabolites in media with or without algae. Therefore, the polar metabolites were most likely formed inside the algae.

There were significantly greater amounts of polar label metabolites present in either live or freeze-killed algae under Vita Lite[®] lamps. While relative amounts of polar metabolites from the live and freeze-killed algae were equal, the total amount of label compound was greater in live algae than in freeze-killed algae. Therefore, fenitrothion accumulation was enhanced in live algae, yet fenitrothion degradation yielded identical relative amounts of metabolites and the metabolites had similar Rf values.

This suggests that the process was photophysically mediated in both the live and freeze-killed algae. Zepp and Schlotzhauer (1983) found the organophosphorus insecticide, methyl parathion,

was susceptible to algal phototransformation even when the alga *Chlorogonium* was heat killed. However, they did not analyze for metabolites. Kikuchi et al. (1984b) found polar metabolites of fenitrothion in the algae *A. flos-aquae*, *C. vulgaris* and *N. closterium*. The highest absorption of fenitrothion was by dead cells (irradiated by UV light for 12 hours), again implying a photophysical process. Based on the metabolites found, 3-methyl-4-nitrophenol, demethylfenitrothion, fenitrooxon and demethylfenitrooxon, they concluded the degradative pathways for algae were through oxidation of P=S to P=O, hydrolysis of P-O-aryl linkage and O-demethylation. All of these particular metabolites would have Rf's of less than 0.5 if developed with the 3:1 cyclonhexane : ethylacetate on silica gel plastic plates (On glass plates the Rf values of the mentioned metabolites were less than 0.35 (Greenhalgh & Marshall, 1976)). The polar label metabolites from the TLC study could have been the same as those found in the Kikuchi et al. (1984) study. The major portion of the polar label was on or near the origin, with Rf values well below 0.5 (plastic plates), which would correspond to fenitrooxon or demethylfenitrooxon (estimated Rf values (plastic plates) of 0.08 and 0.04, respectively).

The TLC study of fenitrothion degradation under anaerobic (N_2) conditions indicates photooxidation of fenitrothion occurred in the algae. In both live and freeze-killed algae, nitrogen treatments had approximately half the polar metabolites percent present in the air exposed treatments (Figure 11). In the absence of oxygen, degradation of the label was greatly reduced. However, there were

still some polar metabolites (33.2 and 42.8 % in live and freeze-killed cells, respectively) in the anaerobic treatments. The tubes containing the algae were bubbled for ten minutes prior to addition of fenitrothion. There could have been traces of oxygen present inside of the algae when the trial commenced. There might be other processes involved, which will be discussed later. While singlet oxygen is a very potent reactant, other sensitizing pathways could have been responsible for fenitrothion degradation under anaerobic conditions.

The polar metabolites present in the media were most likely from photooxidative degradation of fenitrothion. There were no significant differences between algae-free controls and media with algae.

2.4 Photosensitizers

The hypothesis of the present study was that augmentation of fenitrothion photolysis in algae was the result of photooxidative processes. The alga *Chlamydomonas segnis* did play a significant part in the increased photolysis of fenitrothion and the process is most likely photophysic as suggested by the O₂ removal experiment. Because the phenomenon was paralleled in freeze-killed algae, it would appear algal photosynthetic or metabolic processes contributed little to fenitrothion photolysis.

Algal cell pigment reductions and label accumulation did not occur concurrently in the study. The accumulation studies under sunlight definitely showed chlorosis of algae occurring and in the

case of freeze-killed cells, accumulation of label in algae peaked two hours after the observed chlorosis. These were visual observations of total lack of green colour in the tubes and there could have been other pigments present. However, the pigment analysis of freeze-killed algae under Vita Lites[®] revealed no significant absorption by the spectrophotometer, indicating near total bleaching of pigments. Visual observation of these treatments showed no visible colour near the same time of peak accumulation of label. In contrast, the live cells under Vita Lites[®] had only a 15% decrease in pigment concentration, yet there was a peak accumulation at 25 hours, corresponding to freeze-killed algae. This would indicate that pigment concentration and the uptake of fenitrothion are not directly linked. As mentioned earlier, the accumulation of label was probably a result of light induced changes to the cell membranes.

Chlorosis is pigment bleaching or destruction caused by UV light and high levels of visible light (Powles, 1984). Powles defines this photodestruction as photooxidation which occurs after a time-lag period of photoinhibition (reduction of photosynthesis but no pigment destruction). The active chloroplast has conditions which favour 1O_2 production, i.e. triplet excited molecules and ubiquitous oxygen (Knox & Dodge, 1985). The potential damaging action of 1O_2 is well documented. The chloroplast thylakoid membranes are particularly susceptible to 1O_2 induced lipid peroxidation (Halliwell, 1981). Isolated chloroplast thylakoid membranes have been shown to undergo pigment and lipid breakdown

under conditions favouring 1O_2 production (Takahama, 1975; Percival & Dodge, 1983; Percival & Dodge, 1984).

It follows that the pigments, when exposed to high levels of light and UV light, produced 1O_2 which in turn bleached the pigments. This probably occurred in freeze-killed cells where no electron transport systems were left intact to reduce triplet excited chlorophyll molecules. Live cells under sunlight were most likely overwhelmed by the extreme light intensity and high UV levels. The UV light from the Vita Lites[®] could have caused some photooxidation in live cells as indicated by the 15% reduction of pigments. However, the UV levels were not toxic in this case, since 1O_2 quenching mechanisms such as photosynthetic electron transport and carotenoid pigments do exist in viable cells (Knox & Dodge, 1985).

The production of 1O_2 by light harvesting pigments probably accounted for most of the fenitrothion degradation inside the algae. The evidence of photooxidation of pigments is strongly linked to the presence of polar metabolites of fenitrothion. Further support for this theory is shown by the absence of chlorosis in the anaerobic treatments of freeze-killed algae (Figure 12). There were still chlorophyll (green), and carotenoid (yellow, yellow-orange) pigments present in the freeze-killed algae after 25 hours of light. These treatments contained only 50% the polar metabolites of the aerobic treatments (Figure 11). Freeze-killed algae in aerobic conditions had no pigments and the highest levels of polar metabolites. Therefore, photooxidation of pigments

correlated with increased fenitrothion degradation.

Live algae under aerobic conditions contained equal percentages of polar metabolites as the freeze-killed algae yet pigments were intact (Figures 11 and 12). This would appear to contradict the proposed mechanism of photooxidation. However, there was a 15% destruction of all pigments in fenitrothion treated cells, which would indicate the presence of 1O_2 in the live cells. If there was some fenitrothion inhibition of the photosynthetic ETS of photosynthesis, conditions favouring 1O_2 could prevail. Fenitrothion would undergo photooxidation, although at a slower rate. Quenching mechanisms are still removing some of the 1O_2 . There could be continuous formation of 1O_2 over the whole 25 hour period in the live cells as long as toxic levels of 1O_2 were not produced that would totally destroy the pigments. Perhaps another degradative mechanism was responsible for fenitrothion breakdown in live algae. Excitation energy or electrons could have passed directly from light harvesting pigments to fenitrothion, forming metabolites. Positive identification of the metabolites could clarify possible degradative processes.

Freeze-killed cells had few 1O_2 quenching mechanisms intact and consequently there would be the initial formation of high levels of 1O_2 . The labelled compound moved into freeze-killed cells at a faster rate and photooxidation of fenitrothion should have taken place quickly. 1O_2 levels probably reduced once pigments were destroyed (15 hours under Vita Lites²) and further fenitrothion degradation would decrease. An interesting experiment to confirm

this proposed mechanism would be to compare levels of polar metabolites to levels of pigments over the whole time period using both live and freeze-killed cells. The rates of fenitrothion degradation in the cells could be determined and related to the pigment concentrations.

There are other types of reactions involving oxygen inside algal cells. Oxygen is reduced in respiratory metabolic processes and reduction of oxygen produced from photosynthetic water splitting appears unavoidable upon exposure of O_2 to the strong reductants of the 'Z' scheme of photosynthesis (Rabinowitch & Fridovich, 1983). Reduction of O_2 can result in the formation of the superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH^\cdot), all potentially toxic (Knox & Dodge, 1985). However, there are enzymic defences against both O_2^- and H_2O_2 and cells contain antioxidants which interrupt chain reactions of free radicals such as OH^\cdot (Rabinowitch & Fridovich, 1983).

There is a rising interest in the role of O_2^- , H_2O_2 and OH^\cdot in natural waters. Rapid increases of H_2O_2 and OH^\cdot radical concentrations have been observed from sunlight exposure of waters containing organic constituents such as humic substances (Cooper & Zika, 1983; Choudhry, 1981). Khan and Gamble (1983) have suggested that OH^\cdot radicals generated from UV irradiation of an aqueous solution of humic materials degraded prometryn¹. Zepp *et al.* (1985) proposed that the absorption of sunlight by humic substances in

¹ prometryn: 2-(methylthio)-4,6-bis(isopropylamino)-s-triazine

natural waters led to rapid photosensitized reactions of certain pollutants via energy transfer from molecules in their triplet states. Algal production of H_2O_2 under illumination (Zepp *et al.*, 1986) and darkness (Palenik *et al.*, 1987) is also reported. Zepp *et al.* (1987) have suggested that algal-induced photooxidation of anilines were a result of photogeneration of H_2O_2 .

While these studies are important for the understanding of indirect photooxidative reactions of organic pollutants in aquatic mediums, they ignore processes inside algal cells. There could be production of superoxide, hydrogen peroxide, and/or hydroxyl radicals in the cells, but 1O_2 production would seem to be predominant, as evidenced by pigment denaturation especially in freeze-killed cells. Another factor to consider is the requirement for the presence of oxygen in any photooxidative reaction. There was degradation of fenitrothion under anaerobic conditions as indicated by the presence of polar metabolites (Figure 11). Trace amounts of oxygen could have been available for production of 1O_2 . It is more likely that pigments transferred their excitation energy or electrons directly to fenitrothion. If lipophilic compounds are sequestered into lipid membranes of algae and their chloroplasts, the close proximity to light harvesting pigments would surely facilitate collisions and transfer of excitation energy or electrons. Only the identification of polar metabolites present under anaerobic conditions could determine if fenitrothion was oxidised or degraded in another way.

Thus in reference to Figure 1, it would appear that there are

two indirect photodegradation pathways of fenitrothion in *Chlamydomonas segnis*. One pathway would involve the passing of excitation triplet energy from pigments to the intermediary 1O_2 , which in turn would react with fenitrothion (Equation 4, Figure 1) and was possibly the main pathway. The other pathway would be the direct transfer of excitation energy or electrons from pigments to fenitrothion (Equation 8, Figure 1).

2.5 Fenitrothion Persistence

Chlamydomonas segnis significantly affected the persistence of fenitrothion under lighted conditions. The fenitrothion depuration rates were approximately halved in the presence of either live or freeze-killed algae in the light (Figures 13a and 14a). Fenitrothion disappearance followed first-order kinetics as calculated from regression analysis of the plotted curves. Many natural degradative processes involving low pollutant concentrations follow first- or pseudo-first-order kinetics (Roberts et al., 1981b). One of the properties of a first-order disappearance rate expression is that the $t_{1/2}$ stays the same regardless of the pollutant's concentration (Roberts et al., 1981b). Calculation of the fenitrothion's $t_{1/2}$ at various time periods confirms this assumption.

There are various factors that could affect the rate constants and $t_{1/2}$'s. In a closed system, the algal population would replicate until the carrying capacity of the system was reached. As the algal mass increased, more fenitrothion could be accumulated and the rate constant of fenitrothion depuration would change. As

algae cell numbers increased, shading effects could also occur and subsequent photolysis rates could be changed. Nakumura and Mochida (1988) studied the uptake and degradation of fenitrothion by the alga *Selenastrum capricornutum* and found that the alga-induced rate constants in the steady state of algal growth were 1.3 - 2.5 times larger than those in the exponential growth stage. However, the depuration experiments with *C. segnis* were terminated at 48 hours. It is doubtful that carrying capacity or steady state was reached and the algae were presumably in exponential state (under lower light intensities, the growth curve of *Chlamydomonas segnis* (Figure 16, Appendix II) indicated carrying capacity was reached only after more than 6 days).

The rapid decrease of fenitrothion in the media corresponded to the light-induced accumulation of label observed earlier in the study. Even with peak accumulation of label by 25 hours, fenitrothion continued to disappear from the media. This supported the other experiments showing possible fenitrothion degradation by algae. Movement of fenitrothion from the algae back into the media also appeared limited.

2.6 Relevance to Field Conditions

It is very difficult to extrapolate results and mechanisms from laboratory studies to field conditions. The concentration of fenitrothion used in the laboratory experiments, $10 \mu\text{g mL}^{-1}$, was the maximum concentration calculated on a theoretical basis (Section 1.1 of the Discussion). Under normal conditions, fenitrothion

concentrations would probably never go above $1 \mu\text{g mL}^{-1}$, especially when dilution factors and natural degradative processes would tend to dissipate the pesticide quickly. Other important factors in the field would be light intensity, temperature, pH, and algal concentrations and depth. All these components would greatly influence fenitrothion degradation.

What this kind of study can attempt to do is try to explain some of the observed phenomena in the field. Algae are important primary producers in the aquatic food chain. It is important to study what kinds of effects pollutants can have on algae and how accumulation of these pollutants could pass on to higher trophic levels. Of equal importance is algal-induced changes of pollutants. If algae can function as indirect photosensitizers of organic chemicals, persistence and transport of these chemicals could be influenced by these aquatic microphytes.

In modern environmental management, there is an increasing emphasis on predictive models of pollutant fate in aquatic systems. One such model, developed by Roberts *et al.* (1981a), generalized their view of an aquatic environment to a four compartment model: water, sediment, fish, and a "catchall" compartment. They used the "catchall" compartment to describe suspended particulate matter such as aquatic plants. The purpose of such a model is to predict any possible hazard of a pollutant to the environment. However, such a model does not take into account the importance of algae- and light-induced changes of a pollutant's fate. The significant factor, affecting an organic pollutant such as fenitrothion for

The significant light-induced accumulation of label into algae would affect the $t_{1/2}$ of fenitrothion in the media. In media containing live algae under Vita Lite[®] fluorescent lamps, fenitrothion decreased with a rate constant of $4.3 \text{ E-}02 \text{ hr}^{-1}$, resulting in a $t_{1/2}$ of 16.1 hours as compared to a decrease in rate of $2.2 \text{ E-}02 \text{ hr}^{-1}$ and $t_{1/2}$ of 30.7 hours in media only. Under dark conditions, fenitrothion decreased from the media slowly without any significant differences between media with and without live algae (rate constants of $0.76 \text{ E-}02 \text{ hr}^{-1}$ and $0.49 \text{ E-}02 \text{ hr}^{-1}$, and $t_{1/2}$'s of 91.5 and 140.1 hours, respectively). When freeze-killed algae were combined in fenitrothion in the media, the same phenomena was observed, although at a reduced level. Under Vita lights, fenitrothion decreased at a rate constant of $2.4 \text{ E-}02 \text{ hr}^{-1}$ resulting in a $t_{1/2}$ of 29.1 hours (controls showed fenitrothion depuration with a rate constant of $1.5 \text{ E-}2 \text{ hr}^{-1}$ and a $t_{1/2}$ of 45.8 hours). Under dark conditions, fenitrothion decreased with a rate constant below $0.39 \text{ E-}02 \text{ hr}^{-1}$ and the $t_{1/2}$ was greater than 150 hours (control had a rate constant of $0.44 \text{ E-}2 \text{ hr}^{-1}$ and a fenitrothion $t_{1/2}$ of over 150 hours).

Thin layer chromatography (TLC) analysis of the label in live and freeze-killed algae under Vita Lites[®], revealed 78.7% and 77.6%, respectively, of the label to be polar metabolites of fenitrothion. Of the small amounts of label accumulated by live and freeze-killed algae in darkness, 77% was fenitrothion. The degradation of fenitrothion in the algae in the light was probably a photophysical phenomenon, as identical percentages of polar metabo-

lites were identified in freeze-killed algae with nonfunctioning photosynthetic electron transport systems. TLC analysis of lighted live algae in anaerobic conditions showed that polar metabolites decreased from 77.1% (aerobic control) to 33.2%. Freeze-killed algae showed a decrease of polar metabolites from 65.2% in aerobic conditions to 42.8% in anaerobic conditions. These results indicate that photooxidation of fenitrothion could have occurred.

Total chlorosis of algal pigments was observed in all accumulation experiments under sunlight and freeze-killed algae under Vita Lites[®]. Analysis of pigments indicated a 15% reduction in total pigments in live algae under Vita Lites[®]. Live or freeze-killed algae under anaerobic conditions showed little loss of pigments. Chlorosis of algal pigments seemed to correlate with increases of polar metabolites. As 1O_2 is involved in pigment chlorosis, it might have been responsible for fenitrothion photooxidation. Direct transfer of excitation energy or electrons from light harvesting pigments to fenitrothion could have accounted for the presence of fenitrothion metabolites in anaerobic conditions or when 1O_2 quenching systems in nonchlorotic algae were maintained.

The alga *Chlamydomonas segnis* had a significant effect on fenitrothion persistence and fate under lighted conditions in laboratory microcosms. Additional field studies would be needed to clarify the role of algal indirect photosensitization of lipophilic organic contaminants in aquatic ecosystems. Furthermore, persistence investigations of pollutants and algae should always be carried out under full spectrum light conditions.

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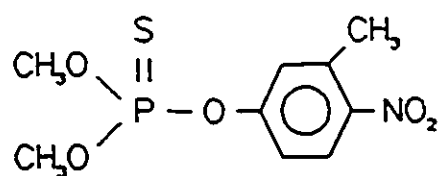
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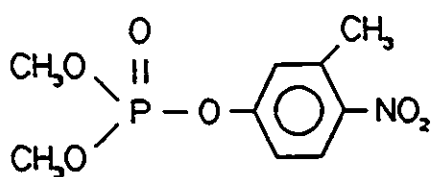
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APPENDICES

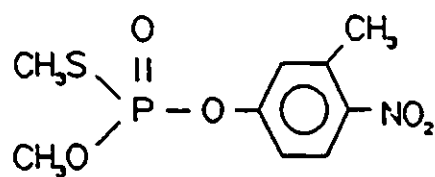
APPENDIX I



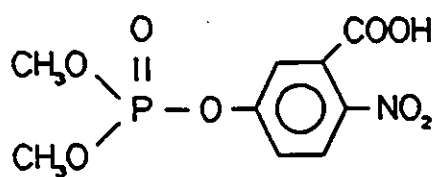
Fenitrothion



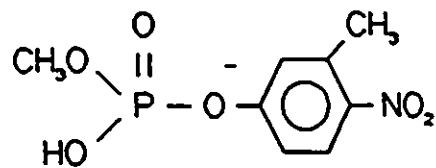
Fenitrooxon



S-Methylfenitrothion

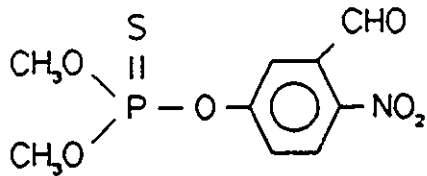


Carboxyfenitrooxon

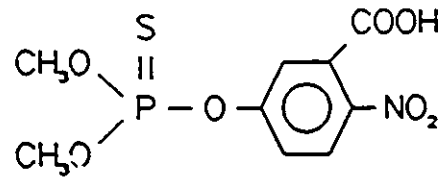


Demethylfenitrooxon

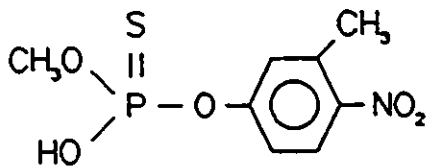
Figure 15. Chemical structures of fenitrothion and its metabolites listed in the text.



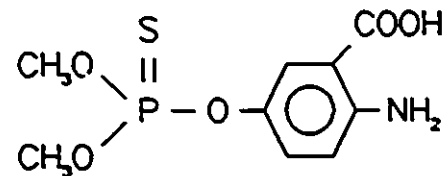
Formylfenitrothion



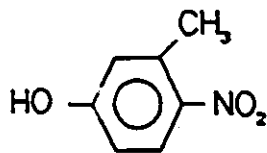
Carboxyfenitrothion



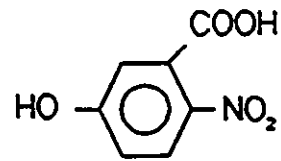
Demethylfenitrothion



Carboxyamino fenitrothion



3-methyl-4-nitrophenol



3-carboxy-4-nitrophenol

Figure 15...cont

Fenitrothion metabolites listed in the text.

APPENDIX II

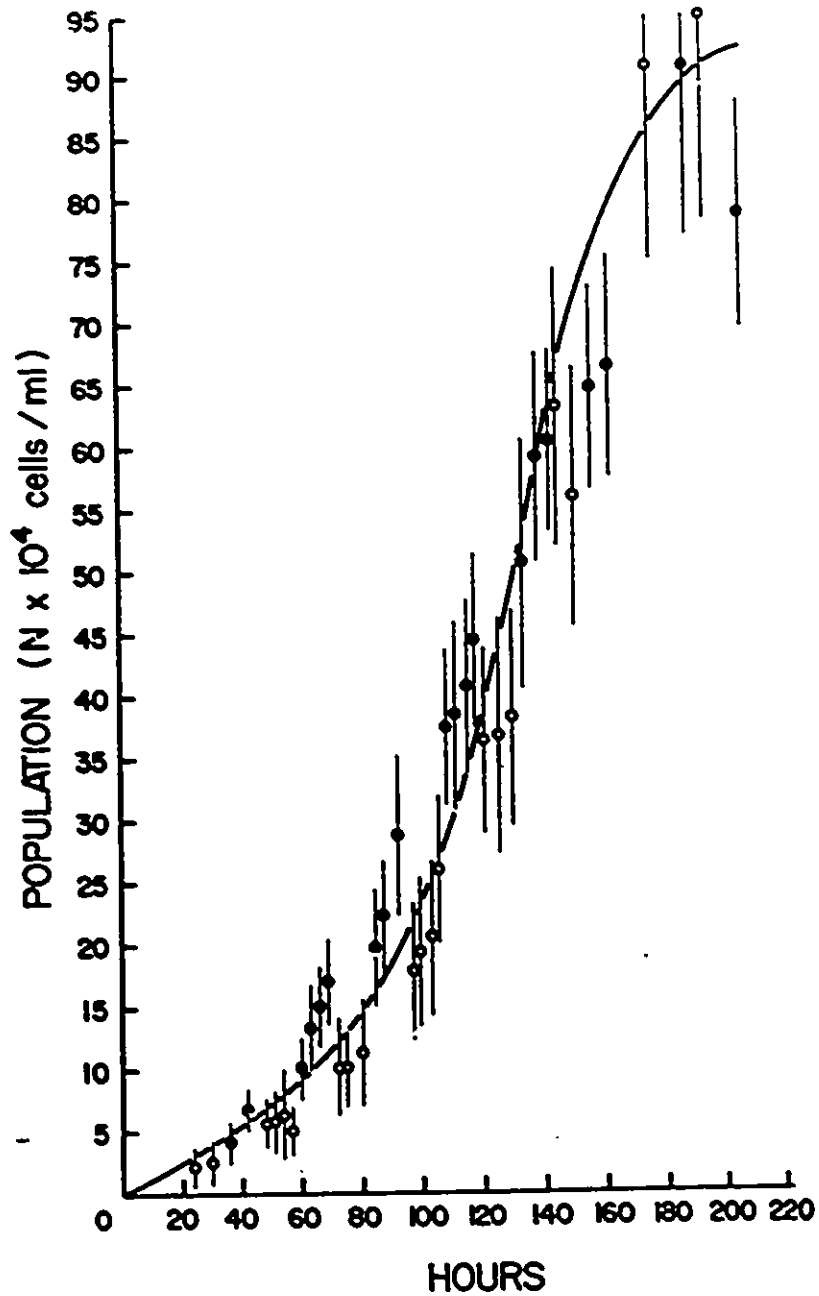


Figure 16.

Growth curve of *Chlamydomonas segnis* maintained at 21-23°C under 16 hour light: 8 hour dark periods provided by 2 cool-white and 2 Grow-lux fluorescent lamps (5 w m⁻²).

APPENDIX III

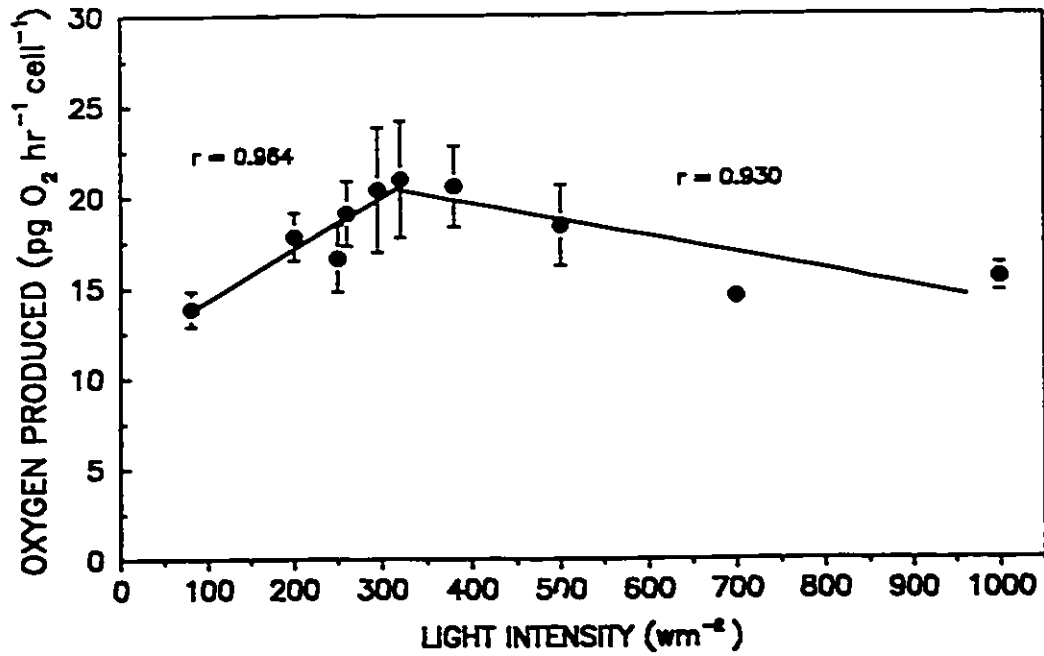


Figure 17a. Calculation of saturating light intensity for the Clark electrode. Photosynthetic rates are compared to varying light intensities. Light source was a 150 w Sunbeam parchlor lamp.

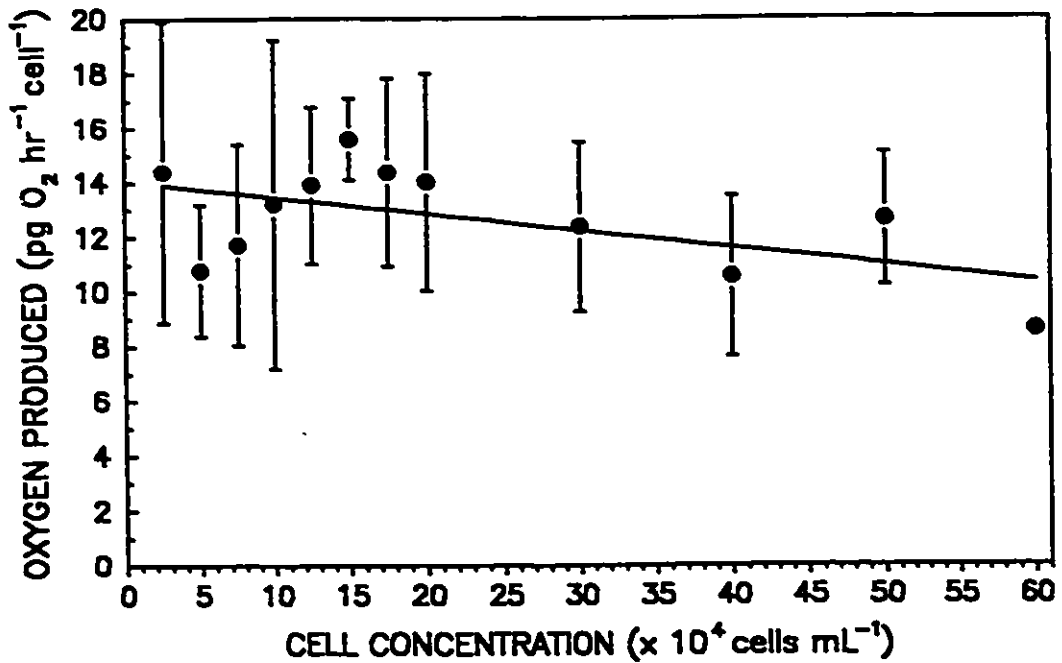


Figure 17b. Shading effects on photosynthesis rates in the Clark electrode apparatus.

APPENDIX IV

Table 1. Algae cell numbers in fenitrothion treated media and media controls.

Time (days)	Treatment ($\times 10^5$ cells mL^{-1})	Control ($\times 10^5$ cells mL^{-1})
0.0	2.11 \pm 0.51	2.11 \pm 0.51
1.0	2.76 \pm 0.63	2.69 \pm 0.72
2.0	* 6.19 \pm 1.19	8.34 \pm 1.52
3.0	* 12.7 \pm 3.2	17.4 \pm 1.4
4.0	* 16.8 \pm 4.0	25.7 \pm 2.7
5.0	* 21.0 \pm 3.4	29.9 \pm 3.4
6.0	* 23.6 \pm 3.0	33.2 \pm 3.3
7.0	* 24.5 \pm 5.7	32.6 \pm 2.9

* Significant at the 0.05 level compared with control.

Table 2. Algae dry weight per cell in fenitrothion treated media and media controls.

Time (days)	Treatment (ng cell $^{-1}$)	Control (ng cell $^{-1}$)
0.0	0.151 \pm 0.038	0.151 \pm 0.038
1.0	* 0.285 \pm 0.054	0.154 \pm 0.002
2.0	0.193 \pm 0.011	0.148 \pm 0.013
3.0	* 0.164 \pm 0.006	0.126 \pm 0.007
4.0	* 0.158 \pm 0.005	0.112 \pm 0.007
5.0	0.146 \pm 0.012	0.126 \pm 0.003
6.0	0.146 \pm 0.008	0.143 \pm 0.005
7.0	0.171 \pm 0.005	0.190 \pm 0.018

* Significant at the 0.05 level compared with control.

Table 3a. Algae chlorophyll a per cell in fenitrothion treated media and media only controls.

Time (days)	Treatment (pg cell ⁻¹)	Control (pg cell ⁻¹)
0.0	1.30 ± 0.05	1.30 ± 0.05
1.0	1.24 ± 0.20	1.27 ± 0.09
2.0	1.49 ± 0.19	1.22 ± 0.04
3.0	1.44 ± 0.13	1.18 ± 0.10
4.0	* 2.01 ± 0.10	1.16 ± 0.02
5.0	* 2.48 ± 0.10	1.72 ± 0.03
6.0	* 2.99 ± 0.11	2.00 ± 0.14
7.0	* 3.27 ± 0.08	2.59 ± 0.02

* Significant at the 0.05 level compared with control.

Table 3b. Algae chlorophyll b per cell in fenitrothion treated media and media only controls.

Time (days)	Treatment (pg cell ⁻¹)	Control (pg cell ⁻¹)
0.0	0.450 ± 0.022	0.450 ± 0.022
1.0	0.467 ± 0.152	0.454 ± 0.054
2.0	0.590 ± 0.097	0.464 ± 0.017
3.0	0.501 ± 0.033	0.410 ± 0.042
4.0	* 0.716 ± 0.030	0.422 ± 0.001
5.0	* 0.935 ± 0.087	0.638 ± 0.010
6.0	* 1.11 ± 0.06	0.752 ± 0.058
7.0	* 1.21 ± 0.04	0.971 ± 0.007

* Significant at the 0.05 level compared with control.

Table 4. Photosynthetic rates per cell of *C. segnis* in media treated with $10.3 \mu\text{g ml}^{-1}$ fenitrothion and media only controls.

Time (days)	Treatment ($\text{pg min}^{-1} \text{cell}^{-1}$)	Control ($\text{pg min}^{-1} \text{cell}^{-1}$)
0.0	30.8 ± 2.1	30.8 ± 2.1
1.0	23.7 ± 6.0	26.3 ± 3.5
2.0	19.9 ± 3.2	20.7 ± 1.2
3.0	17.7 ± 1.2	17.2 ± 1.2
4.0	* 20.4 ± 0.4	12.7 ± 0.2
5.0	* 18.7 ± 0.3	14.4 ± 0.8
6.0	* 16.6 ± 0.1	11.9 ± 0.3
7.0	13.9 ± 0.9	11.2 ± 0.6

* Significant at the 0.05 level compared with control.

Table 5. ^{14}C ring label uptake in live algae under sunlight and dark controls.

Time (hours)	Sunlight ^{14}C Uptake ($\mu\text{g mg}^{-1}$ algae)	Darkness ^{14}C Uptake ($\mu\text{g mg}^{-1}$ algae)
0.25	0.676 ± 0.060	0.723 ± 0.518
0.75	* 0.958 ± 0.042	0.375 ± 0.078
1.5	* 2.95 ± 0.66	0.448 ± 0.046
2.5	* 2.33 ± 0.32	0.952 ± 0.104
3.75	* 9.41 ± 1.96	0.692 ± 0.060
5.0	* 6.88 ± 0.04	0.291 ± 0.005

* Significant at the 0.05 level compared with darkness control.

Table 6. ^{14}C ring label uptake in live algae under Vita Lites² and dark controls.

Time (hours)	Vita Lite ² ^{14}C Uptake ($\mu\text{g mg}^{-1}$ algae)	Darkness ^{14}C Uptake ($\mu\text{g mg}^{-1}$ algae)
1.5	* 0.537 \pm 0.012	0.204 \pm 0.014
3.0	* 0.797 \pm 0.036	0.160 \pm 0.004
7.0	* 1.84 \pm 0.04	0.107 \pm 0.005
11.0	* 6.14 \pm 0.60	0.480 \pm 0.049
15.0	* 9.23 \pm 0.36	0.466 \pm 0.025
19.0	* 10.97 \pm 0.20	0.317 \pm 0.020
22.0	* 10.88 \pm 1.10	0.351 \pm 0.037
25.0	* 16.57 \pm 0.87	0.834 \pm 0.233
28.0	* 15.62 \pm 0.99	0.416 \pm 0.041
38.0	* 13.97 \pm 0.46	0.339 \pm 0.012
48.0	* 11.73 \pm 0.51	0.378 \pm 0.017
72.0	* 9.78 \pm 0.15	0.163 \pm 0.012

* Significant at the 0.05 level compared with darkness control.

Table 7. ^{14}C ring label uptake in freeze-killed algae under sunlight and dark controls.

Time (hours)	Sunlight ^{14}C Uptake ($\mu\text{g mg}^{-1}$ algae)	Darkness ^{14}C Uptake ($\mu\text{g mg}^{-1}$ algae)
0.25	* 2.08- \pm 0.15	1.06 \pm 0.02
0.75	* 2.00 \pm 0.10	0.744 \pm 0.008
1.5	* 5.65 \pm 0.45	1.04 \pm 0.08
2.5	* 8.31 \pm 0.96	1.60 \pm 0.20
3.75	* 15.72 \pm 1.56	1.56 \pm 0.04
5.0	* 5.74 \pm 0.67	0.638 \pm 0.086

* Significant at the 0.05 level compared with darkness control.

Table 8. ^{14}C ring label uptake in freeze-killed algae under Vita Lites* and dark controls.

Time (hours)	Vita Lite* ^{14}C Uptake ($\mu\text{g mg}^{-1}$ algae)	Darkness ^{14}C Uptake ($\mu\text{g mg}^{-1}$ algae)
1.5	* 2.87 \pm 0.19	1.27 \pm 0.02
3.0	* 1.34 \pm 0.19	0.433 \pm 0.012
7.0	* 7.93 \pm 0.48	0.515 \pm 0.027
11.0	* 14.33 \pm 0.91	0.874 \pm 0.075
15.0	* 26.95 \pm 2.38	0.870 \pm 0.078
19.0	* 24.94 \pm 1.52	1.93 \pm 0.05
22.0	* 28.00 \pm 1.65	1.69 \pm 0.02
25.0	* 28.75 \pm 0.88	2.28 \pm 0.06
28.0	* 24.11 \pm 0.84	0.226 \pm 0.068

* Significant at the 0.05 level compared with darkness control.

Table 9. Fenitrothion concentration in media with live algae and media controls under Vita Lites*.

Time (hours)	Algae in Media Fenitrothion ($\mu\text{g mL}^{-1}$)	Media Control Fenitrothion ($\mu\text{g mL}^{-1}$)
0.0	10.30 \pm 0.00	10.30 \pm 0.00
3.0	8.95 \pm 0.49	9.95 \pm 0.04
11.0	* 5.78 \pm 0.25	8.37 \pm 0.13
19.0	* 3.84 \pm 0.12	7.69 \pm 0.03
25.0	* 3.21 \pm 0.40	6.38 \pm 0.28
38.0	* 2.48 \pm 0.16	3.87 \pm 0.34
48.0	* 1.09 \pm 0.28	3.88 \pm 0.35

* Significant at the 0.05 level compared with media only control.

Table 10. Fenitrothion concentration in media with live algae and media controls under dark conditions.

Time (hours)	Algae in Media Fenitrothion ($\mu\text{g mL}^{-1}$)	Media Control Fenitrothion ($\mu\text{g mL}^{-1}$)
0.0	10.30 \pm 0.00	10.30 \pm 0.00
3.0	* 8.02 \pm 0.51	10.06 \pm 0.13
11.0	7.95 \pm 0.44	9.14 \pm 0.07
19.0	7.58 \pm 0.37	8.16 \pm 0.14
25.0	* 6.97 \pm 0.45	8.93 \pm 0.35
38.0	7.37 \pm 0.41	7.75 \pm 0.51
48.0	6.13 \pm 0.56	8.35 \pm 1.35

* Significant at the 0.05 level compared with media only control.

Table 11. Fenitrothion concentration in media with freeze-killed algae and media controls under Vita Lites[®].

Time (hours)	Algae in Media Fenitrothion ($\mu\text{g mL}^{-1}$)	Media Control Fenitrothion ($\mu\text{g mL}^{-1}$)
0.0	10.30 \pm 0.00	10.30 \pm 0.00
3.0	* 8.81 \pm 0.22	9.83 \pm 0.21
11.0	7.14 \pm 0.67	8.59 \pm 0.31
19.0	* 6.02 \pm 0.30	7.52 \pm 0.37
25.0	5.05 \pm 0.24	6.49 \pm 0.58
38.0	3.64 \pm 0.21	5.54 \pm 0.70
48.0	* 3.35 \pm 0.40	5.18 \pm 0.45

* Significant at the 0.05 level compared with media only control.

Table 12. Fenitrothion concentration in media with freeze-killed algae and media controls under darkened conditions.

Time (hours)	Algae in Media Fenitrothion ($\mu\text{g mL}^{-1}$)	Media Control Fenitrothion ($\mu\text{g mL}^{-1}$)
0.0	10.30 \pm 0.00	10.30 \pm 0.00
3.0	9.74 \pm 0.33	10.06 \pm 0.13
11.0	9.68 \pm 0.26	9.74 \pm 0.27
19.0	8.86 \pm 0.59	8.68 \pm 0.45
25.0	8.81 \pm 0.52	9.72 \pm 0.46
38.0	9.48 \pm 0.51	8.44 \pm 0.60
48.0	7.97 \pm 1.01	8.35 \pm 1.34

* Significant at the 0.05 level compared with media only control.

APPENDIX V

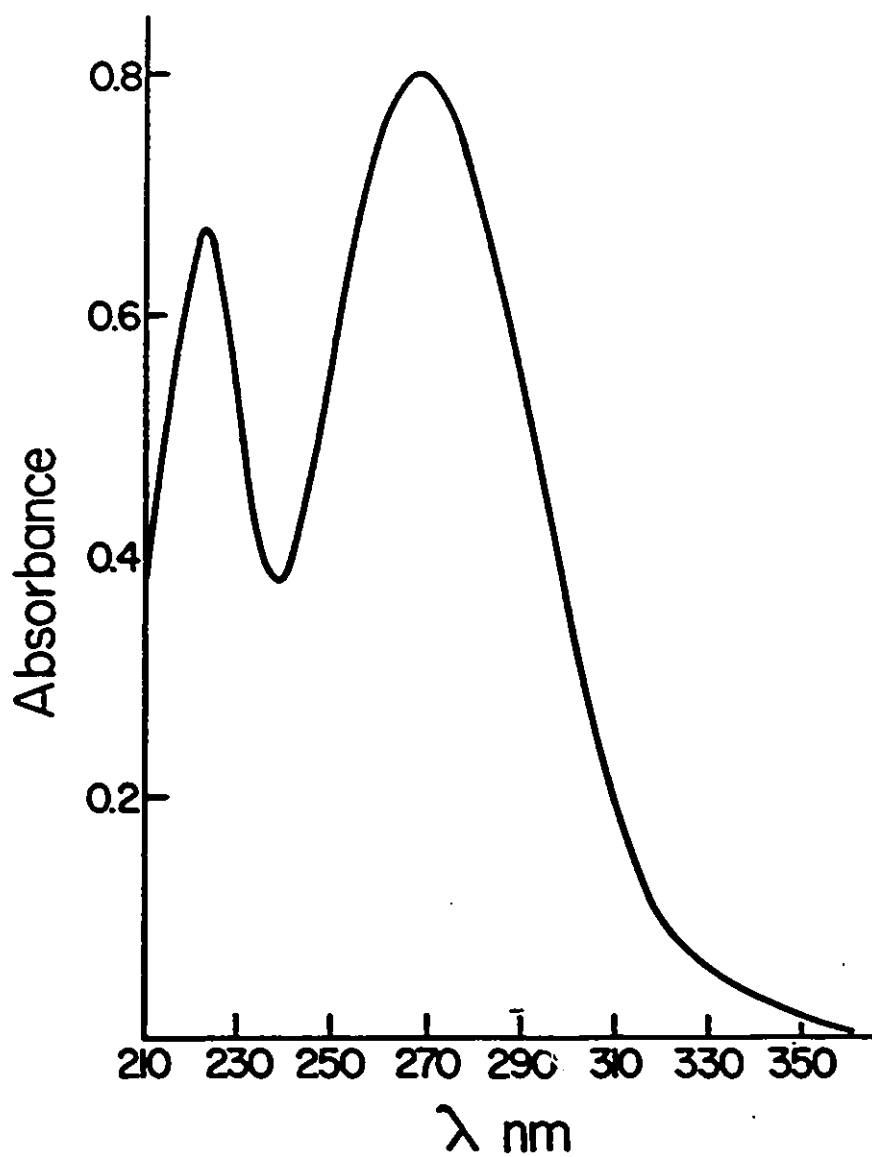


Figure 18.

U.V. absorption spectrum of fenitrothion in methanol (Weinberger *et al.*, 1981b).

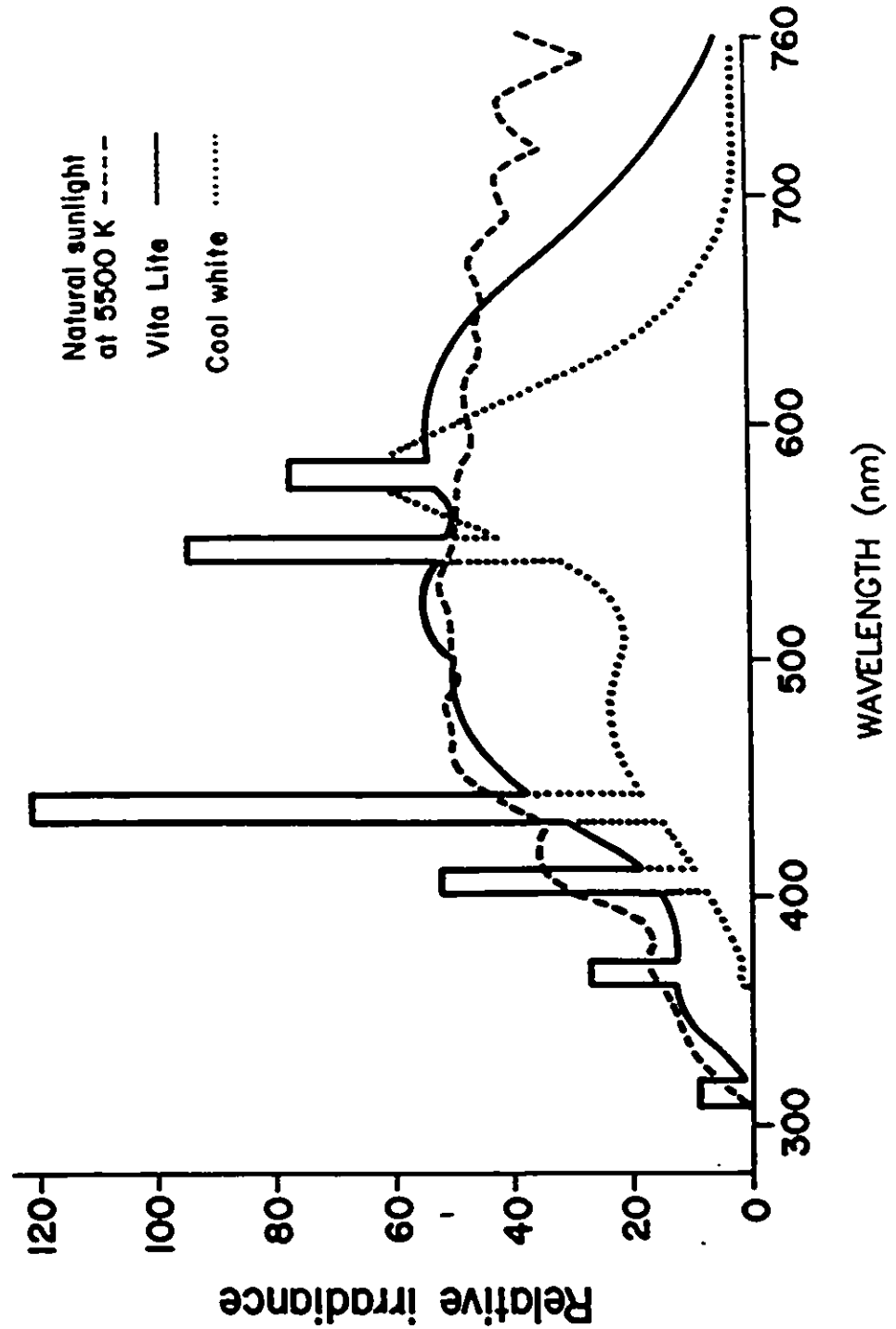


Figure 19.

Relative spectral irradiance distribution of the Vita Lite[®] fluorescent lamp compared with Cool white fluorescent lamps and natural sunlight at 5500 K (Taken from Hughes & Neer, 1981).

APPENDIX VI

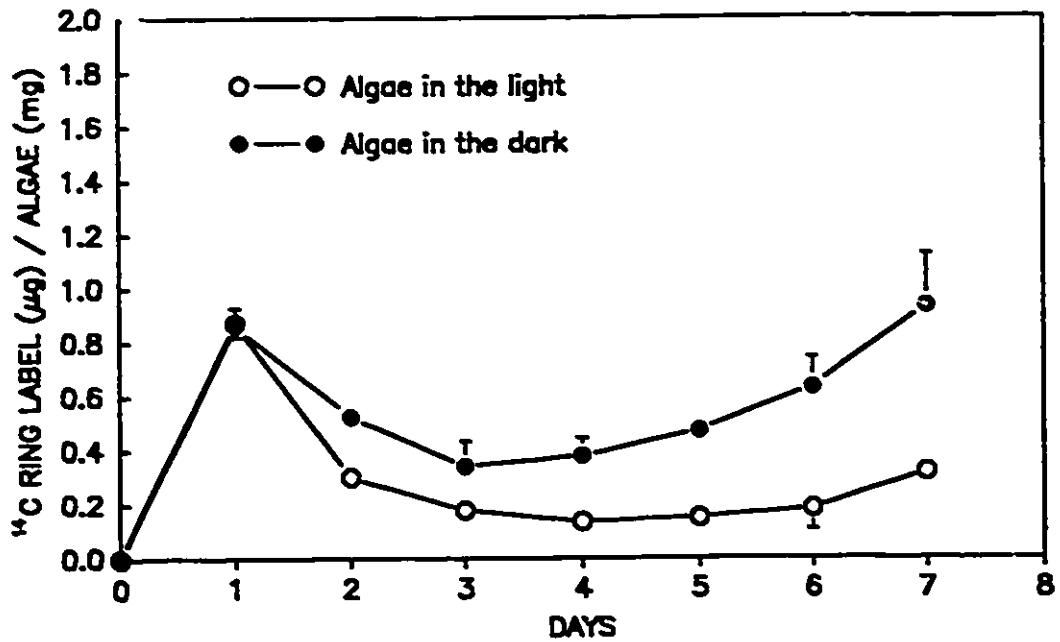


Figure 20. Preliminary experiment showing uptake of ^{14}C ring label fenitrothion in *Chlamydomonas segnis* under cool white fluorescent lamps (5 w m^{-2}).