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ISBN 0-315-70495-0

The thesis is dedicated to the loving memory of

my mother Catherine, may she forever be a part of our lives.

ACKNOWLEDGEMENTS

The author would like to express his gratitude to his research advisory committee: Dr. Pearl Weinberger, Dr. David Currie and Dr. Roy Greenhalgh. Thanks are extended to Mandy Hockey for her assistance in electronic particle enumeration, France Duval and Jacques Helie for drafting figures and André Richer for his innovative technical help. Special thanks are given to my laboratory colleagues especially Nicole Bertrand for helpful suggestions and technical advice over the course of this work. Acknowledgment is made to National Science and Engineering Research Council Grant A1737 for funding for this research.

To Pearl Weinberger, whose confidence and encouragement will never be forgotten. To my father Edmund William who instilled in me the determination and perseverance to finish this work and my wife France who sustained the love, support and encouragement throughout. Finally to my son Jacob William, who supplied the final spark.

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ABSTRACT

Axenic batch cultures of twelve freshwater phytoplankton species were used to study the molecular, cellular and population effects of the organophosphorus insecticide fenitrothion (*O,O*-Dimethyl-*O*-nitro-*m*-tolyl phosphorothioate) on algae. The unicellular chlorophytes *Ankistrodesmus falcatus*, *Chlamydomonas segnis*, *Chlamydomonas reinhardtii*, *Chlorella pyrenoidosa*, *Chlorella vulgaris*, *Cosmarium sp.*, *Pediastrum sp.*, *Scenedesmus obliquus*, *Selenastrum capricornutum* and *Staurastrum sp.*, the bacillariophyte *Navicula sp.*, and the cyanophyte *Anabaena sp.* were exposed to three treatment levels (0.1, 1.0 and 10.0 mg/L) of fenitrothion. The response parameters monitored represent three levels of biological organization from molecular - organism - population and included fatty acid composition, total cellular protein, carbohydrate, nucleic acids, lipids, cell dry weight, cell volume, growth, and population size spectra.

Differential algistasis was observed among the species exposed to fenitrothion over short (96 hours) and long-term (14 day) durations. Effects on growth included an extension in lag-phase and/or alterations in growth rate and/or final standing crop. Fenitrothion concentrations eliciting a 50% growth reduction (EC_{50}) ranged over 3 orders of magnitude, from 0.84 - 109.9 mg/L. At the molecular level, alterations in the fatty acid composition of total lipids suggest that fenitrothion disrupts lipid synthesis and membrane structure. A significant augmentation of macromolecules was observed. This biochemical accrual coupled with elevated cell dry weight and reduction in cell density was a common response pattern. We hypothesize that fenitrothion prevented normal mitotic divisional processes from occurring. Uninhibited biomolecular synthesis resulted in an accumulation of macromolecules and subsequent cell weight augmentation. Modal cell volumes increased and normalized cell distribution spectra displayed a shift towards larger cells in treated algal populations. The ecological implications of the observed effects were discussed.

In addition to effects studies, selected properties of the test algae (cell size functions, lipid content and fenitrothion bioaccumulation capacity) were measured and examined for their relationship to fenitrothion sensitivity. Strong correlations were obtained between fenitrothion sensitivity and cell surface area/volume ratios (S/V), cell lipid content and fenitrothion bioaccumulation capacity of the cells suggesting that these attributes may be useful predictors of differential sensitivity to lipophilic xenobiotics in unicellular phytoplankton. A positive relationship observed between cell S/V ratio and the bioaccumulation of fenitrothion further implicates algal cell size as a significant factor in algal/xenobiotic interaction and subsequent vulnerability to lipophilic xenobiotics.

RESUME

Des cultures axeniques de douze espèces de phytoplancton d'eau douce ont été utilisées dans une étude de l'effet de l'insecticide organophosphoré, fénitrothion (*O,O*-Dimethyl-*O*-nitro-*m*-tolyl phosphorothioate) sur la composition moléculaire, cellulaire et des effets sur la population des algues. Les chlorophytes unicellulaires *Ankistrodesmus falcatus, Chlamydomonas segnis, Chlamydomonas reinhardtii, Chlorella pyrenoidosa, Chlorella vulgaris, Cosmarium sp., Pediastrum sp., Scenedesmus obliquus, Selenastrum capricornutum* et *Staurastrum sp.* le bacillariophyte *Navicula sp.*, et le cyanophyte *Anabaena sp.* ont été exposés à trois concentrations (0,1, 1,0 et 10,0 mg/L) de fénitrothion. Les paramètres-réponses étudiés représentent trois niveaux d'organisation biologique: la molécule, l'organisme et la population et ils incluent la composition en acides gras, en protéines cellulaires totales, en hydrates de carbones, en acides nucléiques, et en les lipides ainsique le poids cellulaire sec, le volume cellulaire, le taux de croissance et le spectre de la population.

Les effets ont été observés et quantifiés pour les trois niveaux d'organisation biologique. Les courbes de croissance ont révélé des différences algistatiques substancielles parmi les espèces observées sur une courte (96 heures) et longue durée (14 jours) d'exposition. Les effets sur la croissance ont été mesurés comme un accroissement dans la "lag-phase" et/ou une altération dans le taux de croissance et/ou le rendement final. Les concentrations en fénitrothion provoquant une réduction de 50% de le croissance (EC_{50}) s'étendent sur trois niveaux de magnitude, soit 0,84 - 109,9 mg/L. Au niveau moléculaire, les changements da la composition des acides gras et des lipides totaux suggèrent qu'il y a eu une désorganisation de la synthése des lipides et de la structure de la membrane. Les indications biomoleculaires de la phytotoxicité se sont manifestés par une augmentation significative des macromolécules. Cette accumulation biochimique couplée au poids sec cellulaire éléve et une réduction de la densité cellulaire était un model commun de réponses. Nous postulons que le fénitrothion empêche le processus normal de division mitotique. La synthèse biomoléculaire non-inhibée a résulté en une accumulation de macromolécules avec des conséquences directes sur l'augmentation du poids cellulaire. Le volume cellulaire moyen a augmenté et le spectre de distribution cellulaire normalisé a exhibé une tendance vers des cellules plus grosses dans les populations d'algues traitées. Les implications écologiques des effets observés sont discutées.

En plus des effets étudiés, des propriétés choisies des algues testées (dimension des cellules, teneur en lipides et capacité d'accumulation en fénitrothion) ont été mesurées et examinées pour leur sensitivité au fénitrothion. Une forte corrélation obtenue entre la sensitivité au fénitrothion et le ratio de la superficie cellulaire/volume, le contenu cellulaire en lipides et la capacité cellulaire d'accumulation en fénitrothion suggère que ces qualités pourraient être d'utiles prédicateurs de sensitivité différentielle aux xénobiotiques lipophiliques chez les phytoplanctons unicellulaires. Une corrélation positive entre le ratio de l'aire de surface/volume et l'accumulation en fenitrothion implique davantage que la dimension cellulaire de l'algue est un facteur significatif dans l'interaction algue/xénobiotique et sa vulnérabilité subséquente aux xénobiotiques lipophiliques.

CHAPTER 1.0

INTRODUCTION

Aquatic biota are constantly subjected to a variety of natural and anthropogenic stresses. Synthetic chemical contaminants constitute a considerable proportion of the latter category in natural systems today and of this, pesticides are a significant component.

The escalating use of pesticides to ensure adequate food and fibre resources and human health protection has prompted great concern regarding their injurious effects on non-target organisms and destabilizing influence on ecosystems. In theory, pesticides should assault the target organism(s) while incurring no significant effects on surrounding biota. In practice, this is all too frequently not the case.

Of the myriad of non-target organisms at risk to pesticide impact, algae constitute a major component. Their ecological position at the base of most aquatic food webs and essential roles in nutrient cycling and oxygen production is critical to all ecosystems. It has been estimated that 70% of the earth's atmospheric oxygen is generated by phytoplankton (Reynolds 1984). Cairns (1968) pointed out that although a fish kill as a result of pesticide contamination is far more dramatic than a crash in the diatom community, the survival of the fish is meaningless if their food resources are disrupted or eliminated. It is therefore important to clearly examine the potential hazard that pesticides pose to this ubiquitous group of primary producers.

The pervasive distribution of algae in all environmental media (air, water, soil and sediment) ensures a great likelihood for exposure regardless of the nature of pesticide application. Their characteristic high surface area-volume properties and large membrane proportion of microflora implicates a high potential for sorption of, and subsequent interactions with xenobiotics, particularly lipophilic compounds.

A fundamental goal in ecotoxicology is to predict ecological effects from xenobiotic stressors (Cairns and Pratt 1989). Since ecosystem injury should be averted rather than corrected, scientists and regulators should work proactively to refine their predictive capacity to anticipate vulnerable components (i.e. taxonomic groups, species, etc.) of ecosystems under stress as well as the potential outcomes.

Healthy living systems are characterized by a balanced and dynamic set of physiological processes. Interference (via chemical perturbance) in a particular process or mechanism often results in the indirect disruption of others. One of the principle difficulties in ecotoxicology is identifying the primary inhibitory (or in some cases stimulatory if the end result is potentially deleterious) nature of chemical stressors on an organism, since numerous processes are undoubtedly affected (Conner 1981).

The presence of pesticides (and other contaminants) in aquatic systems represent multi-level perturbances which in the process of evoking changes at the molecular and cellular level can also alter the structure and function of phytoplankton assemblages. These may lead to more complex effects on community dynamics.

To date, the emphasis in ecotoxicological testing has largely focused on standardized whole organism or population responses (survival, growth, reproductive capacity). This has been attributed in part to insufficient knowledge regarding a stressor's biochemical behaviour and mode of action at lower levels of biological organization (Caux 1989). A major disadvantage to this approach has been to overlook potentially sensitive sub-acute indicators of xenobiotic stress which likely manifest before whole organism or population responses. In addition, the latter approach provides insight into linking biochemical effects to whole organism and population responses.

1.1 Differential Algal Sensitivity to Xenobiotic Stress

Considerable diversity in the sensitivity of freshwater and marine phytoplankton to xenobiotics has been documented (Luard 1973; Cullimore 1975; Blanck *et al.*, 1984). The phenomena of differential algal sensitivity to contaminant stress described by Wurster (1968), asserts that certain species or groups within phytoplankton assemblages or communities, possess the inherent ability to resist pollutant stress far better than others. Differential sensitivity has since been a central concept in a number of laboratory and field studies with marine (Fisher *et al.*, 1974; Fisher 1977; Maly and Ruber 1983) and freshwater phytoplankton (Conner 1981; Sloof *et al.*, 1983; Blanck *et al.*, 1984; Harrass *et al.*, 1985; Munawar et al., 1987; Wangberg and Blanck 1988). For example, organic contaminants have induced differential growth inhibition in natural algal assemblages and thereby disrupted community structures and trophic pathways via dominance shifts, successional alterations and size spectrum effects (Mosser and Wurster 1972; O'Connors et al., 1978; Mahoney and Haskin 1980; De Noyelles *et al.*, 1982; Maly and Ruber 1983).

Few single-species experiments yielding wide differences in sensitivities to chemical stressors have been employed to predict community structure changes and dominance profiles in laboratory mixed assemblages (Mayasich *et al.*, 1987; Harrass *et al.*, 1985). There is limited evidence suggesting that differential algal sensitivity patterns and rankings in laboratory have been validated *in situ* with marine species (Ostgaard et al., 1984).

The degree of sensitivity to xenobiotics is related to many factors: the uptake rate and total burden, the ability to regulate, detoxify and/or safely sequester these compounds, the physical and chemical nature of the cell barriers, and the phylogeny of the algae (Hollister and Walsh 1973; Luard 1973; Fisher 1977; Wangberg and Blanck 1988). Studies of marine diatom clones have shown that sensitivity is related to the physical/chemical stability (Fisher

1977) and pollution history (Murphy and Belastock 1980) of their environmental origin. Estuarine clones, isolated from nutrient-sufficient habitats characterized by large-scale physical fluctuations (temperature, salinity, light levels) were systematically more tolerant than open ocean clones. It was advanced that the latter diatoms, which are considered more specialized in exploiting the minimal nutrient resources typical of oceanic habitats (e.g. lower K_s values for nitrogen) have as a result evolved highly efficient, sensitized membrane systems, which in turn makes them less tolerant to xenobiotics (particularly lipophilic compounds).

1.2 Fenitrothion Use in the Canadian Environment

The large scale aerial pesticide programs in eastern Canada to control lepidopterous defoliators constitutes a significant anthropogenic stress to aquatic non-target biota of forest habitats (Weinberger *et al.*, 1981). Fenitrothion (O,O-Dimethyl-O-nitro-*m*-tolyl phosphorothioate), CAS No. 122-14-5, [trade names Folothion, Novathion and Sumithion], the test stressor in these investigations, is an organophosphorus broad-spectrum insecticide which has been (and currently remains in some regions) the principal chemical pesticide used in these pest control programs. Used extensively throughout the world for the control of forest and agricultural pests, fenitrothion is used in Canada primarily in fir and spruce forests to combat the spruce budworm *(Choristoneura fumiferana)* and hemlock looper *(Lambdina fiscellaria)*. It is also registered in Canada for controlling infestations by specified budworms, loopers and other insect pests in tree nurseries, woodlots and Christmas tree plantations.

Fenitrothion was introduced in Canada in the early 1960s as a replacement for the highly persistent organochlorine insecticide, DDT. Although other organophosphorus (phosphamidon) and carbamate (aminocarb) insecticides have been significant components of the spruce budworm control programs, fenitrothion has been the most heavily used since the mid 1960s. In the mid 1980s, its prominence began to decline as some provinces discontinued (or drastically reduced) spray programs or opted for what was perceived as a less environmentally harmful pest control using the microbial insecticide B.t. *(Bacillus thuringiensis)*. Today, public opinion has largely dictated that B.t. remain the principal or sole control agent in most provincial forest insect pest control programs (e.g. Alberta, Manitoba, Ontario, Quebec, Newfoundland).

Though its once widespread use in silviculture has declined considerably, fenitrothion still remains among the 10 most heavily used insecticides in Canada (Environment Canada / Agriculture Canada 1987) with over 1200 tonnes of formulated and technical material imported into Canada from 1983-1987 (Statistics Canada 1987). Over the last several years, large-scale spraying programs employing fenitrothion in Canada were limited to New Brunswick and Newfoundland, where fenitrothion represented the largest percentage of insecticides used (Carr 1988). The latest national figures for operational spray programs indicate that fenitrothion use over the 1989 and 1990 spray seasons has been restricted entirely to New Brunswick. Its use remains relatively large and constant over this period with 196,014 and 190,596 kg being applied over a total forest area of 466,700 and 453,800 ha respectively (CAPCO 1990).

As a result of its once nationally intensive and widespread usage and its current regional significance, fenitrothion is subject of one of the largest environmental monitoring, fate and effects databases in Canada. Its use in the Canadian environment and the hazards posed to human and non-human receptors have been the subject of several major reviews (NRCC 1975; NRCC 1977 and Ernst *et al.*, 1989). The most recent review by the federal environment department focuses on the three most documented environmental hazards posed by fenitrothion use including effects on: (1) insect pollinators and pollination; (2) forest avifauna and (3) aquatic biota. Noted in the review is the overwhelming lack of consistency between operational dosage rates and biological effects. Insufficient knowledge regarding differences in species susceptibilities and unevenness of localized pesticide deposits (spray accountability) were given as the principle determinants of this variability (Ernst *et al.*, 1989).

This critical mass of environmental data together with the continued public unease has prompted Agriculture Canada, currently the lead authority responsible for pesticide regulation in Canada, to institute a special re-evaluation of fenitrothion's use in the Canadian environment (Agriculture Canada 1990). Until then, it is likely however, that its intensive use will continue in New Brunswick (and periodically in Newfoundland) until political pressures force its eventual phase-out in favour of B.t.-based formulations.

1.3 Fenitrothion in the Aquatic Environment

Contamination of aquatic habitats by fenitrothion is an unavoidable characteristic of aerial spraying of forests (Fairchild *et al.*, 1989). Fenitrothion enters the aquatic system primarily via direct application by spray aircraft or indirectly from spray drift and surface runoff (NRCC 1975). Despite implementation of regulatory no-spray buffer zones since 1978 and increased environmental awareness on the part of the spray applicators, there is no quantitative evidence that suggests a significant reduction in deposit in typical small non-target aquatic habitats (Sexsmith 1987). Current regulations in New Brunswick do not require spray-free zones for non-designated rivers and lakes less than 40 ha in size (Ernst *et al.*, in press) . Spray drift, foliar washoff and surface runoff also contribute to off-target contamination. Deposition of significant aquatic residues up to 75 km away from the nearest spray zone have been reported (Pearce *et al.*, 1979).

Due to the nature of aerial pesticide applications over large areas of diverse terrain, spray deposits are seldom uniform. Given the magnitude of the treatment zones each spring, overspraying of boundary areas of spray blocks frequently occurs (Symons 1977). Recent evidence on conifer foliage residues indicate that spray deposits 6 - 8 times the mean level occur regularly over 5% of a spray area (Pearce and Ernst 1988).

Surface water concentrations of fenitrothion following operational programs are highly variable, dependent upon such factors as: habitat type (lotic vs lentic), canopy cover, use of buffer zones and presence of organic surface films. With the exception of one recent study, all routine monitoring work during operational programs has been in lotic systems. Typical maximum concentrations detected in running waters following (2 hours) operational spraying range from 0.0013 to 0.701 mg/L (Mallet and Cassista 1984; Moody et al., 1978). Limited experimental studies on lentic waters report maximum concentrations ranging from 0.00038 to 1.1 mg/L (Maguire and Hale 1980; Fairchild *et al.*, 1989). Most of these studies, however, were from large lentic bodies such as lakes and therefore most likely protected by buffer zones or at least not directly oversprayed.

Recent monitoring work during the 1989 spray program in New Brunswick investigated levels in small (< 0.5 ha) acidic ponds immediately following a typical spray operation (two treatments of 210 g active ingredient/ha, approximately 5 to 10 days apart)(Ernst *et al.*, in press). Surface water concentrations ranged from 0.0006 to 2.5 mg/L with maximum mean concentrations of 1.5 mg/L in one pond two hours following spraying.

Many of the highest maximum concentrations recorded were associated with surface layers or slicks (Maguire and Hale 1980; Morin *et al.*, 1986). These may be natural films of lipid-rich neustonic communities and/or buildups of insoluble formulation adjuvants and are known to play a significant role in the concentration and persistence of hydrophobic chemical contaminants (Duce *et al.*, 1972; Maguire and Tkacz 1987). Small ponds or stream pools are the most vulnerable to fenitrothion contamination as they are usually not avoided by spraying, have low capacity for dilution, limited flushing rates and often limited canopy protection (NRCC 1982). In addition, these water bodies are often used as boundary markers for spray blocks and as a result are exposed to spray overlap and double swathing (Symons 1977).

Although it is not the purpose of this study to provide a complete review of the aquatic fate and behaviour of fenitrothion (several detailed reviews already exist: NRCC 1975; 1977; Fairchild *et al.*, 1989), a brief overview of its degradation and persistence provides the necessary context for its exposure and duration profile and subsequent potential toxic hazard to algal receptors.

Following entry into the aquatic environment, fenitrothion dissipates relatively rapidly with residues usually unable to be detected beyond 40 days (NRCC 1975). Aquatic persistence or half-life ($t_{½}$) under field conditions has been measured as being 2 days (Greenhalgh *et al.*, 1980; Malis and Muir 1984) or less (Maguire and Hale 1980). Comparatively, laboratory persistence studies (Greenhalgh *et al.*, 1980; Caunter and Weinberger 1988; Kent and Weinberger 1991) generally indicate a half-life of fenitrothion at least one order of magnitude greater than field studies.

Several key fate processes are principally associated with the aquatic dissipation of fenitrothion under field conditions. These include hydrolysis, photolysis, surface volatilization and microbial degradation and are associated with the formation of various process-specific breakdown products.

Chemical hydrolysis and the resultant formation of 3-methyl-4-nitrophenol has been shown to be pH and temperature dependent (Zitco and Cunningham 1974). Generally this reaction proceeds very slowly under environmental conditions and is not expected to occur appreciably where pH ranges from 6.3 - 8.0. The rate of hydrolysis however, has been demonstrated to increase rapidly at high pH (where pH > 8.0) (Greenhalgh *et al.*, 1980).

Photodecomposition is considered one of the primary routes of *in vitro* dissipation of fenitrothion (Mikami et al., 1985; Weinberger et al., 1982; Greenhalgh et al., 1980) with the principle photolytic breakdown product being carboxyfenitrothion. A host of secondary photo-oxidative products some of which include *p*-nitro-*m*-cresol, carboxy-fenitro-oxon, fenitro-oxon, formylfenitrothion, 3-carboxy-4-nitrophenol and S-methyl-fenitrothion have also been identified (Mikami *et al.*, 1985; Greenhalgh and Marshall 1976). Under sunlight, photolysis is expected to occur rapidly with reports of 80% of the parent compound degrading to polar derivatives within 6 hours (Weinberger *et al.*, 1982). This degradation route is dependent upon light intensity and spectrum.

Anaerobic and aerobic microbial metabolism is considered the other primary degradative route for fenitrothion in natural aquatic systems (Zitco and Cunningham 1974; Moody *et al.*, 1982; Greenhalgh *et al.*, 1980). The predominant biological metabolites include aminofenitrothion and desmethyl-aminofenitrothion.

Volatilization from deposition areas has also been demonstrated to contribute to the dissipation of fenitrothion from aquatic sites (Metcalfe et al., 1980; Maguire and Hale 1980). Whereas evaporation from true aqueous solutions may not be significant under field situations, experimental evidence suggests that volatilization from surface layers may be very rapid (t½ < 1 hour)(Maguire and Hale 1980).

In addition to abiotic and biotic modes of chemical degradation, adsorption to suspended and bottom sediments has also been shown to play a significant role in the fate and short-term persistence of fenitrothion (Weinberger *et al.*, 1981; Baarschers *et al.*, 1983; Eidt et al., 1984). The significance of fenitrothion adsorption to aquatic plants is discussed in section 1.4.

Whereas most evidence indicates a general rapid decline of fenitrothion to below detectable levels usually within a few days of application, recent studies on small lentic habitats show a return to measurable levels in water the following spring (Fairchild and Eidt 1988). Findings of significant residues being sequestered in biological sinks (i.e. aquatic plants) and remobilized following winter accounted for the increased persistence of fenitrothion in stagnant New Brunswick bog ponds. This work demonstrates small stagnant ponds of low pH (e.g. bog ponds) to be particularly susceptible to fenitrothion persistence and subsequent toxic impacts.

1.4 Fenitrothion and Aquatic Microflora

Owing to the lack of adequate studies, there was a tendency to assume that the presence of fenitrothion is of minor consequence in aquatic systems, because of its relatively rapid disappearance from aquatic environments. However, field studies have demonstrated accumulation and short-term persistence of fenitrothion in sediments and particularly in aquatic plants (Lakshminarayana and Bourque 1980; Weinberger *et al.*, 1982; Fairchild and Eidt 1988). Weinberger *et al.*, (1982) demonstrated that common rooted macrophytes such as *Myriophyllum, Elodea*, and *Sagittaria* represent effective sinks for fenitrothion as bioaccumulation ratios after 5 days ranged from 370 to 488. This phenomena of rapid partitioning by aquatic flora was also observed in the floating macrophyte *Lemna minor*. Ten hours following experimental aerial applications in Manitoba, *Lemna minor* plants were found to contain 4000 ppb fenitrothion versus 44 ppb in the ambient water (Moody *et al.*, 1978).

Due a variety of factors (e.g. small size, high surface to volume ratios, limited cell barriers, high lipid content and effective adsorptive capacity) it is expected that algae in general should possess a high capacity for sequestering and concentrating lipophilic pesticides from the ambient medium. Numerous investigations reviewed by Butler (1977) and Baughman and Paris (1981) on the uptake of aquatic contaminants by algae have supported this assumption.

When the first detailed environmental hazard reviews of fenitrothion were released (NRCC 1975; 1977; Symons 1977) it seemed incomprehensible that they failed to address the potential for adverse effects on algae. Although the current state of knowledge regarding fenitrothion/algal interactions and resulting effects has expanded somewhat since then, it is again not surprising that after twelve years, the most recent hazard assessment on fenitrothion use in Canada (Ernst *et al.*, 1989) provides an equally inadequate analysis of this subject. This is likely a result of the continued view of many researchers to regard algae as unaffected carriers that simply facilitate the passage of contaminants such as pesticides through the food chain until incorporated by higher trophic level biota considered more worthy of study.

Like higher aquatic plants, microalgae have also displayed a high potential for partitioning fenitrothion from ambient medium under a variety of operational spray program and experimental exposure regimes (Lakshminarayana and Bourque 1980; Weinberger *et al.*, 1982; 1983; Moody 1982; Fisher 1985). In comparison, investigations on the toxic effects of fenitrothion exposure on aquatic microflora has received far less attention.

Fenitrothion can be lethal to marine diatoms at 50 mg/L (Butler 1977). Further details regarding parameters measured were however, not provided. A similar effects threshold concentration of ≥ 56 mg/L fenitrothion was needed to elicit a measurable decrease in standing crop and induce a lag-phase in growth in the common freshwater chlorophyte *Chlorella vulgaris* (Kikuchi *et al.*, 1984a). In the only study found which statistically examined fenitrothion's effects on algal growth, Couture *et al.*, (1982) determined short (24 - 50 hours) and long-term (14 days) IC 50s (concentration inhibiting growth by 50%) for *Chlamydomonas variabilis* and *Selenastrum capricornutum* to be 75 mg/L and 1.2 mg/L respectively.

Fenitrothion has also been reported to inhibit photosynthesis (as measured by oxygen evolution) in the freshwater diatoms *Navicula* and *Fragilaria* (Rama Rao *et al.*, 1979). Exposures of 2.0, 5.0 and 10.0 mg/L elicited reductions of 31, 57 and 62% respectively in primary production rates. Conversely, Kikuchi *et al.*, (1984a) indicates *Chlorella vulgaris* cells exhibited a marked (1.4 - 7.0 X) amelioration in photosynthesis (^{14}C fixation) following 30 and 100 mg/L fenitrothion exposures.

Since growth is the culmination of many biochemical events, it is expected that biochemical changes occur before reductions in growth are observed. Related phosphorothioate pesticides, guthion, malathion (Lal and Saxena 1980) and methyl parathion (Saroja and Bose 1982) have been shown to affect macromolecular synthesis at levels as low as 10 mg/L in protists and unicellular chlorophytes. There are indications to suggest a fenitrothion-induced decrease in precursor incorporation into DNA, RNA and protein in *Chlorella vulgaris*, however these apparent effects on macromolecular biosynthesis were not statistically supported (Kikuchi *et al.*, 1984a). Whereas fenitrothion is known to induce nuclei deformations and anomalous protein deposits in vascular plant cells (Weinberger *et al.*, 1981), molecular alterations in algae have not been reported. None of these studies, however, were found, have indicated detrimental effects on algal receptors from environmentally-relevant concentrations of fenitrothion.

1.5 Research Approach and Objectives

In examining algal/fenitrothion interactions and toxic responses, this research had two main goals: (I) elucidate the mechanisms by which fenitrothion affects phytoplankton, and (II) to develop a model that can predict the sensitivity of phytoplankton to fenitrothion.

Within this approach, the following hypotheses were forwarded:

H1. Fenitrothion stress evokes significant differences in sensitivity among algal species.

H2. Toxic responses of freshwater phytoplankton to fenitrothion stress is elicited over multiple levels of biological organization.

H3. Sensitivity to fenitrothion stress in the test phytoplankton is influenced by: (i) physical (size) and chemical (lipid content) attributes of cells; (ii) capacity to accumulate fenitrothion and (iii) taxonomy.

Little attempt has been made to demonstrate the potential relationships between biochemical effects of pesticides on non-target organisms and higher-level biological responses that may ensue (NRCC 1985). The present study was undertaken to elucidate the effect of fenitrothion in freshwater unicellular algae and to examine the possible linking mechanisms to whole organism and population responses.

The specific objectives of this study were:

1. To characterize the differential sensitivity of twelve common phytoplankton species to fenitrothion stress, including a/an:
    a) quantitative examination of growth in batch cultures using linear, exponential and logistic models of population growth
    b) establishment of sensitivity rankings

2. To investigate the following molecular, cellular and population-level indicators of

fenitrothion stress in algae:

a) major cellular macromolecule levels (protein, carbohydrate, RNA and DNA)

b) cell size (volume, surface area), cell dry weight and cell number in culture

c) population growth (maximum growth rate and standing crop) over short-term (96 hours) and long-term (14 day) exposures

d) size spectrum (cell volume distribution) of populations

3. To identify phytoplankton characteristics and algal/pesticide interaction factors which are related to algal sensitivity[1] to fenitrothion and thereby aid in predicting algal vulnerability to pesticide stress. This entails observations and measurements (and subsequent correlative analyses with sensitivity indices) of:

   a) cell size attributes and functions (cell volume, surface area, surface area:volume ratio and transformed ratio[2].

   b) lipid content of cells

   c) bioaccumulation capacity[3]

   d) taxonomic group of each species

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[1] operationally defined as a ranking or indice based on growth effects (see Section 3.2.1 and 3.2.2)

[2] refers to ratio of $\sqrt{\text{Surface Area}} / \sqrt[3]{\text{Volume}}$

[3] capacity of a species to accumulate fenitrothion over a given exposure period

CHAPTER 2.0

MATERIALS AND METHODS

2.1 Materials

2.1.1 Test Organisms

Axenic isolates of the various algal species were obtained from the following culture collections and biological supply houses: University of Texas Culture Collection (UTEX), Austin, TX, U.S.A., Wards Scientific (WS), Mississauga, Ont., Canada and Boreal Laboratories (BL), Toronto, Ont., Canada.

ALGAL ISOLATE	ORIGIN/STRAIN
Cyanophyta	
Oscillatoriales	
Anabaena sp.	BL 67088
Chlorophyta	
Chlorococcales	
Ankistrodesmus falcatus	WS 86W0010
Chlorella pyrenoidosa	BL 67178
Chlorella vulgaris Beijerinck	UTEX 29
Pediastrum sp.	BL 67486
Scenedesmus obliquus (Turp.) Kutz	UTEX 78
Selenastrum capricornutum	UTEX 1648
Volvocales	
Chlamydomonas segnis Ettl	UTEX 1343
Chlamydomonas reinhardtii Dang	UTEX 89
Zygnematales	
Cosmarium sp.	BL 67460
Staurastrum sp.	WS 86W0660
Bacillariophyta	
Pennales	
Navicula sp.	WS 86W1210

2.1.2 Test Compound and Chemicals

Technical fenitrothion (97% pure) was kindly donated by Sumitomo Chemical Co., Takatsukasa, Japan. Bovine serum albumin (BSA), glucose, and salmon sperm DNA standards for the macromolecular quantification of protein, carbohydrate and DNA were obtained from Sigma Chemical Co., St Louis, MO., USA. The DNA standard was purified before use by the phenol extraction method (Maniakis *et al* 1982). Amyl acetate, 2 N Folin Ciocalteau reagent, perchloric acid, phenol, sulfuric acid and growth media ingredients were all reagent grades from Fisher Scientific and BDH Chemical Companies, Toronto, Ont., Canada. The lipid standards (palmitic acid and methylheptadecanoate) used in total lipid and fatty acid analyses were obtained from Supelco Chemical Co., Oakville, Ont., Canada. The carbonylcyanide m-chlorphenylhydrazone (CP) employed in bioaccumulation studies was purchased from Aldrich Chemical Co., Milwaukee, WS. U.S.A. All organic solvents used were purchased from BDH Chemical Co. and were of pesticide grade. The fluor (LSC Cocktail 4698) used in liquid scintillation counting was obtained from BDH Chemical Co.

2.2 Methods

2.2.1 General Culture Conditions

All algae were cultivated under axenic, nutrient sufficient conditions in Kuhl's inorganic liquid medium (pH 6.8) (Kuhl and Lorenzen 1964) (see Appendix II for ingredients). All stock and experimental cultures were incubated in a Sherer Controlled Environment Lab Cabinet (Model CEL 255-6) and Conviron (Model S10H) growth chamber at 20 ± 1°C under a combination cool-white/Grow-lux fluorescent lighting (75 - 95 μEinsteins m^{-2} sec^{-1} ≈ 5 watts m^{-2}). Light intensity and radiometric energy was monitored using a GE light meter (Type 213) and a Li-cor Quantum radiometer-photometer (Model L1-185b) respectively. A 12:12 (L:D) photoperiod was maintained to induce synchronized cell division (occuring at regular intervals and approximately simultaneously). Stock cultures and growth bioassays were maintained in sterile, teflon-capped glass tubes (Pyrex No. 9825, 22 X 150 mm), loosely capped and kept on their sides on an Eberbach reciprocal shaker (90 oscillations per minute).

Experimental systems for cell physical parameters, macromolecular, total lipids, fatty acid content, and bioaccumulation assays consisted of 30.0 - 50.0 ml algal suspensions in sterile foam-plugged 250 mL Erlenmeyer flasks under the conditions noted above. Exponential growth phase cells, maintained in stock cultures by media dilutions every 1 - 2 days and confirmed by microscopic examination and enumeration, were utilized in all assays.

2.2.2 Pesticide Application and Treatments

A primary stock solution of 100,000 µg/mL technical fenitrothion (97% pure) in pesticide grade acetone was used to prepare stock suspensions of 1.0 and 10.0 mg/L fenitrothion in sterile growth media. Suitable aliquots of freshly prepared, thoroughly vortexed fenitrothion/acetone/media suspensions were added to sterilized test vessels containing autoclaved growth medium yielding final concentrations of 0.1, 1.0 and 10.0 mg/L (ppm) of fenitrothion. The first two treatment levels are within the concentration range encountered in surface water following a spray program (Morin *et al.*, 1986; Moody *et al.*, 1978). The latter treatment concentration represents a worst case scenario which may possibly result from over-application or spray overlap. The final concentration of acetone carrier did not exceed 0.01% in any experimental system. Controls with and without the solvent carrier were prepared for all experiments. All pesticide applications and growth media transfers were performed using sterile techniques within a laminar-flow sterile hood.

All glassware used in stock culturing or experimental work was of Pyrex^R or Kimax^R

brand, Fisher Chemical Co. and underwent the following cleaning/decontamination regime prior to use: (1) phosphorus-free detergent wash with brush, (2) minimum 4 hour soak in a concentrated chromic-sulfuric acid bath, (3) three rinses with tap water followed by three rinses with distilled-deionized water (DDW), (4) rinse with pesticide grade acetone, (5) drying in 80°C oven.

2.2.3 Pesticide Analysis

The fate of fenitrothion in the growth media was monitored in *Chlorella pyrenoidosa, Ankistrodesmus falcatus* and media-only systems over the entire growth period of 14 days (336 hours). Aliquots of 5 mL were aseptically sampled at 0, 2, 24, 48, 168 and 336 hours following initiation of growth studies. Cells were separated from media by centrifugation (1000g) for 5 mins. The supernatant was transferred to a separatory funnel and fenitrothion was extracted by partitioning 3 times with 10 mL chloroform. Solvent extracts were filtered through $NaSO_4$ to remove residual water, concentrated on a rotary evaporator (Brinkman, Toronto, Ont., Canada) to near dryness and reconstituted in acetone for GC analysis. Following solvent partitioning described above, a subsample of the aqueous phase was lyophilized and stored in methanol for analysis of polar metabolites.

Fenitrothion residues were quantified with a Hewlett Packard 5880A gas liquid chromatograph (GC) equipped with a flame photometric detector. A 1.0 m X 2.0 mm glass column packed with 3% SE 30 Ultraphase on Chromosorb W (80 - 100 mesh) was operated at 195°C with nitrogen carrier at 40 mL min^{-1}, detector flow 500 mL min^{-1} air and 35 mL min^{-1} hydrogen. Fenitrothion was quantified by peak areas of sample injections compared to those of injected standards. The limit of detection of parent fenitrothion was 0.01 μg/L.

2.2.4 Growth Studies and Analysis

Following preparation of pesticide treatments and controls using fresh sterile media, identical aliquots (100 - 200 μL) of late exponential phase algae were dispensed into 22 X 150 mm screw-cap tubes to inoculate growth cultures. Initial cell densities (2 - 10 X 10^4 cells/mL) varied for each species. The final volume in each experimental system was 15 mL. Population estimates were determined daily for up to 14 days from direct optical density (O.D.) measurements of each culture tube in a Spectronic 21 spectrophotometer at a wavelength of 540 nm according to standard methods (Stein 1973). A standard curve was prepared for *Chlamydomonas segnis* by plotting the biomass estimate (O.D.) vs cell number (determined by enumeration with hemocytometer) (Figure 16).

The effects of fenitrothion on growth were assessed in one series of experiments during which, growth effects were measured over the standard exposure durations of 96 hours (short-term) and 14 day (long-term exposures) (U.S. EPA 1982). Biomass estimates (O.D. 540 nm) were measured daily.

After 96 hours, growth rate and final standing crop (maximum biomass attained) were determined. Growth rates (represented as r) were obtained using the exponential model: ($y = e^{a + bx}$) where y = the population estimate, x = time and a = slope or growth rate. Final standing crops (represented by K) were obtained directly from the final (day 4) O.D. estimates.

Long-term growth effects were quantitatively interpreted using a non-linear regression analysis. The logistic equation was iteratively fitted to the observed growth curve biomass estimates using the statistical software Statgraphics^R. The logistic model-fitting analyses generated best fit parameter estimates for the coefficients *a, r* and *K* represented in the function below:

(Integral form of the Logistic Equation)

$$N_t = \frac{K}{1.0 + e^{(a - rt)}}$$

where N_t = population estimate (O.D.) at time t

e = base of natural log

a = integration constant

r = ***max. rate of population growth***

K = ***upper asymptote or maximum value of N (maximum Biomass)***

The differences between control and treatment coefficient values for r (maximum growth rate) and K (maximum attainable biomass or carrying capacity) were used to quantify growth inhibition responses. These values represented the % total significant difference ($P \leq 0.05$) of r and K between controls and all treatments for each species. In addition to these growth variables, the lag-phase, and its duration was also monitored in control and treated populations. The lag-phase is operationally defined within the context of these studies as the initial period (starting from day 0) of growth over which exponential increase in cell numbers is not apparent.

EC_{50} values, representing fenitrothion concentrations where growth was reduced by 50%, were estimated for those species which elicited significant growth responses (measured as growth rate or maximum attainable biomass) from controls over the treatment range tested using the EPA Probit Analyses Program (Version 1.4).

3.2.5 Cell Dry Weight and Cell Density

Suitable aliquots of log-phase algae were added to media pre-treated with fenitrothion to yield early exponential phase populations. All cultures were treated with the test chemical 1 hour before the dark period commenced. This corresponds to the S phase of the aligned cell cycle (Weinberger *et al* 1987; Chisholm 1981). Four replicate aliquots were sampled for cell dry weight and cell number following 6 days treatment at the end of the dark period (G1 phase) to minimize variability attributable to the cell cycle. Cell numbers were determined using an improved Neubauer-Levy haemocytometer and light microscope (Wild Heerbrugg, Switzerland). A known volume and number of cells were separated from the culture medium by filtration onto previously weighed Whatman GF/C glass microfiber filters under low vacuum (< 5 mm Hg) and rinsed with fresh growth medium. Filters were dried for 24 hrs at 70°C, stored in a dessicator for 24 hrs and algal dry weight (expressed on a per cell basis) was determined gravimetrically.

3.2.6 Cell Volume and Surface Area

Cellular dimensions of test algae were measured using an ocular micrometer-equipped microscope. A minimum of 100 cells from early exponential phase control cultures were randomly selected using a haemocytometer grid for measurements. An appropriate geometric morphology was selected for each test species measured, and cell volume (V) and surface area (A) were calculated using the formulae of Kovala and Larrance (1966).

3.2.7 Modal Cell Volume and Population Size Spectra

Cell size frequency distributions of 7-day control and treatment replicate cultures of *Chlamydomonas segnis, Chlorella pyrenoidosa, Selanastrum capricornutum*, and *Scenedesmus* *obliquus* were determined using a Coulter model ZF electronic particle counter (Coulter Electronics Co., Hialeah, FL. U.S.A.) and channelyzer calibrated with 4.91 and 9.82 μm latex calibration microspheres (Coulter Electronics Co.). The maxima of the distribution curves corresponded to the modal cell volume (MCV). Distributions were graphically represented using a X-Y chart recorder interfaced to the Coulter Counter Channelyzer. Cell volumes were calibrated using both microscopic observations (via an ocular micrometer) and 4.91um diameter latex calibration beads.

Size distributions were obtained following 6 days of fenitrothion treatment. By this time, growth was well into exponential phase. All treatments were analysed at the same time to negate variation attributable to the cell cycle.

Cell volumes and surface area estimations from control cultures were determined microscopically using a graduated ocular micrometer eyepiece. The measurements on which the volume and surface area calculations were based corresponded to the mean values from a sampling of a minimum of 100 cells randomly selected on a haemocytometer grid.

3.2.8 Macromolecular Studies

Extraction and quantification of cellular protein, carbohydrate and nucleic acids in *Chlamydomonas segnis* and *Ankistrodesmus falcatus* were carried out following minor modifications to methods previously described in this laboratory (De Chacin 1984; Weinberger *et al* 1987). Following 48 hour treatments of fenitrothion, replicate aliquots (30 mL) of *Chlamydomonas segnis* and *Ankistrodesmus falcatus* culture were taken for analysis at the end of the dark period. Cells were enumerated microscopically using a hemocytometer and then separated from the medium by centrifugation (1000g, 5 mins.). The resulting algal pellets were extracted at 40°C with 90% acetone (30 mL) and thoroughly homogenized in a polytron

(10 mins.). Following centrifugation (1000g, 10 mins.), the pellet was extracted twice at 40°C with ethanol:ether (1:1). The depigmented residue was collected by centrifugation (1000g, 10 mins.), chilled in ice (10 mins.) then washed (15 mins. each) with 1) 5 mL ice cold 1N perchloric acid (PCA); 2) 5 mL cold ethanol and 3) 5 mL cold ether and then dried under nitrogen. The dried material was then solubilized in 2 mL 1N NaOH at 37°C for 18 hours (Iwamura *et al* 1970). Following the alkaline hydrolysis, samples were centrifuged (1000g, 10 mins.) to separate alkali-insoluble materials from the major macromolecules to be assayed.

3.2.8.1 Protein

An aliquot (0.2 mL) was taken from the clear hydrolyzate for protein estimations following the methodology of Layne (1957). The initial volume (0.2 mL) was diluted to 0.3 mL with 1N NaOH and 3 mL of Protein Reagent (50 mL 2% Na_2CO_3 and 1 mL of 5% $CuSO_4.H_2O$ in 1% sodium tartarate) was added. After 10 mins., 0.3 mL of 1N Folin Ciocalteau Reagent was added and the mixture was left to stand at room temperature for 30 minutes. Absorbance was read at 720 nm using a Bausch and Lomb Spectronic 20 spectrophotometer and protein per sample was quantified from calibration curves using bovine serum albumin (BSA) standards (10 - 200 μg/mL) . Total estimated algal protein was expressed as a per cell basis (pg/cell).

3.2.8.2 Carbohydrate

A volume (0.2 mL) was taken from the clear alkaline hydrolyzate and adjusted to 2.0 mL with 1N NaOH and 0.05 mL of phenol reagent (90 mL 90% phenol/10 mL H_2O) was added and thoroughly mixed. Following this, 5.0 mL of concentrated H_2SO_4 was rapidly and carefully added and the mixture was left to stand for 30 minutes. Absorbance was read at 485 nm and carbohydrate per sample was quantified from calibration curves using glucose standards (10 - 200 μg/mL)(Kochert 1978a). Total estimated soluble carbohydrate was expressed as a per cell basis (pg/cell).

3.2.8.3 Nucleic Acids

1.5 mL of the alkaline hydrolyzate was chilled on ice and neutralized with 0.16 mL if ice-chilled 60% PCA. To this was added 0.1 mL $MgCl_2$ (1M sol.) and 0.1 mL 0.5% BSA. The solution was thoroughly mixed and allowed to stand for several minutes in an ice bath, acidified with 0.2 mL ice-chilled 60% PCA followed by a further 1.94 mL of PCA. The sample mixture was then centrifuged and the supernatant (removed for RNA determination) was measured for absorbance at 260 nm and 320 nm. RNA was quantified according to the following equation:

RNA (μg/mL) = 29.5 X (Abs. 260 nm - 320 nm) X 4 (Iwamura *et al.*, 1980).

The blank was prepared containing 1.5 mL 1N NaOH and 2.5 mL 1N PCA. Total estimated algal ribonucleic acid was expressed on a per cell basis (pg/cell).

Following RNA extraction, the centrifuged residue was washed with 5.0 mL of cold ethanol, followed by 5.0 mL ether and then dried under nitrogen. The residue was then diluted with 2.0 mL 0.5N PCA, after which 1.0 mL 2.5N HCl and 1 mL 0.06% indole solution were added. The mixture was placed in a boiling water bath for 10 minutes to develop colour and extracted in a separatory funnel with 4.0 mL amyl acetate. The absorbance of the extracted solution was measured at 490 nm against the blank and total DNA per sample was quantified from calibration curves using purified (Maniakis *et al* 1982) salmon sperm DNA standards which were prepared in the same way as the unknown samples (Keck 1956). Total estimated algal deoxyribonucleic acid was expressed as a per cell basis (pg/cell).

3.2.9 Lipid Studies

3.2.9.1 Total Lipid Content

A quantitative determination of total cellular lipids in untreated *Ankistrodesmus falcatus, Chlamydomonas segnis, Chlamydomonas reinhardtii, Chlorella pyrenoidosa, Chlorella vulgaris, Cosmarium sp., Navicula sp., Pediastrum sp., Scenedesmus obliquus, Selanastrum capricornutum* and *Staurastrum sp. cells* were undertaken following a modified version of Kochert's dichromate reduction method (Kochert 1978b).

A known volume and mass of exponential phase culture was separated from the growth medium by centrifugation to yield a final packed cell volume of approximately 0.5 mL. Due to the presence of tough cell walls in several species, solvent extraction with chloroform/methanol (2:1 v/v) was enhanced by sonication of the cell/solvent suspension for 10 minutes using a Tronic sonicator Model TJ1026 (Tronic Corp., Bellemont, CA., USA). Dichromate reduction was measured spectrophotometrically at 350 nm against palmitic acid standards.

Following the method stated above, the effects of fenitrothion on the total lipid content in *Ankistrodesmus falcatus* was investigated. Replicate samples were taken from control and following 48 hours treatment at 0.1, 1.0 and 10.0 mg/L fenitrothion.

3.2.9.2 Fatty Acid Content of Total Lipids

The effects of fenitrothion on the composition and quantity of fatty acids in total lipids were examined in *Ankistrodesmus falcatus* cells. Replicate samples were harvested from control and treated cultures (following 48 hours exposure to fenitrothion) and separated from the growth medium by centrifugation. Prior to this step, subsamples were taken for cell enumeration and weight measurements. Using a vortex mixer, cells were rinsed 3 times with 10 mL of fresh growth medium to remove surface contaminants. Extraction and purification of total lipids was carried out following modifications to established procedures (Bligh and Dyer 1959; Kates 1970; Caux 1989). Following the rinse, total lipids were extracted by adding 8.0 mL methanol-chloroform-hydrochloric acid solution CH_3OH:$CHCl_3$:0.2N HCl (2:1:0.8 v/v/v), sonicating for 10 minutes and leaving the suspension undisturbed at 22°C for 1 hour. The suspension was then centrifuged (700g, 10 mins.). The extraction procedure was repeated once on the debris pellet and the supernatants combined.

Water and non-lipid contaminants were removed through a two-phase purification step. Chloroform (2.0 mL) and DDW (2.0 mL) were added to the combined supernatants to obtain a methanol:chloroform:water ratio of 1:1:0.9 (v/v/v). The mixture was agitated with a vortex and centrifuged (700g, 10 mins.). The chloroform phase (bottom layer) was carefully removed with a pasteur pipette and neutralized to pH 7.0 with 0.3N NH_4OH in methanol. The purified lipids were concentrated under N_2, resuspended in chloroform ($\approx$ 2.0 mL) and transferred to teflon-capped glass vials (4.0 mL) stored under N_2 in the freezer.

Total lipids were then transesterified and methylated (Metcalfe *et al* 1966) and the FAME (fatty acid methyl esters) were quantified by gas chromatography following an internal standard procedure (Grenier *et al* 1979) using methylheptadecanoate. A Hewlett Packard model 5880 gas chromatograph equipped with a hydrogen flame ionization detector was employed. FAME extracts were injected directly into a 2-mm i.d. glass column packed with Supelco GP SP-2330 on 100/120 Chromosorb WAW. Oven temperature was maintained at 175°C. The injection port and detector temperatures were 210°C and 240°C respectively. Peak areas were determined using a Hewlitt Packard digital integrator Model 5880A.

3.2.10 Bioaccumulation Studies

Fenitrothion uptake experiments were carried out in 125 mL erlenmeyer flasks under conditions identical to growth experiments. Exponential phase inoculum were added to 30.0 mL of medium to yield final cell densities ranging from 20.0 - 25.0 X 10^4 cells / mL. Each 30.0 mL system contained a mixture of ^{14}C ring-labelled fenitrothion (2.730 μg, approx. 600,000 DPM) and unlabelled fenitrothion (27.3 μg) yielding a final combined pesticide concentration of 1.0 ppm (mg/L). Each experiment consisted of four replicates for each of the following two treatments:

1. live exponentially growing cells and fenitrothion
2. dead cells and fenitrothion

A metabolically deactivated or dead treatment was run concurrent to elucidate the nature of fenitrothion uptake in freshwater phytoplankton (i.e. abiotic vs. biotic uptake processes). A 1.0 mM (204.6 mg/L) treatment of carbonylcyanide m-chlorophenylhydrazone (CP) was employed according to the method of Tarapchak *et al.*, (1981) to effectively inhibit photophosphorylation and oxidative phosphorylation (ATP production) of algae in culture. Complete inhibition of cellular metabolism in algal cultures following a 30 minute treatment with CP was confirmed by oxygen evolution measurements with a Clarke Electrode following the method of Caunter (in prep.). Oxygen production was completed inhibited in all the species tested when exposed to 1.0 mM CP treatment.

Radiolabelled fenitrothion was determined in cells and media at four exposure periods (2, 8, 24 and 48 hours) for *Chlamydomonas segnis* and *Chlamydomonas reinhardtii* to determine exposure duration associated with bioaccumulation maxima. Samples (5.0 mL) of algal culture treated with radiolabelled fenitrothion were harvested from the replicate treatment flasks and dispensed into conical centrifuge tubes. Cells were separated from the medium by centrifugation (1000g, 8 min.), and initial 1.0 mL aliquots of supernatant were drawn off and dispensed into liquid scintillation counting (LSC) vials containing fluor to be analysed by LSC. These samples were referred to as *primary supernatant* (PS). The remainder of the initial supernatant was removed using vacuum (< 5 mm Hg) and fresh growth media (3.0 mL) was added to the algal pellet. The sample was then thoroughly resuspended with a vortex shaker for 2 minutes. Cells were again separated by centrifugation (1000g, 8 mins.) and 333.0 μL was removed and placed into LSC vials containing fluor. This was repeated for a total of three times so that the final volume of supernatant removed from the algal sample was 1.0 mL, which then constituted the *secondary supernatant* (SS). Following the final rinse, the remaining algal pellet was resuspended in fresh growth media and filtered through pre-weighed Whatman GF/C glass microfibre filters, which were then dried, enclosed under nitrogen, weighed and dispensed with fluor into vials for LSC. The radiocarbon identified with the final filter (algal biomass) and the secondary supernatant was combined and represented the algal-associated fraction. The radiocarbon measured in the primary supernatant represented the fraction remaining in the growth media.

All LSC was performed using a Packard Liquid Scintillation Counter (model 2000 CA, Downers Grove, Ill., USA). Sample counts were automatically corrected for background and quenching. Counting efficiency (dpm) was automatically determined by a ^{14}C external standard program supplied by Packard.

3.2.11 Statistical Analysis of Data

A variety of statistical tests were employed to determine significance of difference between treatments and algal species including Analysis of Variance (1- and 2-way ANOVA), Least Significant Difference Test (95% LSD) Student's t-Test and Tukey's Multiple Range Test

(Tukey 1949, Zar 1974). Bartlett's procedure was employed to determine homogeneity of variance (Bartlett 1937). Results were deemed significantly different at the level $\alpha \leq 0.05$).

Linear and non-linear regression analysis were also utilized in line fitting certain data. The results of each regression analysis (including the coefficient of correlation and model equation) are reported in the Results section following the pertinent table or figure.

Correlation coefficients of each model were calculated as well as coefficients corresponding to the maximum growth rate (r) and final standing crop (K) and their standard errors. An analysis of variance was performed for each growth curve-fitting exercise. Significant differences in r and K coefficients between treatments were determined using the Student's t-test.

All experiments were repeated at different time periods for a minimum of two replicates. All analyses were performed on a IBM-compatible personal computer using the commercial software packages Statgraphics^{R} (Statistical Graphics Corp.) and SigmaPlot^{R} (Jandel Scientific).

CHAPTER 3.0

RESULTS

3.1 Fenitrothion Fate Studies

Under the conditions provided, the parent fenitrothion compound was relatively stable in the media-only systems with a mean half-life of 37.7 days (Figure 1). Persistence of fenitrothion however, was substantially affected by the presence of algae in the media, as t½ decreased over 6-fold to 5.9 days for *Ankistrodesmus falcatus* and more than 4-fold to 8.9 days for *Chlorella pyrenoidosa*. Using the analytical methods outlined in Section 2.2.3, fenitrothion degradative products (non-polar and polar) were not detected in the growth media in both algae and media-only systems.

3.2 Growth Studies

Figures 2A - L compare the growth of 12 algal species under 0.1, 1.0 and 10.0 mg/L exposures of technical fenitrothion with their growth in Kuhl's liquid growth medium alone (control). The results indicate a broad range of sensitivity within species to the pesticide stressor fenitrothion.

Maintenance of a 12:12 light dark regime during all experiments yielded synchronously dividing cultures in most cases. Microscopic observations of unicellular cultures immediately prior to the dark and light periods confirmed that vegetatively growing cells and autospores were never markedly dissimilar in size. This was taken as an indicator of relative age and cell cycle synchronicity of the cultures. The cells of some species which divided sexually in culture (e.g. *Pediastrum sp.*, *Staurastrum sp.* and *Cosmarium sp.*) responded less favourably to synchrononized lighting and therefore were comparably more variable in age and size. Size observations among the different cell types in the colonial *Anabaena* sp. revealed that although individual cellular dimensions were very similar each day, trichome length was highly variable.

In all cases, the growth of untreated algae (controls) conformed reasonably well to the logistic model characteristic of algal growth in batch cultures (Fogg 1975). Various algistatic response patterns were observed in fenitrothion treatments. Fenitrothion inhibited the growth of 9 of 12 of the species investigated to varying degrees: *Anabaena sp., Ankistrodesmus falcatus, Chlamydomonas reinhardtii, Chlamydomonas segnis, Chlorella pyrenoidosa, Chlorella vulgaris, Cosmarium sp., Navicula sp., Pediastrum sp, Scenedesmus obliquus, Selenastrum capricornutum* and *Staurastrum sp.* Toxic effects were evidenced by extended lag-phases, decreased growth rates and/or final standing crop suppressions.

A lag-phase was induced or significantly extended by 10.0 mg/L fenitrothion exposure in six species (Table 1). Compared to controls, this period of suspended growth was induced in *Ankistrodesmus falcatus, Chlamydomonas segnis, Navicula sp.* and *Selenastrum capricornutum*. In this group, lag-phase duration ranged from 3 days in *Chlamydomonas segnis* to a complete suppression of exponential growth (14 days) in the more sensitive *Ankistrodesmus falcatus*. In addition, significant extensions (4 - 5 days) of existing (controls) lag-phases were observed in *Scenedesmus obliquus* and *Staurastrum sp.*. In the latter species, lag-phases were noted in all fenitrothion treatments.

Analyses of growth bioassays over 96 hours revealed that the growth of most species (8 of 12) was significantly inhibited by fenitrothion at concentrations of 1.0 mg/L or above. A high range of differential sensitivity was observed among the twelve test species (Table 2). Over this exposure duration, responses ranged from a 65% decrease in growth rate in *Anabaena sp.* at 1.0 mg/L to no significant effects on final biomass and growth rate in three species exposed to up to 10.0 mg/L fenitrothion (*Chlorella pyrenoidosa, Cosmarium sp.* and *Pediastrum sp.*). Whereas no effects were observed in the lowest treatment (0.1 mg/L), five species (*Anabaena sp., Ankistrodesmus falcatus, Chlamydomonas segnis, Staurastrum sp.* and *Scenedesmus obliquus*) elicited significant changes from controls in 96 hour growth endpoints at the 1.0 mg/L treatment concentration. These changes varied from 8.8% reduction in growth rate of *Ankistrodesmus falcatus* to over 77% decrease in the same parameter in *Staurastrum sp.* Most species (9 of 12) exhibited some form of growth inhibition when exposed to 10.0 mg/L over 96 hours, ranging from a 15% growth rate reduction in *Chlorella vulgaris* to a 96% drop in final standing crop in *Chlamydomonas reinhardtii*.

Similarly, for longer 14 day exposures, it was found that a fenitrothion exposure threshold of 1.0 mg/L and greater was also consistently associated with growth impairment in the algae investigated (Table 3). Although fewer species and treatments displayed significant growth alterations over this extended treatment duration, 3 and 7 species exhibited growth inhibition in 1.0 and 10.0 mg/L treatments respectively. Like short-term growth tests, 0.1 mg/L fenitrothion had no significant effect on growth rate or maximum attainable biomass. Only the maximum standing crop in *Ankistrodesmus falcatus* and *Chlamydomonas reinhardtii* and growth rate of *Staurastrum sp.* were significantly suppressed by 11, 20 and 49% respectively at 1.0 mg/L. The highest treatment level evoked even more marked responses, with a greater than 50% reduction in growth rate or final biomass in all but one of the seven species.

A comparison of growth rate and maximum standing crop responses indicate that neither parameter was a consistently more sensitive indicator of the effect fenitrothion has on the growth of freshwater phytoplankton. Of a total of 36 treatments (12 species X 3 positive treatment levels) conducted, 33% (9 species) and 31% (7 species) yielded significant effects based on growth rate and final standing crop respectively for short-term (96 hour) bioassays. Similarly for long-term (14 day) bioassays, 17% (5 species) and 25% (7 species) of all treatments elicited significant growth impairment. In addition, 75, 64, 67 and 56% of the responses represented > 50% deviations from controls for 96 hour and 14 day growth rate and maximum standing crop respectively. In most treatments where growth effects were manifested (18 of 22 for 96 hour; 10 of 15 for 14 day), both parameters were significantly different from controls.

With the exception of 79 and 96% stimulations in 96 hour final standing crop of *Anabaena sp.* and *Chlamydomonas reinhardtii* respectively, all growth effects (37 cumulative accounts) observed in 12 species over 4 and 14 day exposures to concentrations of 0.1 - 10.0 mg/L fenitrothion, constituted significant inhibitions compared to controls.

EC_{50} values were estimated for those species eliciting significant growth responses from controls over the treatment exposures tested (Table 4). EC_{50} values, obtained using EPA Probit Analyses Program (Version 1.4), represent effective concentrations of fenitrothion producing a 50% difference from controls in a given response parameter. EC_{50} for growth ranged over three orders of magnitude from 0.84 mg/L based on *Staurastrum sp.* 96 hour growth rate to 109.0 mg/L for *Chlamydomonas reinhardtii* 14 day standing crop. Although a high degree of variability was observed among species, the majority of EC_{50} values (85%) were in the range of 1 - 10 mg/L.

3.2.1 Sensitivity Indices - Growth

For the purposes of quantitatively correlating the overall growth effects of fenitrothion on phytoplankton to their various physical, physiological, biochemical and taxonomic properties, four separate sensitivity indices were defined and measured. These include for each species, cumulative % significant ($P \leq 0.05$) differences from controls for (i) maximum growth rate and (ii) maximum attainable biomass (standing crop) based on (a) 96 hour growth bioassays and (b) 14 day growth bioassays. The values for each indice are summarized in Tables 2 and 3.

3.2.2 Sensitivity Rankings - Growth

Sensitivity indices for all 12 species were calculated based upon significant growth responses ($P \leq 0.05$) observed in short-term (96 hours) and long-term (14 days) bioassays. These indices were later correlated against various physical and physiological attributes (Section 3.8). Several rankings were established based on bioassay length (96 hour or 14 day) and measured growth endpoint (growth rate or maximum biomass). These are summarized in Table 5.

3.3 Cell Density, Cell Dry Weight and Cell Volume

Following six days of exposure to 0.1, 1.0 and 10.0 mg/L fenitrothion, several distinct dose-response patterns in cell number, dry weight and cell volume were observed in the four species tested (Figures 3A - D and Appendix IV).

An inverse trend between cell numbers and dry weight was consistently noted among *Chlorella pyrenoidosa, Chlamydomonas segnis, Scenedesmus obliquus* and *Selenastrum capricornutum* at treatment levels above 0.1 mg/L. In addition to this trend, an increase in cell volume was observed with increasing fenitrothion concentrations. Apart from a 24% stimulation in *Selenastrum capricornutum* cell number at the 0.1 mg/L treatment level, all effects on these parameters occurred at and above 1.0 mg/L. Maximum augmentation in cell dry weight was 249% from controls in *Chlamydomonas segnis* whereas cell numbers were as much as 71% lower than controls in *Selenastrum capricornutum* following 7 days exposure to 10.0 mg/L fenitrothion. In addition, significant increases in cell volume of 2.5, 4.6, 18 and 30% were observed in *Chlamydomonas segnis, Scenedesmus obliquus, Selenastrum capricornutum* and *Chlorella pyrenoidosa* respectively. This effect manifested itself in sizeable shifts in the size structure of algal populations (Section 3.4).

Further examination of alterations in cell size in *Selenastrum capricornutum* following an additional 3 days exposure indicated that cell volume augmentation had increased an additional 11% (corresponding to a 27% gain compared to controls).

3.4 Population Size Spectra

Size distributions were obtained following six days of fenitrothion treatment. All treatments were analysed at the same time to negate variation attributable to the cell cycle. The results indicate a shift in cell size spectra at the 10.0 mg/L level as treated cultures of *Chlamydomonas segnis, Chlorella pyrenoidosa, Scenedesmus obliquus* and *Selenastrum capricornutum* displayed a tendency towards larger cells becoming more abundant (Figures 4A - D). Although size spectrum shifts are not apparent at 0.1 mg/L treatments in any of the test algae, deviations from controls are evident in treatments as low as 1.0 mg/L in *Selenastrum capricornutum*.

3.5 Macromolecular Studies

The biomolecular indicators of phytotoxicity examined in *Ankistrodesmus falcatus* and *Chlamydomonas segnis* were total cellular protein, carbohydrate, RNA and DNA. A trend of elevated levels of these biomolecules was evident in fenitrothion treatments above 0.1 mg/L (Figures 5A and B and Appendix IV). In *Chlamydomonas segnis*, a significantly increased carbohydrate level was observed at 10 mg/L. *Ankistrodesmus falcatus* was appreciably more sensitive. Cellular protein levels were significantly augmented by 34 and 50% at 1 and 10 mg/L treatment levels, while carbohydrate increased 52% from control at 10 mg/L treatment. Multiple range tests (Tukey's) supported these results at the 95% confidence level.

3.6 Lipid Studies

3.6.1 Total Lipid Content in Selected Phytoplankton

The total lipid contents in unstressed (control) cells were determined for all 11 unicellular species and the results are summarized in Table 6. Lipid contents ranged from 1.5% of cell dry weight in *Chlamydomonas segnis* to 23.1% in *Navicula sp.*

3.6.2 Effects on Total Lipids

The total lipid contents of *Ankistrodesmus falcatus* cells following 48 hours treatment of 0.1, 1.0 and 10.0 mg/L fenitrothion are summarized in Figure 6. Although the total lipids per cell increased by more than a factor of 2 at the 10.0 mg/L level, the percentage of total lipids per dry weight of cell did not significantly change. This is explained by the corresponding increase (from 19.2 ± 3.4 to 39.9 ± 7.2 pg/cell) in cell dry weight following 48 hours treatment at the 10.0 mg/L level.

3.6.3 Effects on Fatty Acids of Total Lipids

The fatty acid content of the total lipids in *Ankistrodesmus falcatus* was determined and summarized in Table 7. These data were compared to recent work (Kauss 1987) on the fatty acid composition of the genus *Ankistrodesmus*. In general, the present results reflected similar composition with the exception that a minor *Ankistrodesmus* fatty acid, stearic (18:0), was not detected here.

The lipids of unstressed (control) *Ankistrodesmus falcatus* cells were typically composed of 35.7% linolenic (18:3)[4], 25.2% oleic (18:1), 22.2% palmitic (16:0), 10.1% palmitoleic (16:1) and 6.8% linoleic (18:2) acid. It is evident that a 48 hour 10.0 mg/L treatment of fenitrothion significantly altered the fatty acid composition of *Ankistrodesmus falcatus* lipids. A saturation occurred in *Ankistrodesmus* membranes represented by the 5.4 and 4.3% increase in palmitic (16:0) and oleic (18:1) acids and a 1.8 and 7.6% decrease in linoleic (18:2) and linolenic (18:3) acids respectively. This resulted in a shift from a linolenic (18:3) acid dominated profile to a more saturated oleic (18:1) acid one.

3.7 Bioaccumulation Studies

The initial ^{14}C fenitrothion uptake experiments with *Chlamydomonas reinhardtii* and *Chlamydomonas segnis* identified 24 hours as the uptake maxima for fenitrothion (Figure 7). Although it was not the purpose of this inquiry to detail the kinetics and mechanisms of algal uptake of fenitrothion, the methods employed yielded several notable observations regarding the bioaccumulation of fenitrothion by algae under laboratory conditions. Firstly, although the algae did bioconcentrate fenitrothion above the ambient concentrations, most (> 97%) of the radiolabelled fenitrothion remained in solution (primary supernatant) over the time periods monitored. Those fractions associated with algal biomass (secondary supernatant and filtered cells) generally accounted for less than 3 % of the total radiocarbon mass in the system. Of these fractions, significantly more (8.0 - 12.8 times for live cells; 7.2 -7.5 times for dead cells) ^{14}C fenitrothion was found in the secondary supernatant. This was interpreted as that fraction loosely associated or sorbed on/into the cells or cell material (i.e. able to be removed

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[4] Numbers represent ratio of normal carbon chain length to number of double bonds.

by rigorous rinse).

Bioconcentration factors (BCF), or the ratio of the total radiocarbon associated with algae (filter and secondary supernatant) over the total radiocarbon detected in the media (primary supernatant), calculated for viable *Chlamydomonas reinhardtii* and *Chlamydomonas segnis* cells were 293.1 ± 17.3 and 124.2 ± 12.8 respectively. Similarly, BCF's for dead cells were 1260.7 ± 107.4 and 1024.5 ± 80.2 for the same species respectively.

3.8 Sensitivity Correlations

Correlation analyses were performed between the following measured attributes of the phytoplankton investigated and their corresponding sensitivity indices and rankings. A total of 7 significant relationships ($P \leq 0.05$) were found and these are reported below.

3.8.1 Cell Size

Cellular dimensions obtained microscopically corresponded well (within ± 5.7%) with the cell volumes calibrated from the Coulter Channelyzer. Mean cellular size attributes of all twelve species are listed in Table 9.

Upon examining the four functions of cell size, and their relationship to phytoplankton sensitivity to fenitrothion, it is apparent that the transformed ratio of cell surface area : volume provided the strongest predictor of sensitivity. This ratio accounted for 77 and 72% of the variability in 14 day growth rate and maximum standing crop response to fenitrothion (Figures 8 and 9). These results also explained the significant correlation with sensitivity ranking for all 12 species (Figures 10 and 11).

3.8.2 Total Lipid Content

A significant relationship ($r^2 = 0.57$) with 96 hour growth rate sensitivity index (Figure 12) indicates that this variable may play a important role in determining sensitivity of phytoplankton to fenitrothion stress over shorter exposures.

3.8.3 Bioaccumulation Capacity

This predictor (live cells) accounted for 56% of the variability in 14 day growth rate responses to fenitrothion (Figure 13). Also of notable significance is that the transformed ratio of cell surface area : volume explained a significant percentage ($r^2 = 0.68$) of the variability in bioaccumulation capacity in live cells (Figure 14).

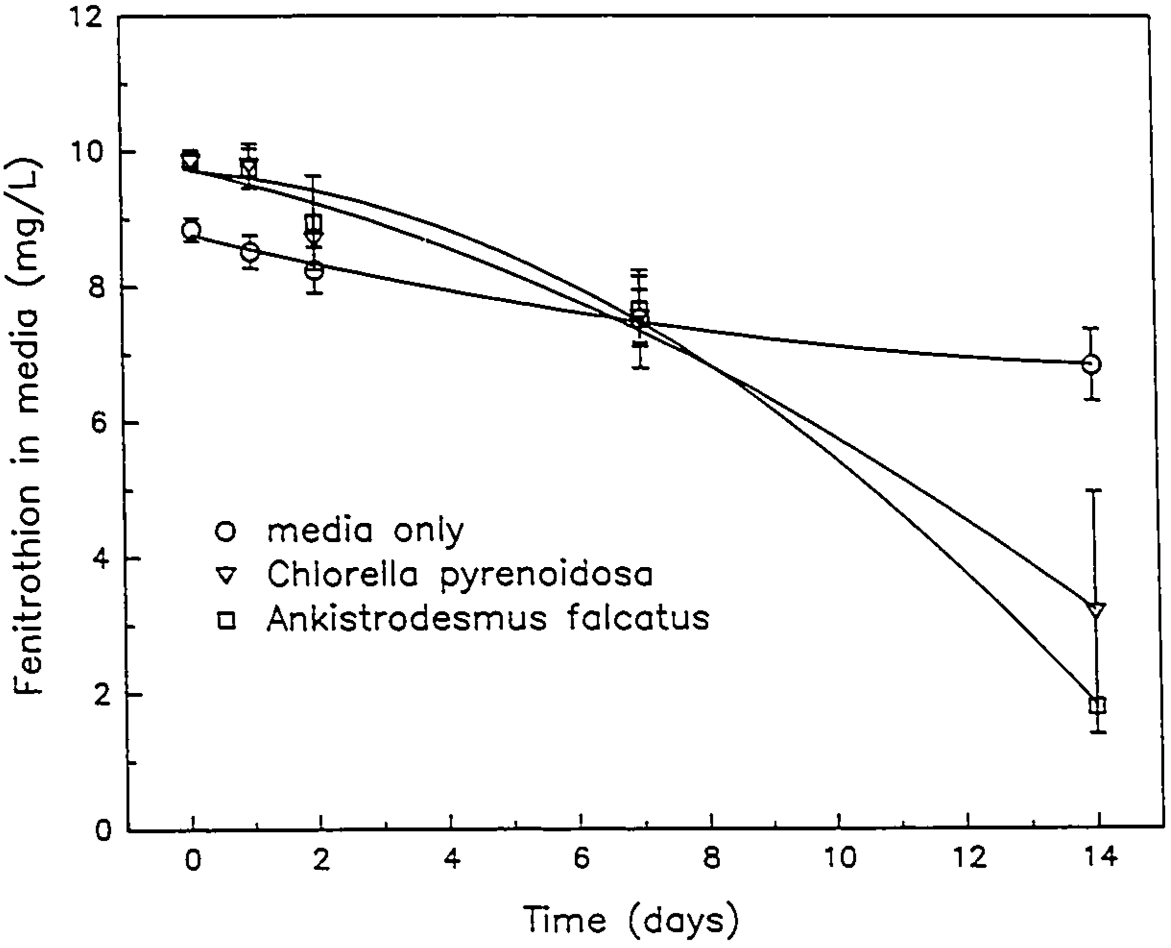


FIGURE 1. Degradation of parent fenitrothion (10.0 mg/L) in algal and media-only laboratory microcosms at 20°C (75-95 μEinsteins m^{-2} sec^{-1} , 12:12 photoperiod). Data presented are means and standard deviations of four replicates.

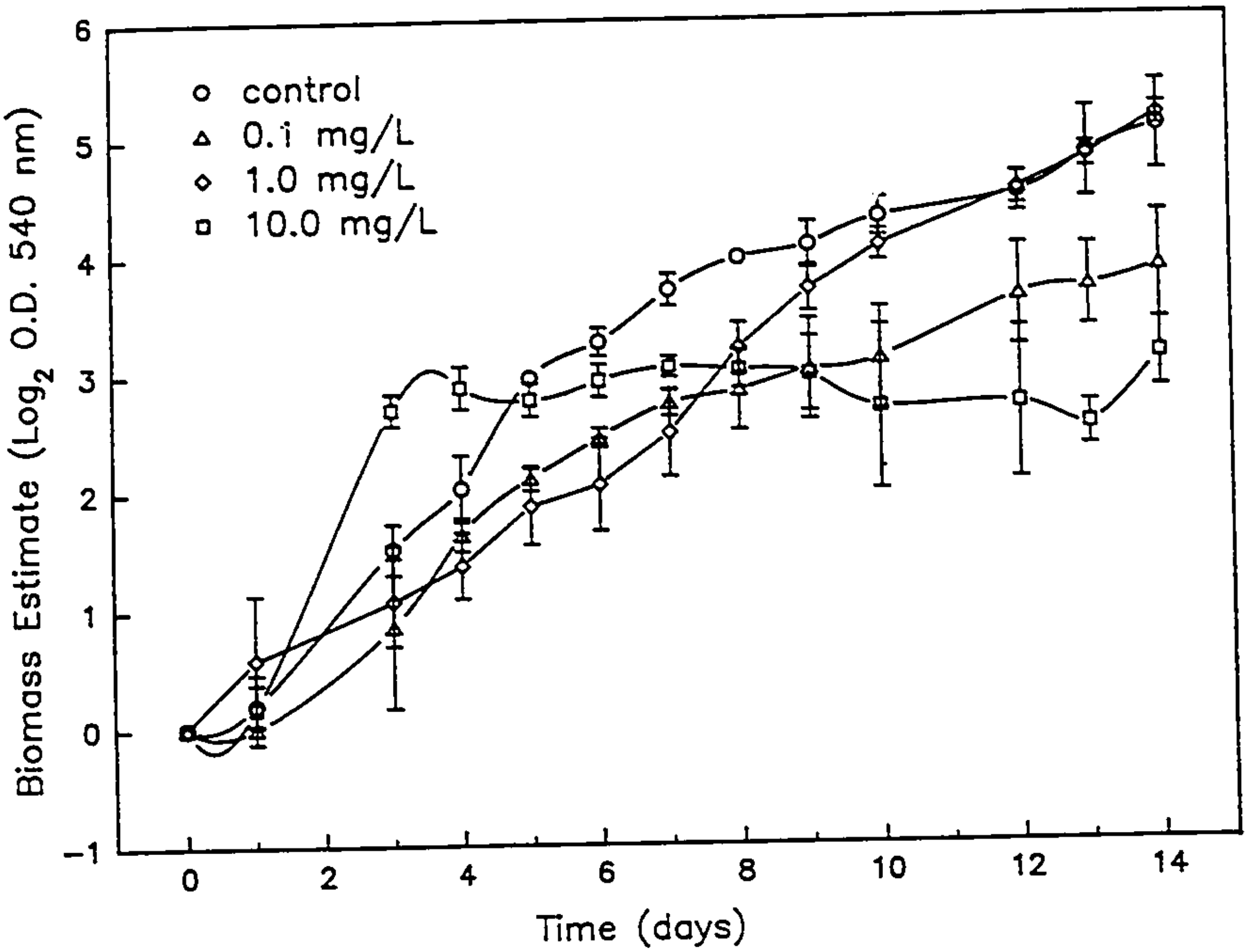


FIGURE 2A. Growth of the alga *Anabaena sp.* in the absence (control) and presence of 0.1, 1.0 and 10.0 mg/L of fenitrothion. Means and standard deviations of four replicates are shown.

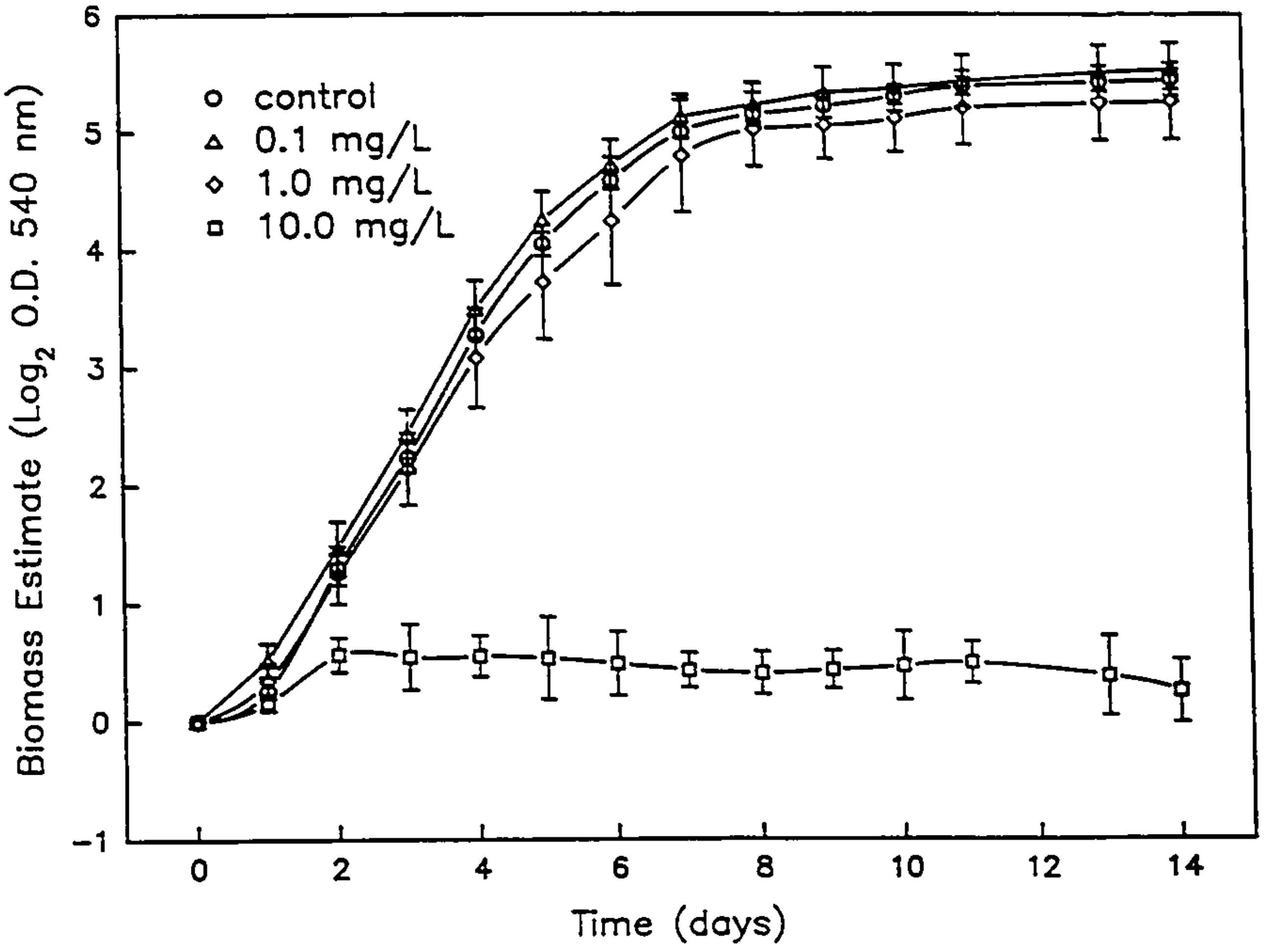


FIGURE 2B. Growth of the alga *Ankistrodesmus falcatus* in the absence (control) and presence of 0.1, 1.0 and 10.0 mg/L of fenitrothion. Means and standard deviations of four replicates are shown.

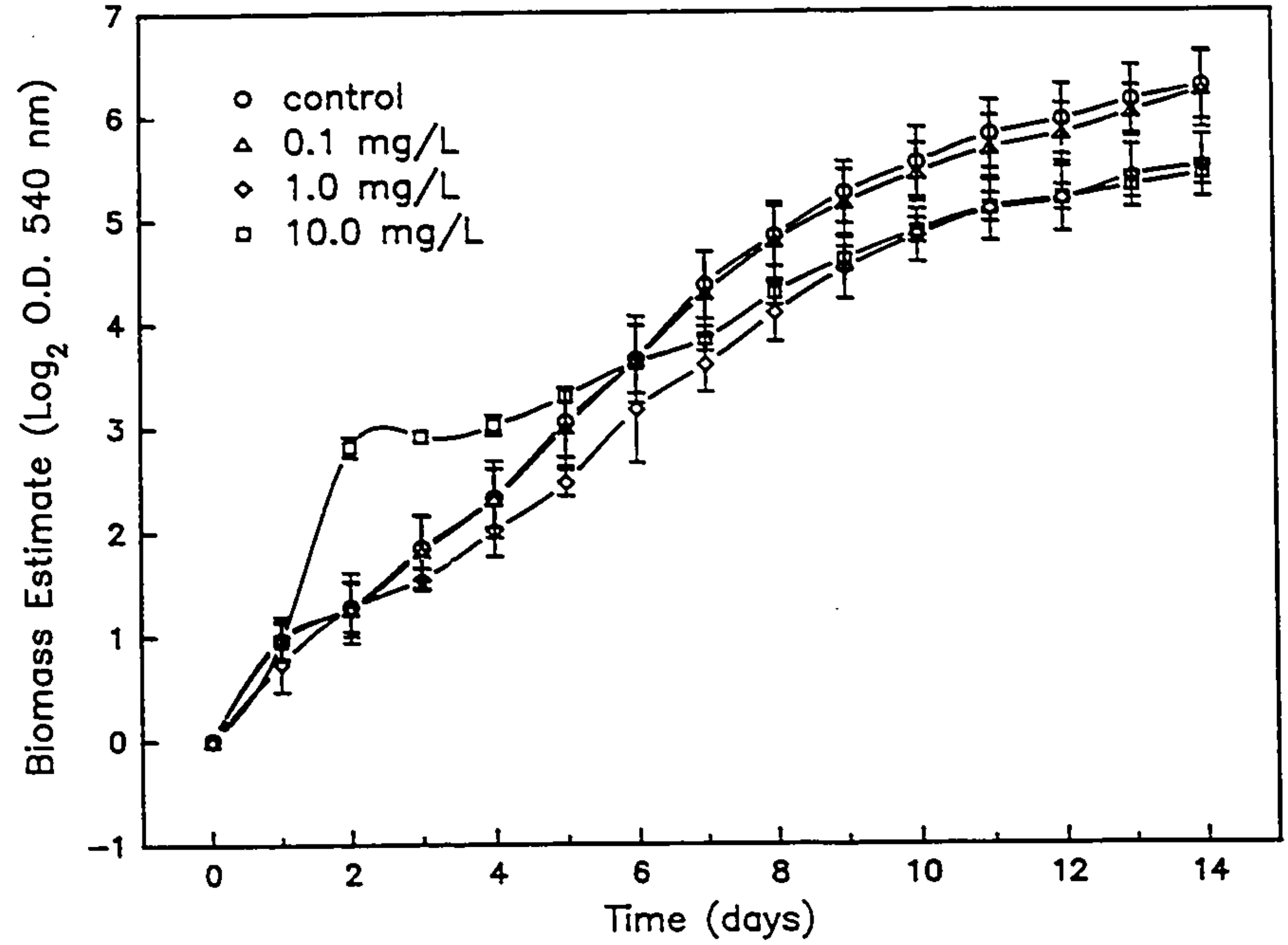


FIGURE 2C. Growth of the alga *Chlamydomonas reinhardtii* in the absence (control) and presence of 0.1, 1.0 and 10.0 mg/L of fenitrothion. Means and standard deviations of four replicates are shown.

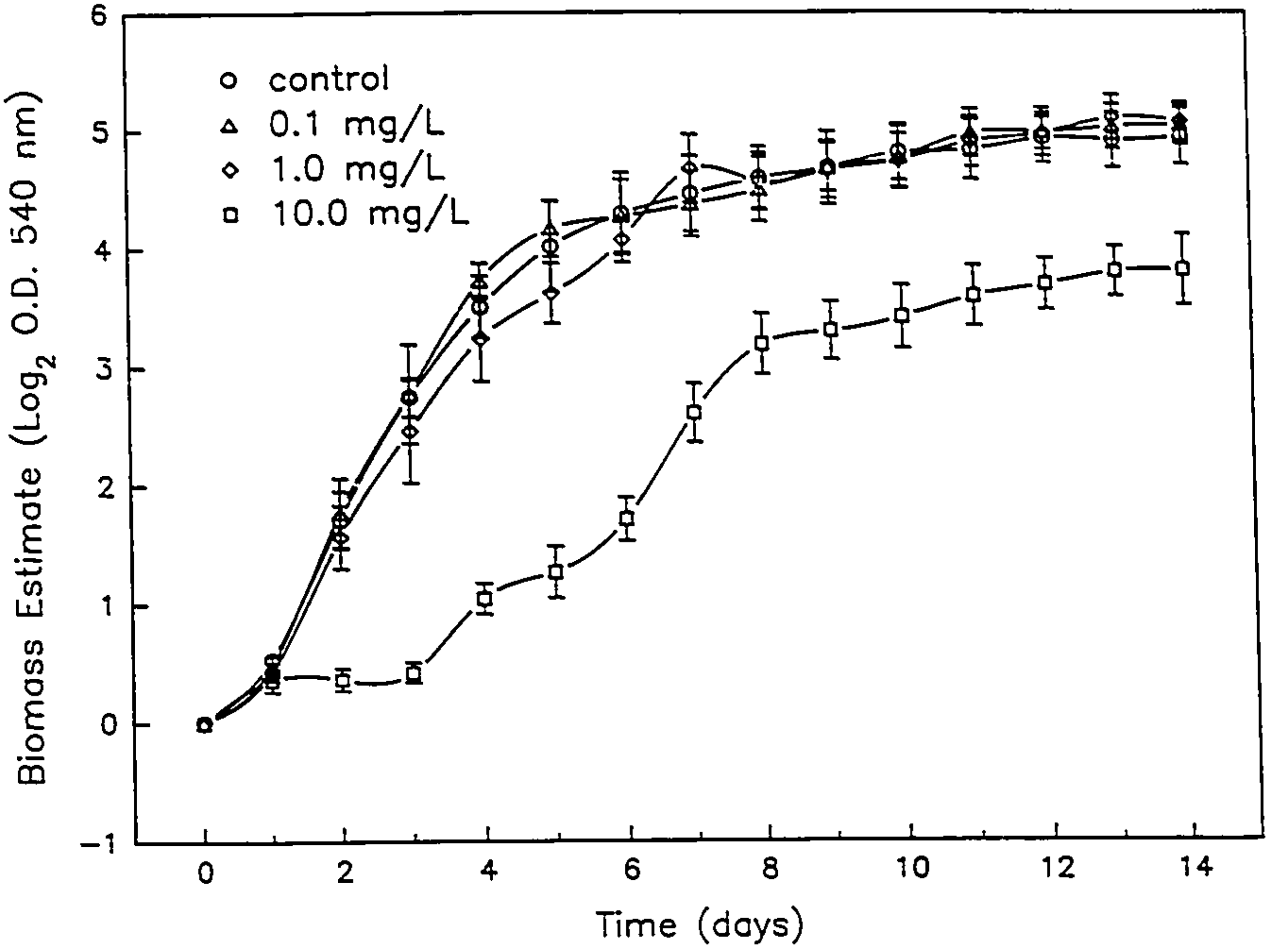


FIGURE 2D. Growth of the alga *Chlamydomonas segnis* in the absence (control) and presence of 0.1, 1.0 and 10.0 mg/L of fenitrothion. Means and standard deviations of four replicates are shown.

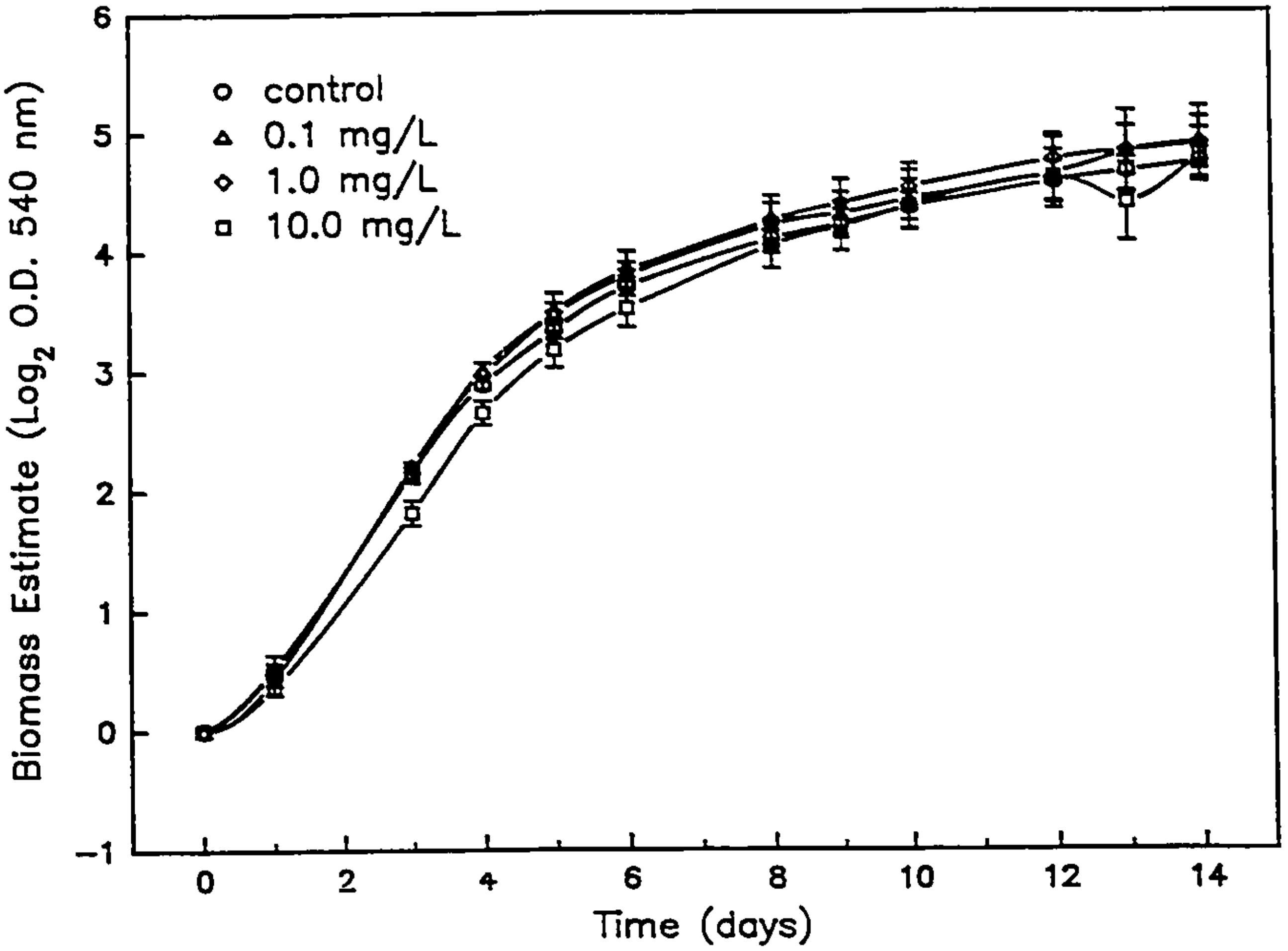


FIGURE 2E. Growth of the alga *Chlorella pyrenoidosa* in the absence (control) and presence of 0.1, 1.0 and 10.0 mg/L of fenitrothion. Means and standard deviations of four replicates are shown.

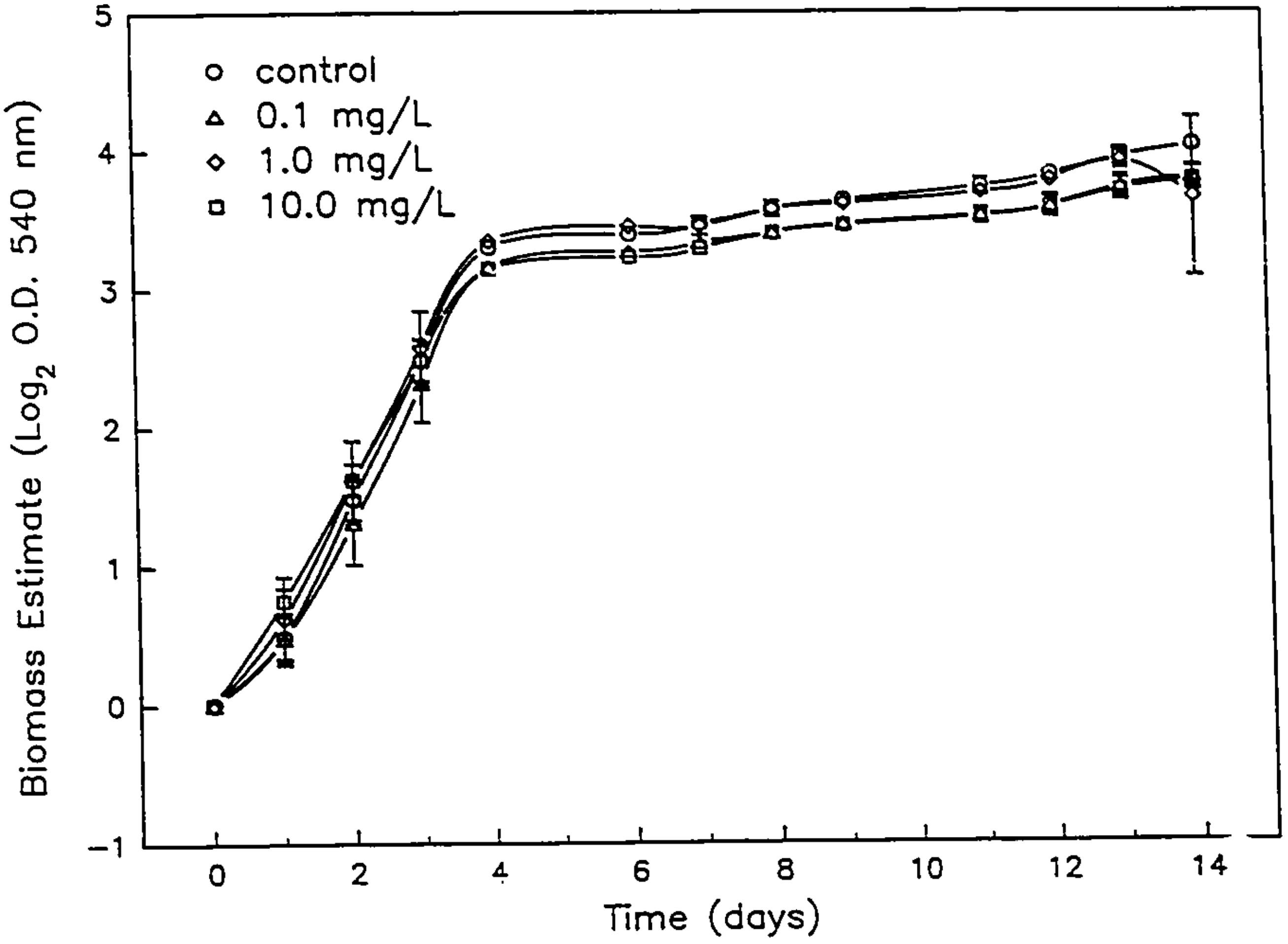


FIGURE 2F. Growth of the alga *Chlorella vulgaris* in the absence (control) and presence of 0.1, 1.0 and 10.0 mg/L of fenitrothion. Means and standard deviations of four replicates are shown.

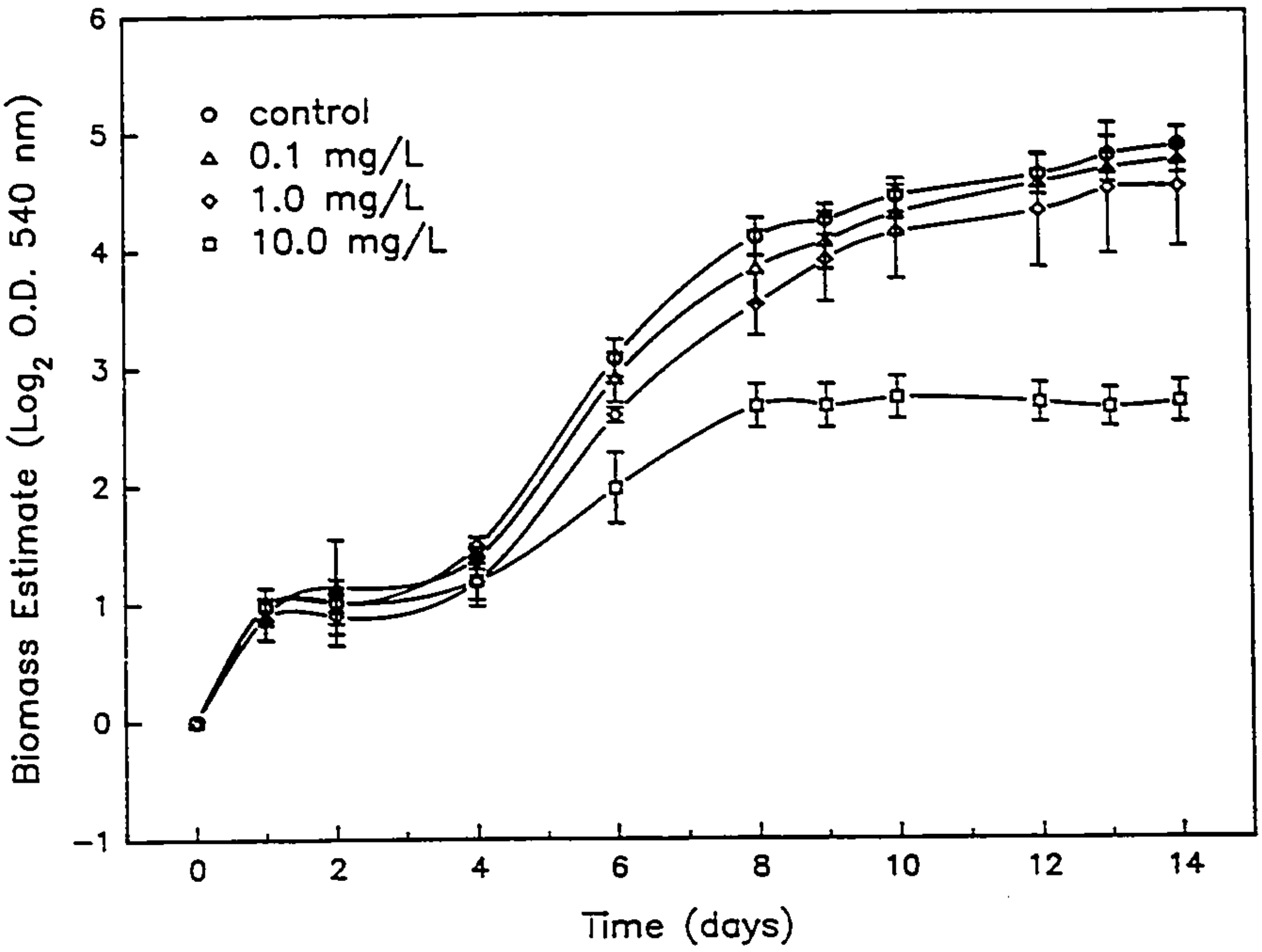


FIGURE 2G. Growth of the alga *Cosmarium sp.* in the absence (control) and presence of 0.1, 1.0 and 10.0 mg/L of fenitrothion. Means and standard deviations of four replicates are shown.

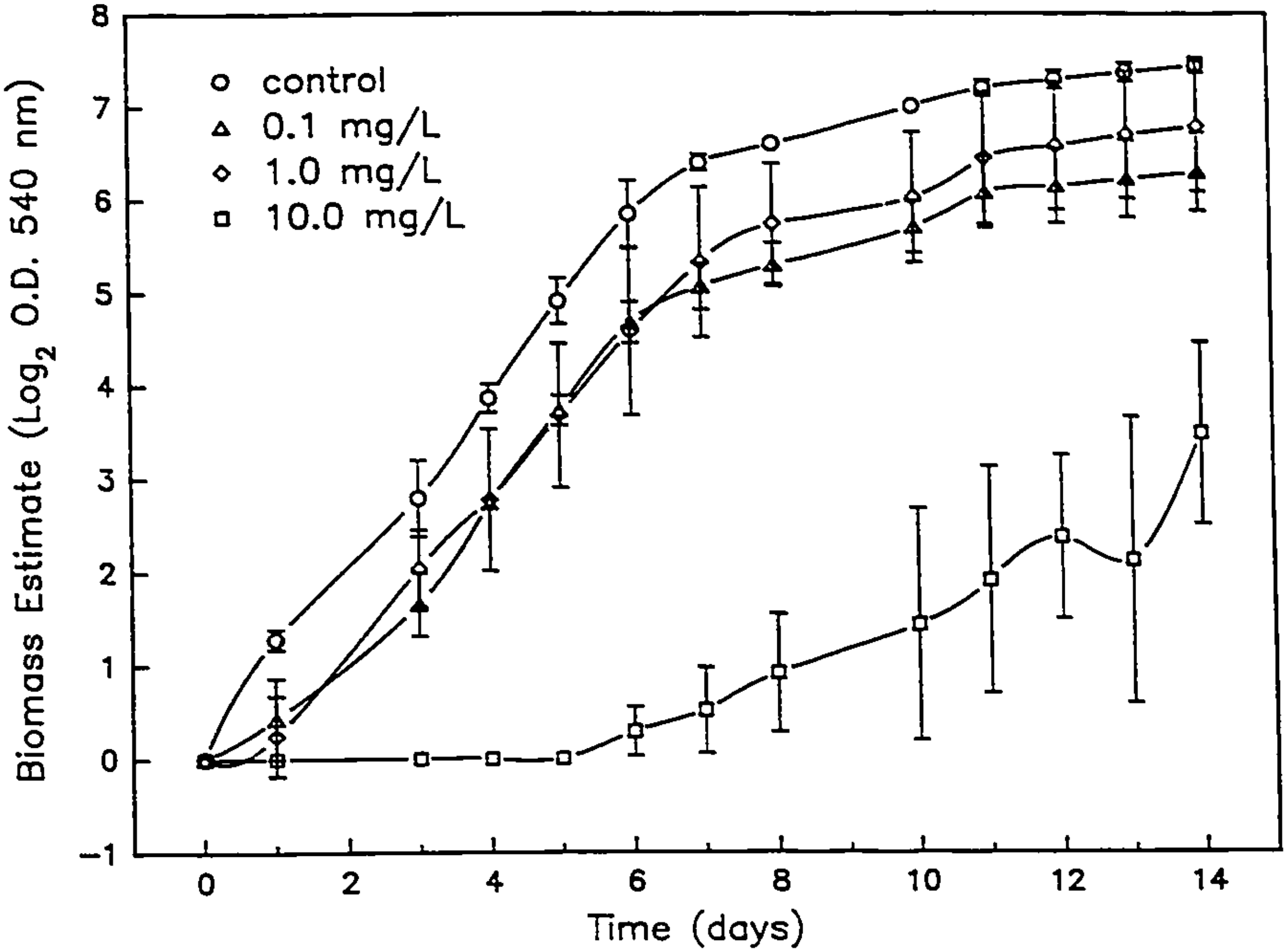


FIGURE 2H. Growth of the alga *Navicula sp.* in the absence (control) and presence of 0.1, 1.0 and 10.0 mg/L of fenitrothion. Means and standard deviations of four replicates are shown.

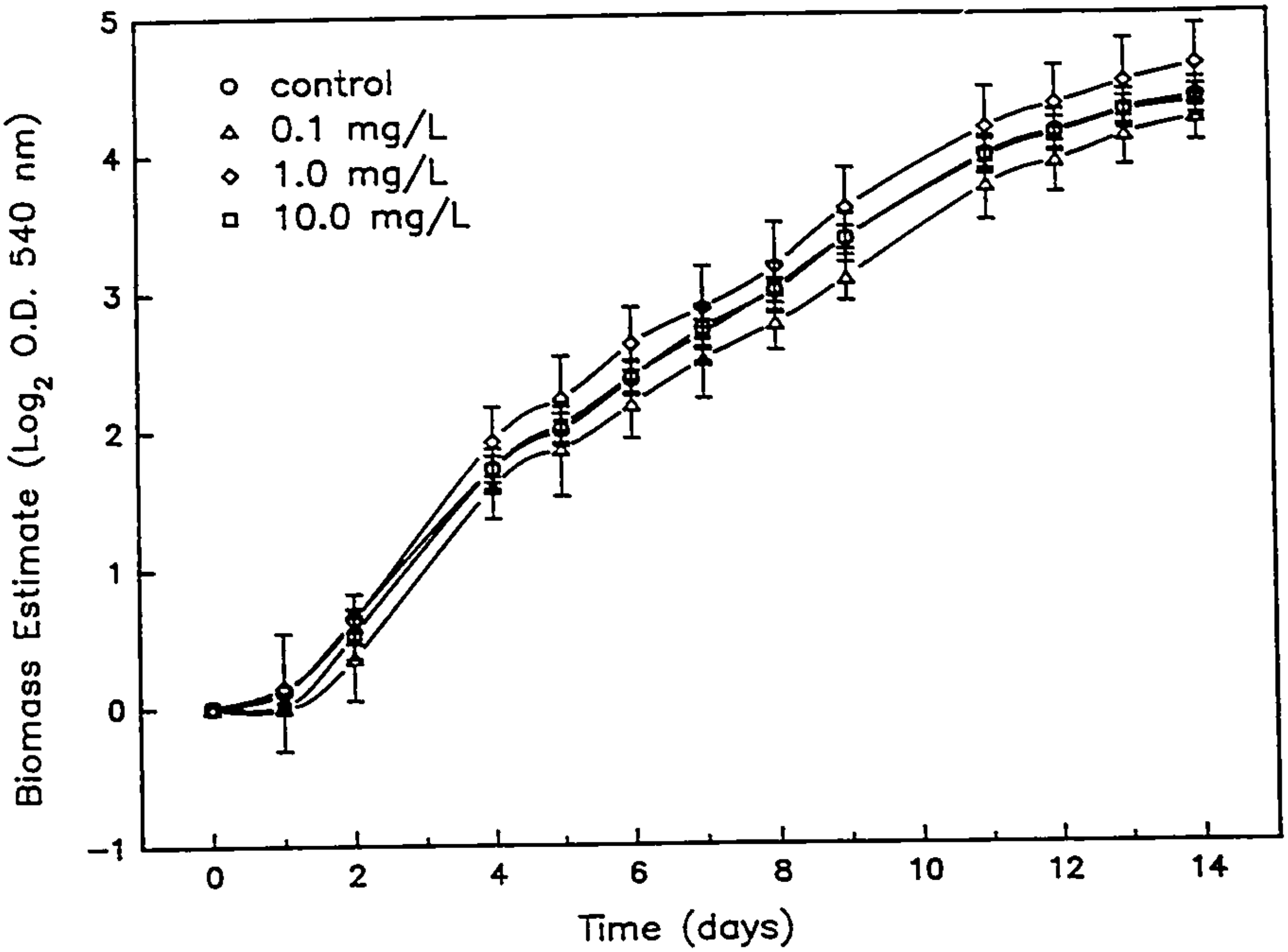


FIGURE 21. Growth of the alga *Pediastrum sp.* in the absence (control) and presence of 0.1, 1.0 and 10.0 mg/L of fenitrothion. Means and standard deviations of four replicates are shown.

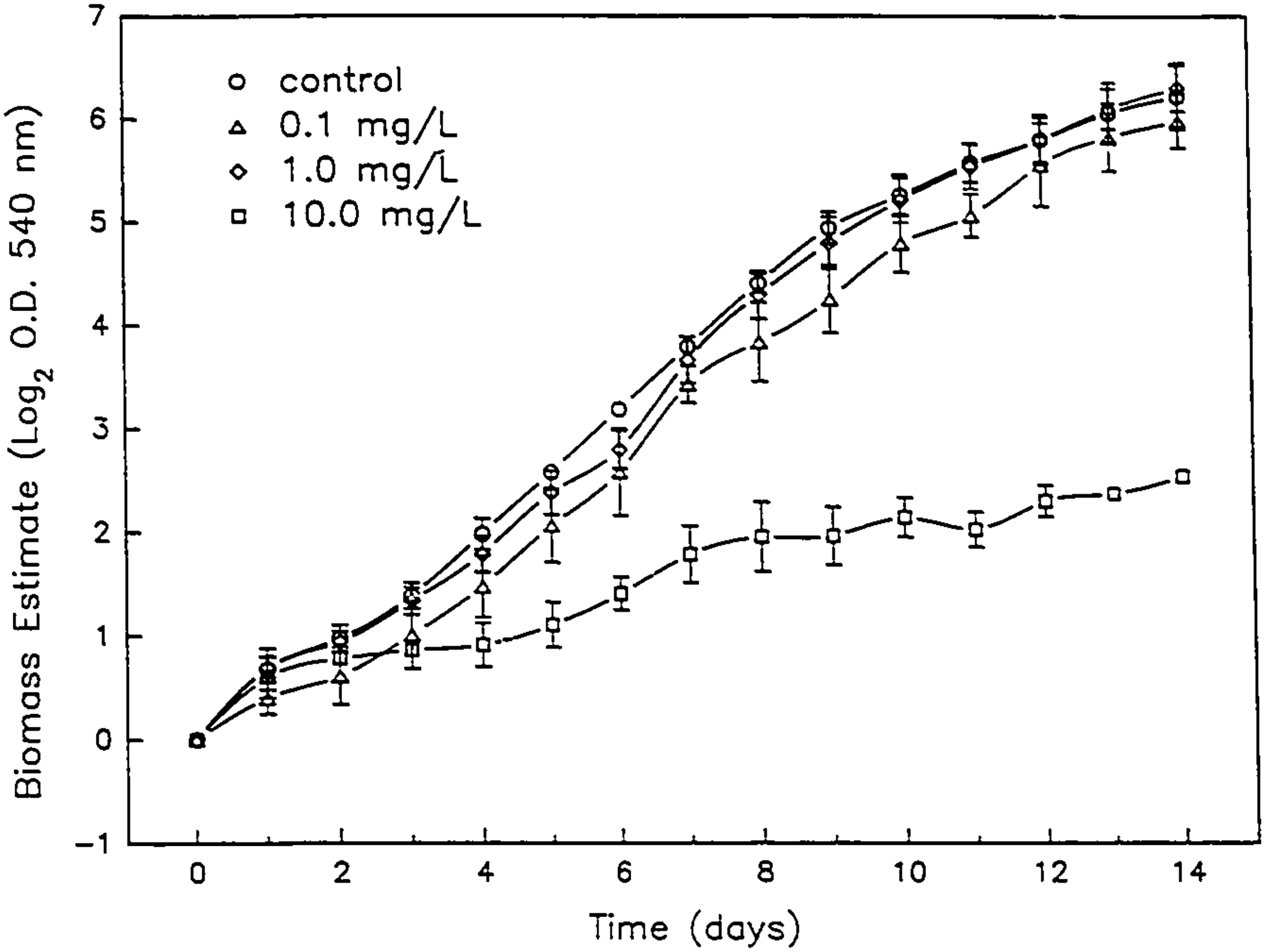


FIGURE 2J. Growth of the alga *Scenedesmus obliquus* in the absence (control) and presence of 0.1, 1.0 and 10.0 mg/L of fenitrothion. Means and standard deviations of four replicates are shown.

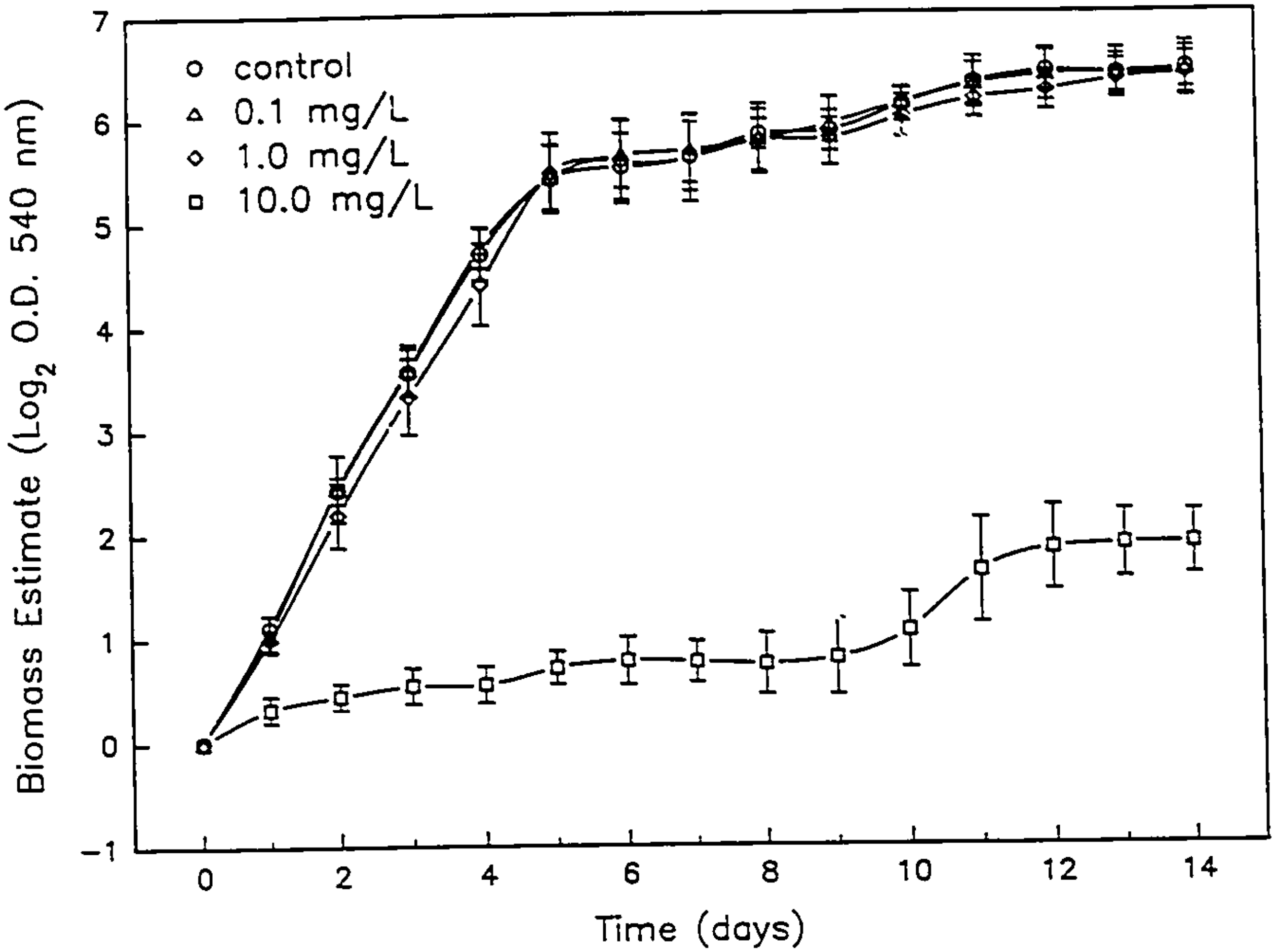


FIGURE 2K. Growth of the alga *Selenastrum capricornutum* in the absence (control) and presence of 0.1, 1.0 and 10.0 mg/L of fenitrothion. Means and standard deviations of four replicates are shown.

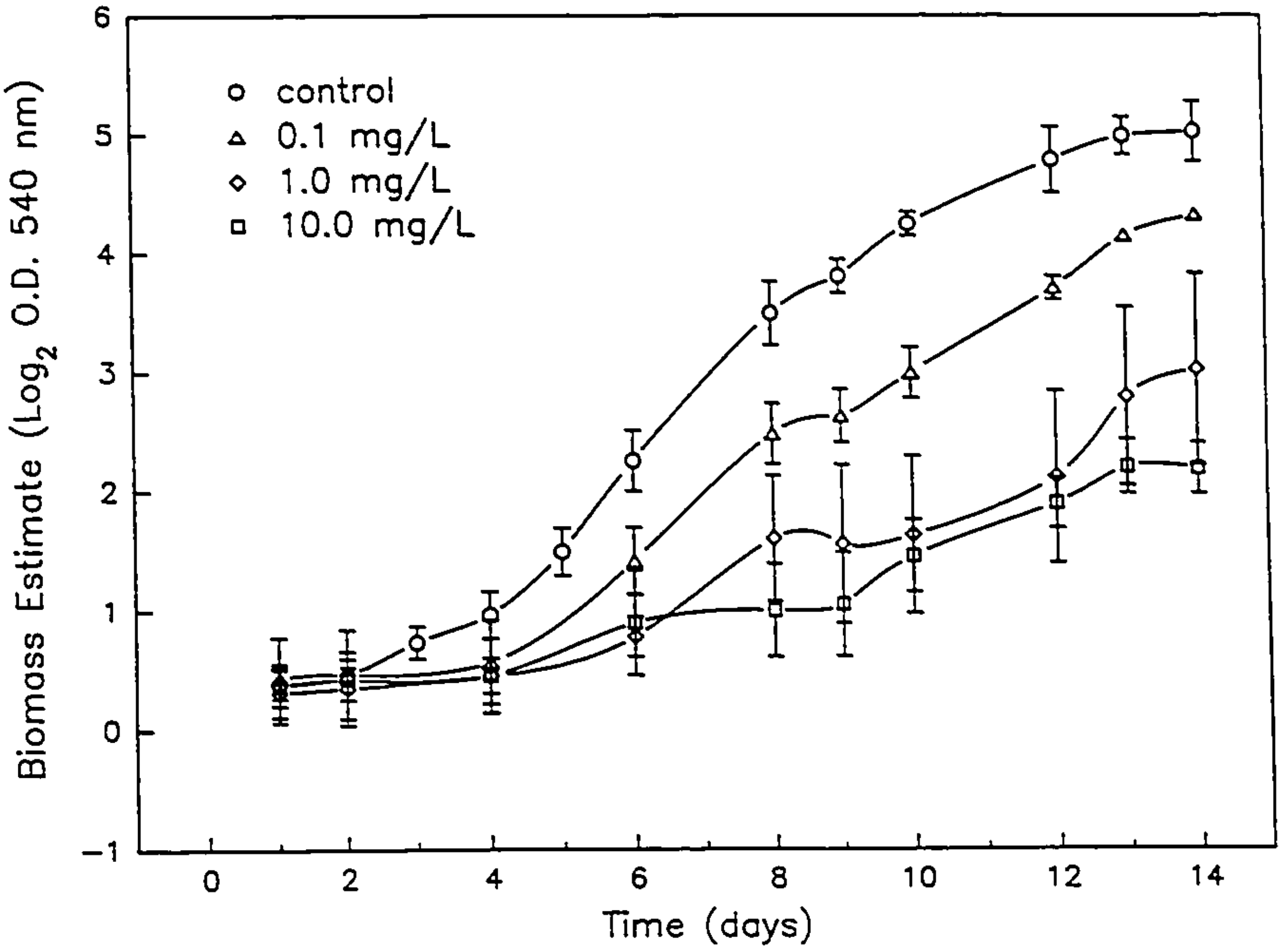


FIGURE 2L. Growth of the alga *Staurastrum sp.* in the absence (control) and presence of 0.1, 1.0 and 10.0 mg/L of fenitrothion. Means and standard deviations of four replicates are shown.

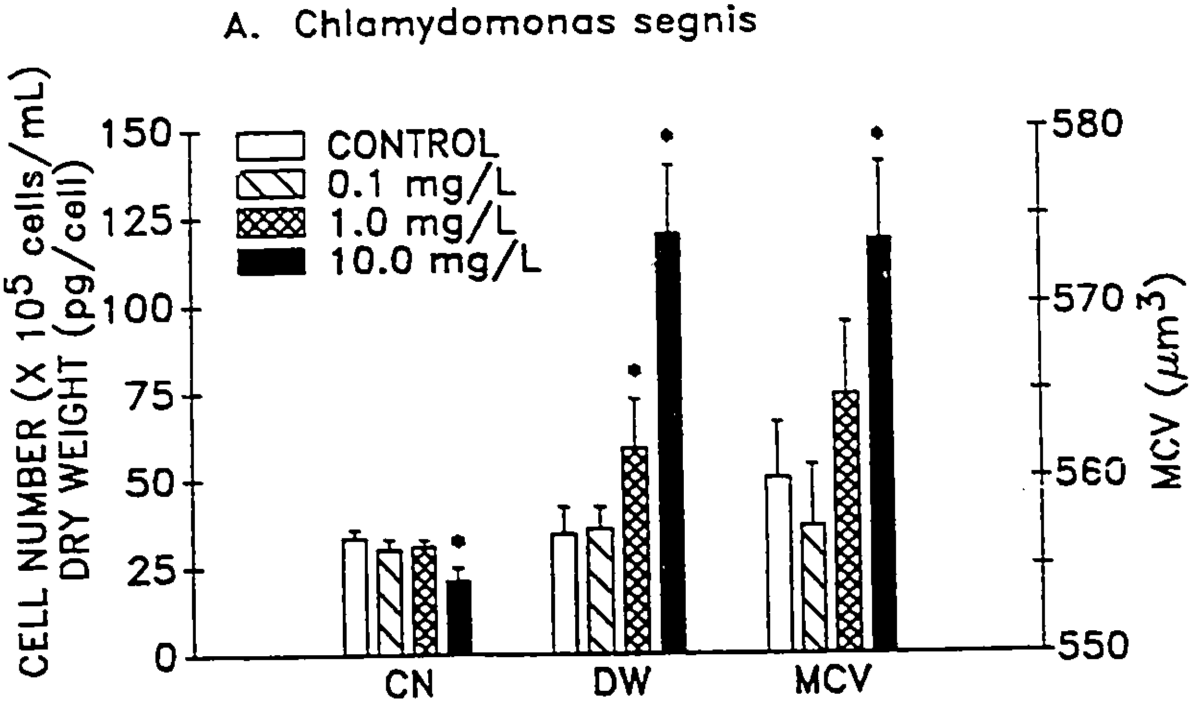


FIGURE 3A. Effects of fenitrothion (0.1, 1.0 and 10.0 mg/L) on cell density, cell dry weight and modal cell volume of *Chlamydomonas segnis* following 6 days treatment. Data presented are means and standard deviations of four replicates. An asterisk denotes significant difference from controls ($P \leq 0.05$) determined by Tukey's Multiple Range test.

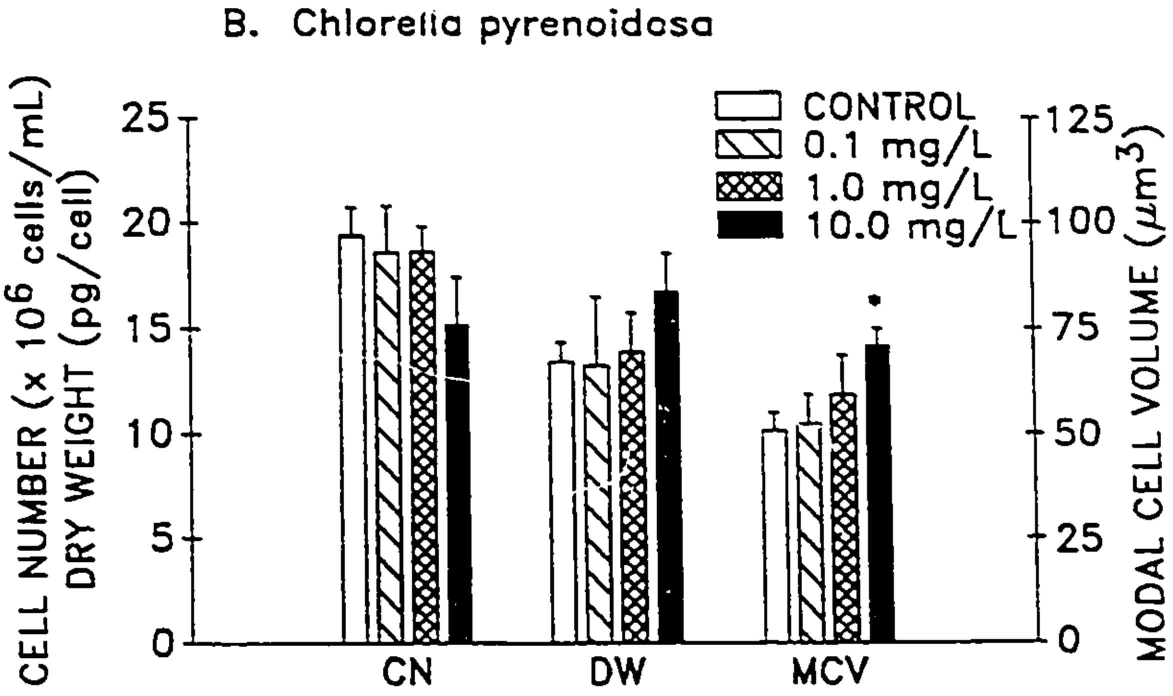


FIGURE 3B. Effects of fenitrothion (0.1, 1.0 and 10.0 mg/L) on cell density, cell dry weight and modal cell volume of *Chlorella pyrenoidosa* following 6 days treatment. Data presented are means and standard deviations of four replicates. An asterisk denotes significant difference from controls ($P \leq 0.05$) determined by Tukey's Multiple Range test.

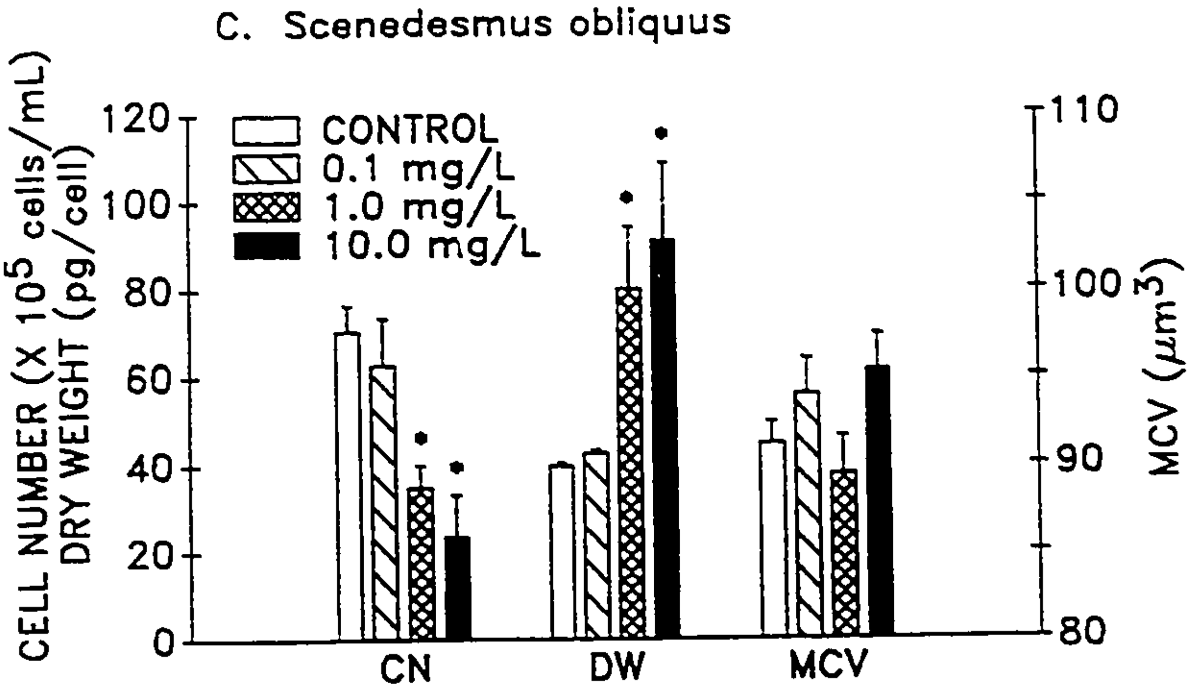


FIGURE 3C. Effects of fenitrothion (0.1, 1.0 and 10.0 mg/L) on cell density, cell dry weight and modal cell volume of *Scenedesmus obliquus* following 6 days treatment. Data presented are means and standard deviations of four replicates. An asterisk denotes significant difference from controls ($P \leq 0.05$) determined by Tukey's Multiple Range test.

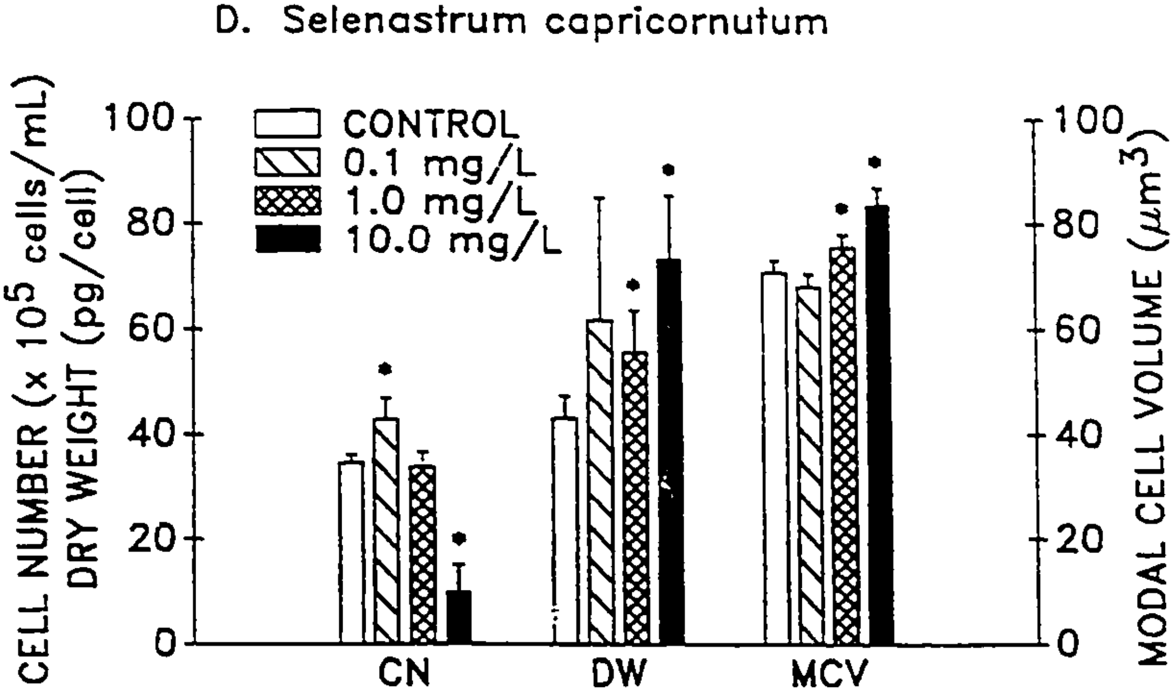


FIGURE 3D. Effects of fenitrothion (0.1, 1.0 and 10.0 mg/L) on cell density, cell dry weight and modal cell volume of *Selenastrum capricornutum* following 6 days treatment. Data presented are means and standard deviations of four replicates. An asterisk denotes significant difference from controls ($P \leq 0.05$) determined by Tukey's Multiple Range test.

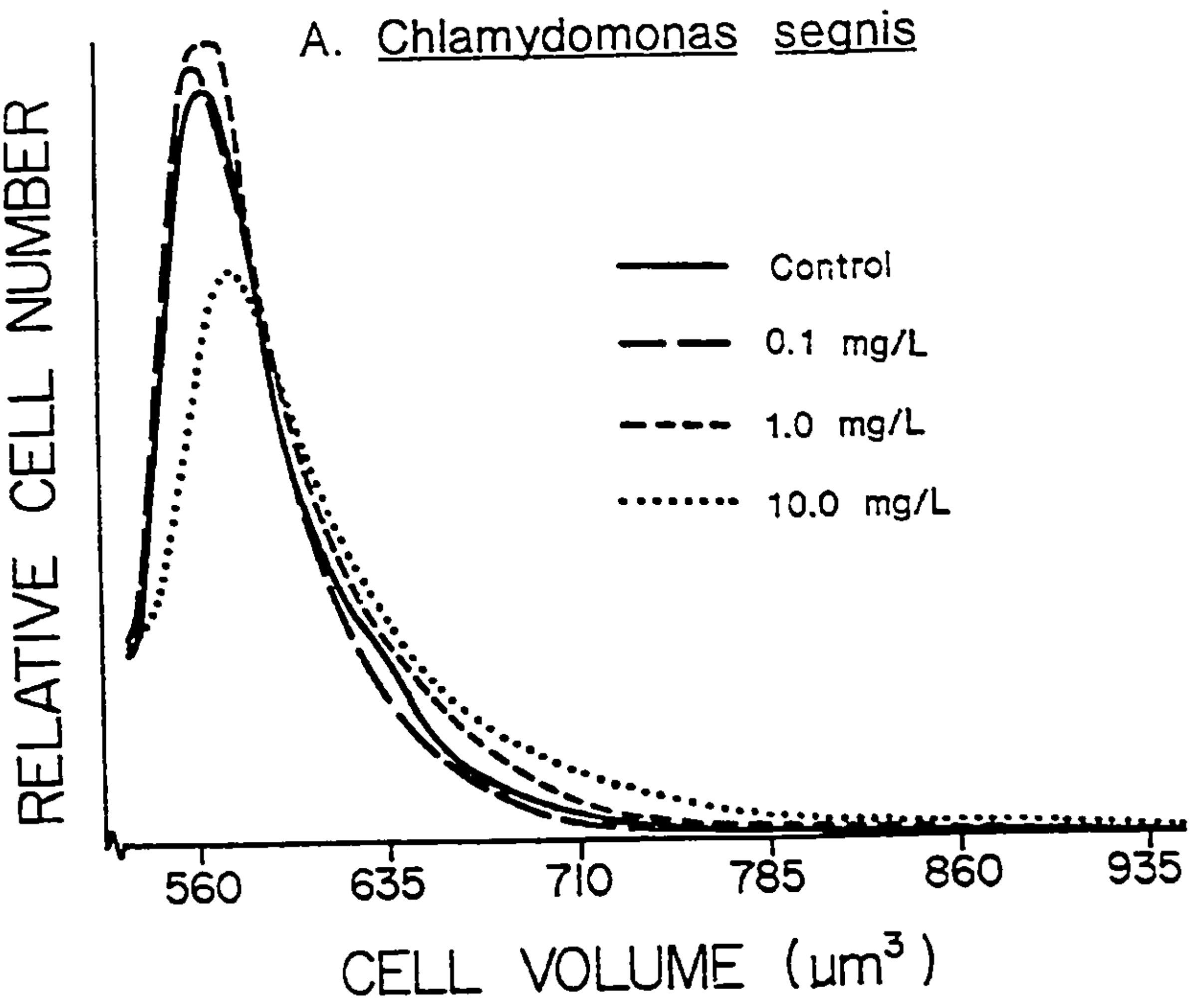


FIGURE 4A. The effects of fenitrothion (0.1, 1.0 and 10.0 mg/L) on the cell size spectra of *Chlamydomonas segnis*. Normalized mean cell volume frequency distributions following 6 days treatment are presented.

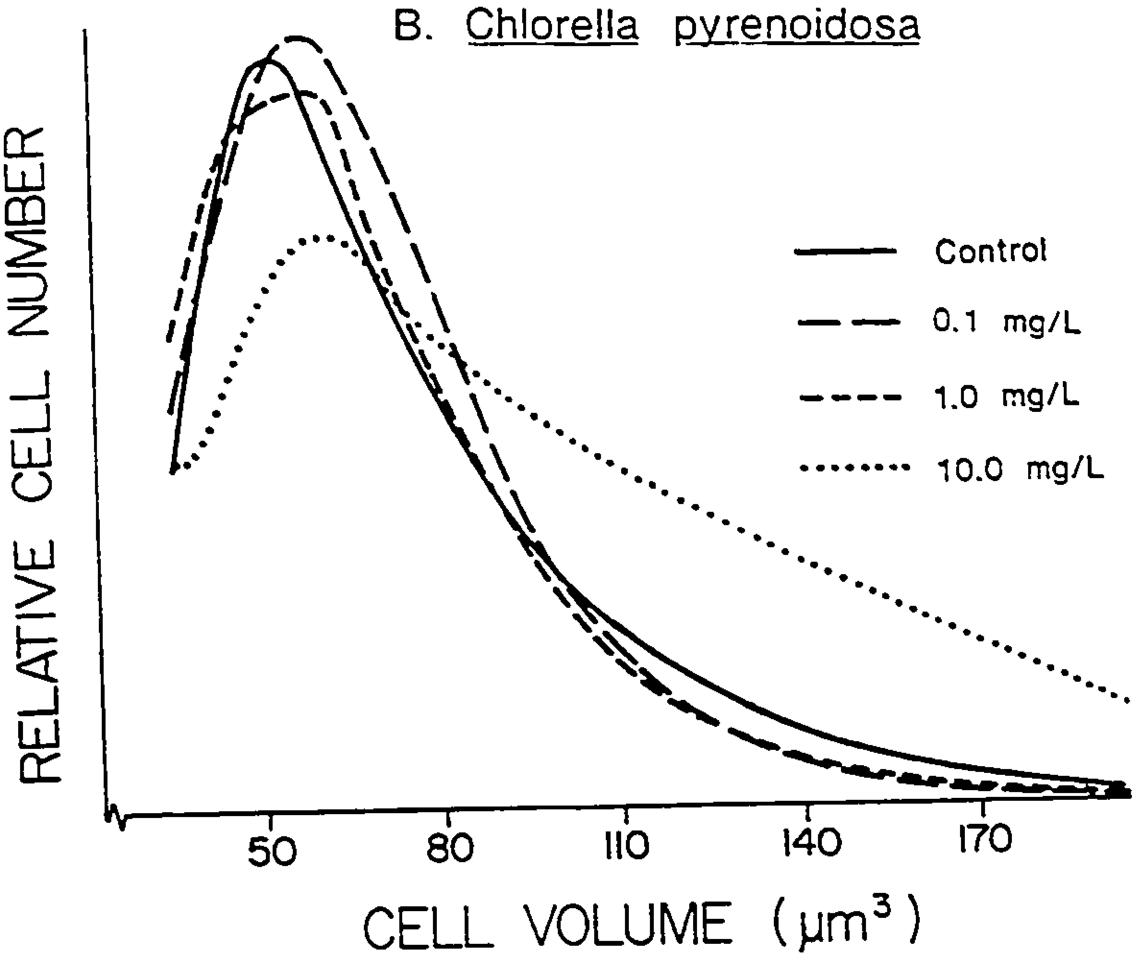


FIGURE 4B. The effects of fenitrothion (0.1, 1.0 and 10.0 mg/L) on the cell size spectra of *Chlorella pyrenoidosa*. Normalized mean cell volume frequency distributions following 6 days treatment are presented.

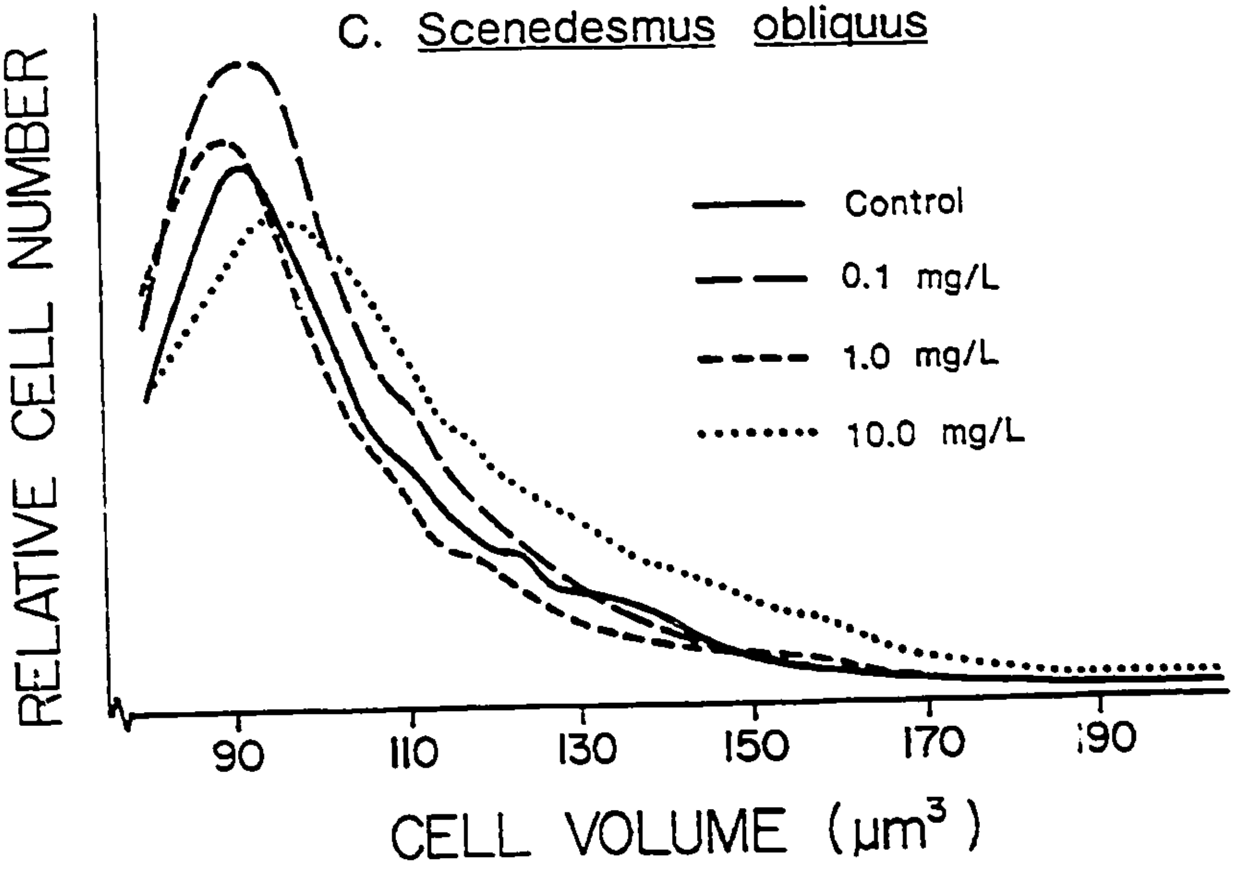


FIGURE 4C. The effects of fenitrothion (0.1, 1.0 and 10.0 mg/L) on the cell size spectra of *Scenedesmus obliquus*. Normalized mean cell volume frequency distributions following 6 days treatment are presented.

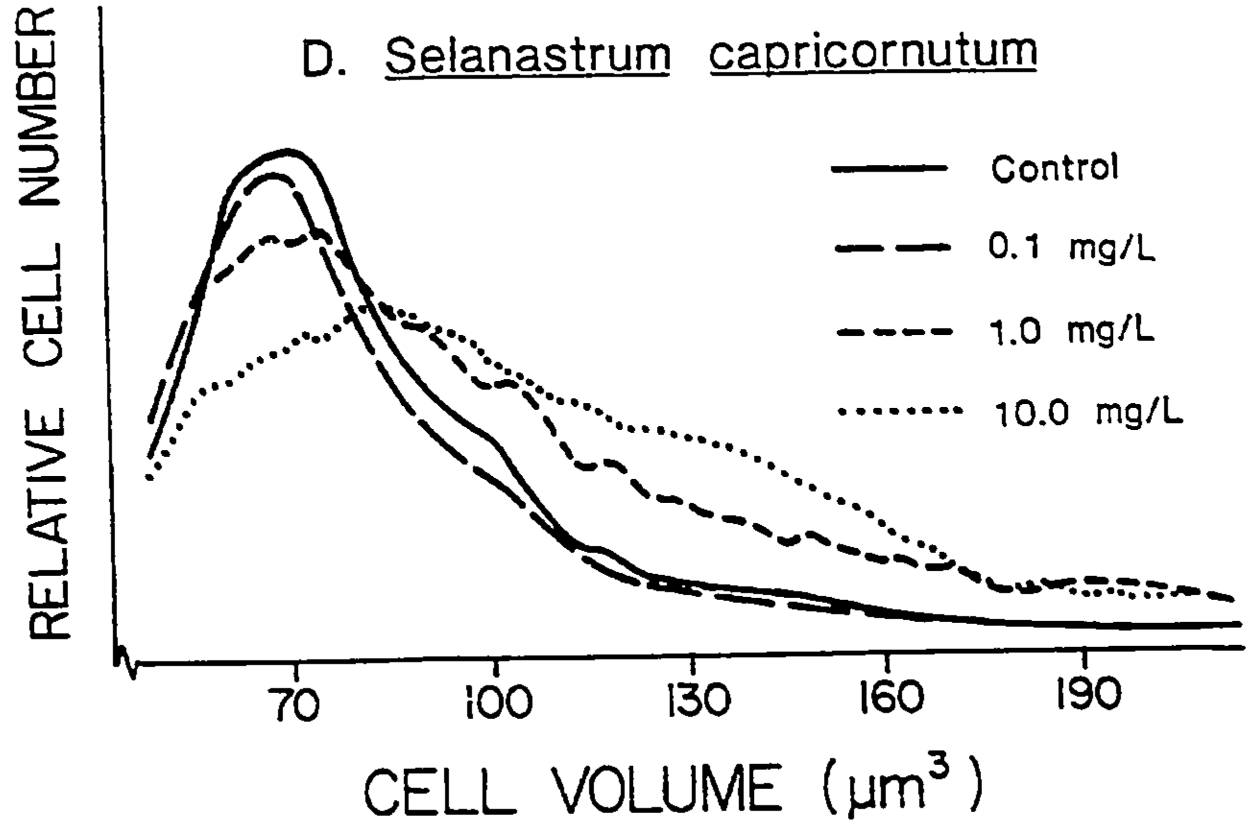


FIGURE 4D. The effects of fenitrothion (0.1, 1.0 and 10.0 mg/L) on the cell size spectra of *Selenastrum capricornutum*. Normalized mean cell volume frequency distributions following 6 days treatment are presented.

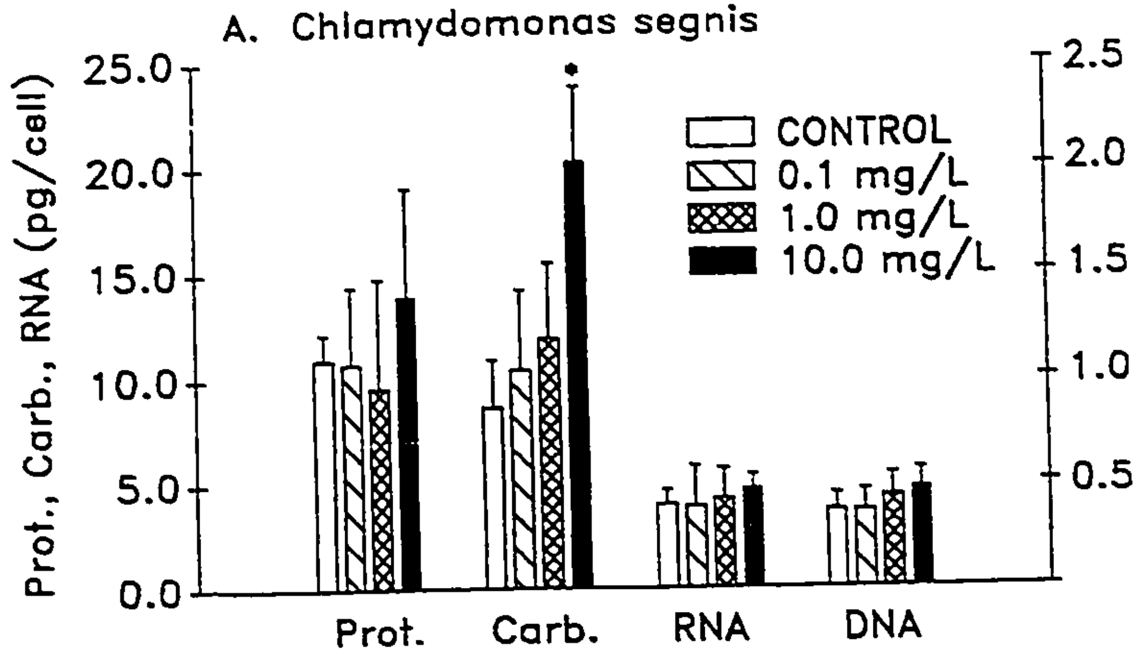


FIGURE 5A. Effects of 48 hour treatment of fenitrothion (0.1, 1.0, and 10.0 mg/L) on cellular protein, carbohydrate, ribonucleic acid and deoxyribonucleic acid content in *Chlamydomonas segnis*. Data graphed are means and standard deviations. An asterisk denotes significant difference from controls ($P \leq 0.05$) determined by Tukey's Multiple Range test.

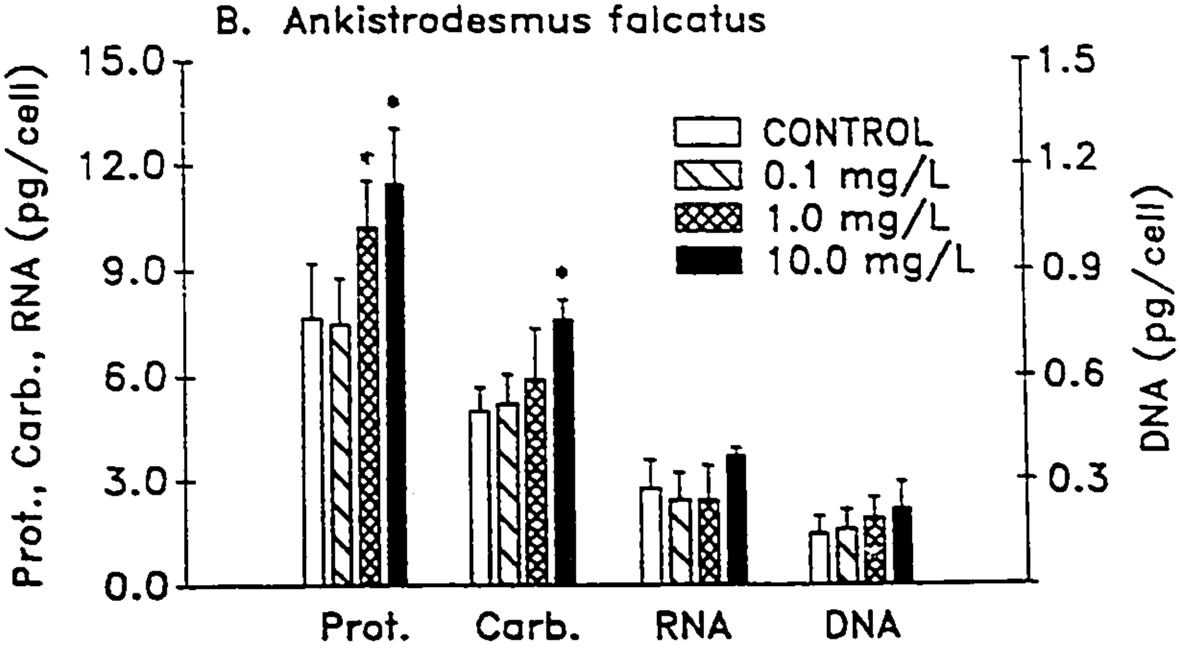


FIGURE 5B. Effects of 48 hour treatment of fenitrothion (0.1, 1.0, and 10.0 mg/L) on cellular protein, carbohydrate, ribonucleic acid and deoxyribonucleic acid content in *Ankistrodesmus falcatus*. Data graphed are means and standard deviations. An asterisk denotes significant difference from controls ($P \leq 0.05$) determined by Tukey's Multiple Range test.

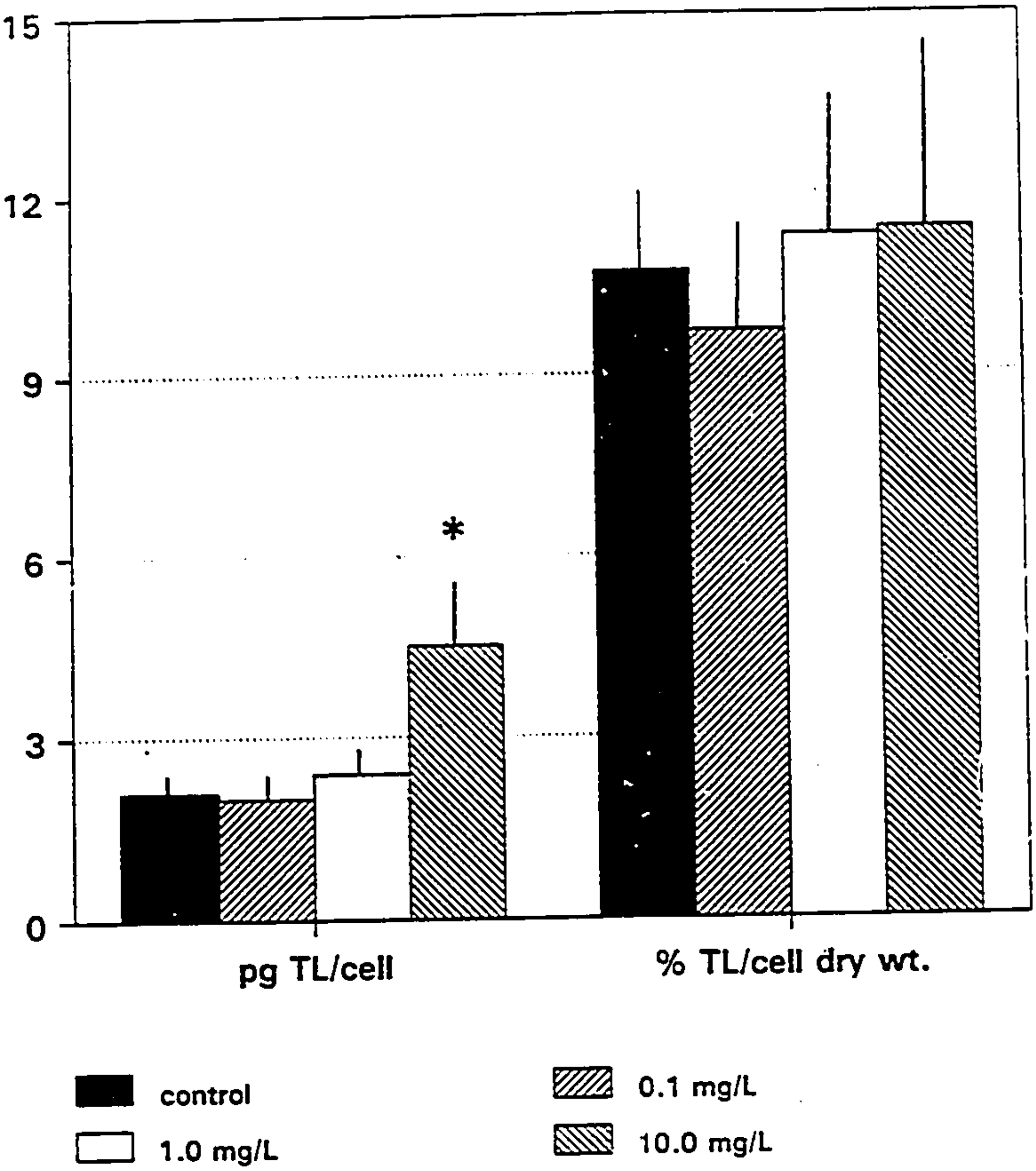


FIGURE 6. Effects of 48 hour treatment of fenitrothion (0.1, 1.0 and 10.0 mg/L) on the total lipid content of *Ankistrodesmus falcatus* cells. Values, expressed on a dry weight basis, are presented as the mean and standard deviation of three replicates. The Y-axis scales both total lipids per cell in pg and the percentage of cell dry weight representing total lipids.

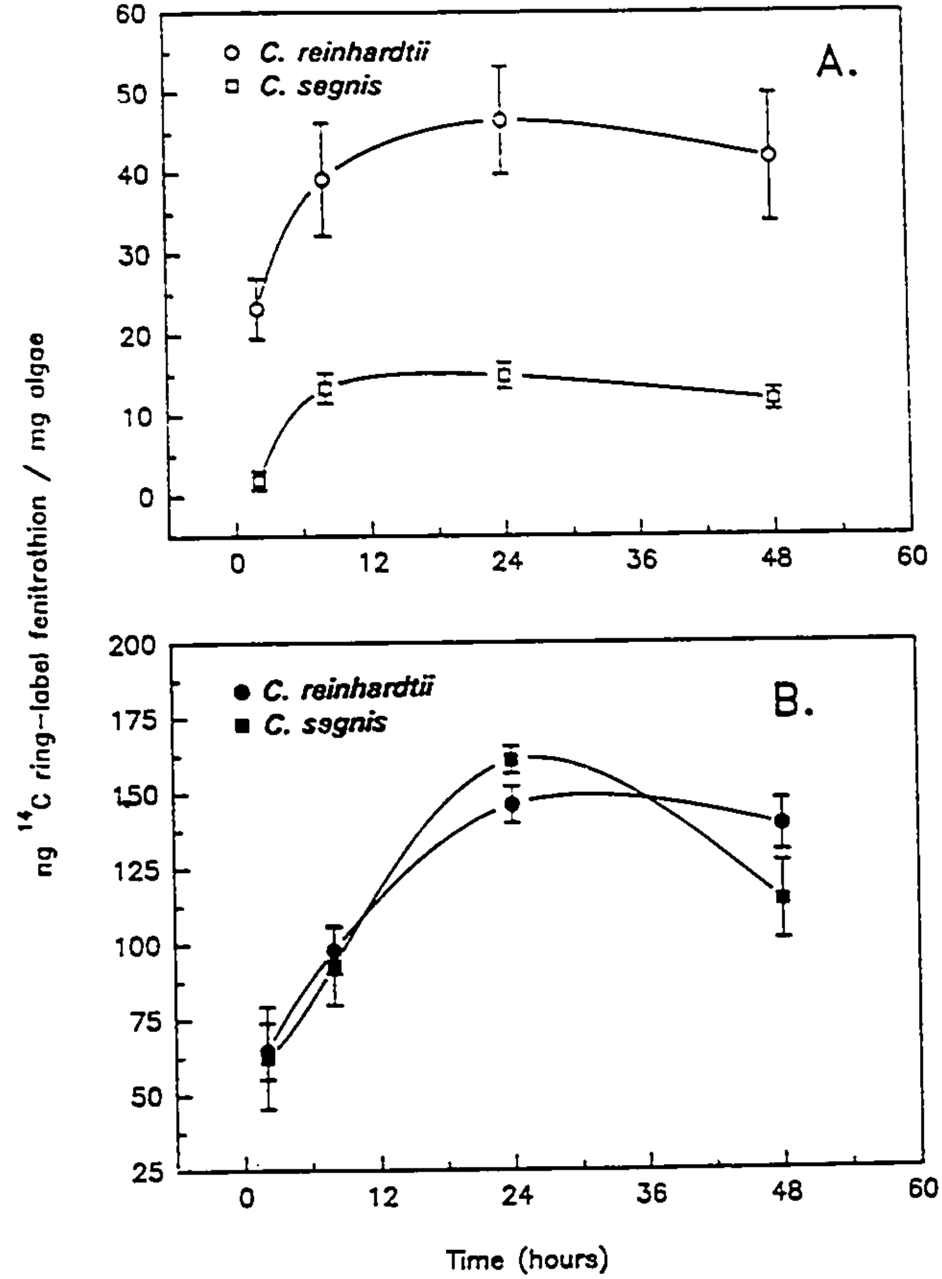


FIGURE 7. Bioaccumulation of ^{14}C ring-label fenitrothion over 48 hours by *Chlamydomonas reinhardtii* and *Chlamydomonas segnis* live (A) and dead (B) cells at 20°C under cool-white/Grow-lux fluorescent lighting (75-95 μEinsteins m^{-2} sec^{-1}, 12:12 photoperiod). Data presented are means and standard deviations of four replicates.

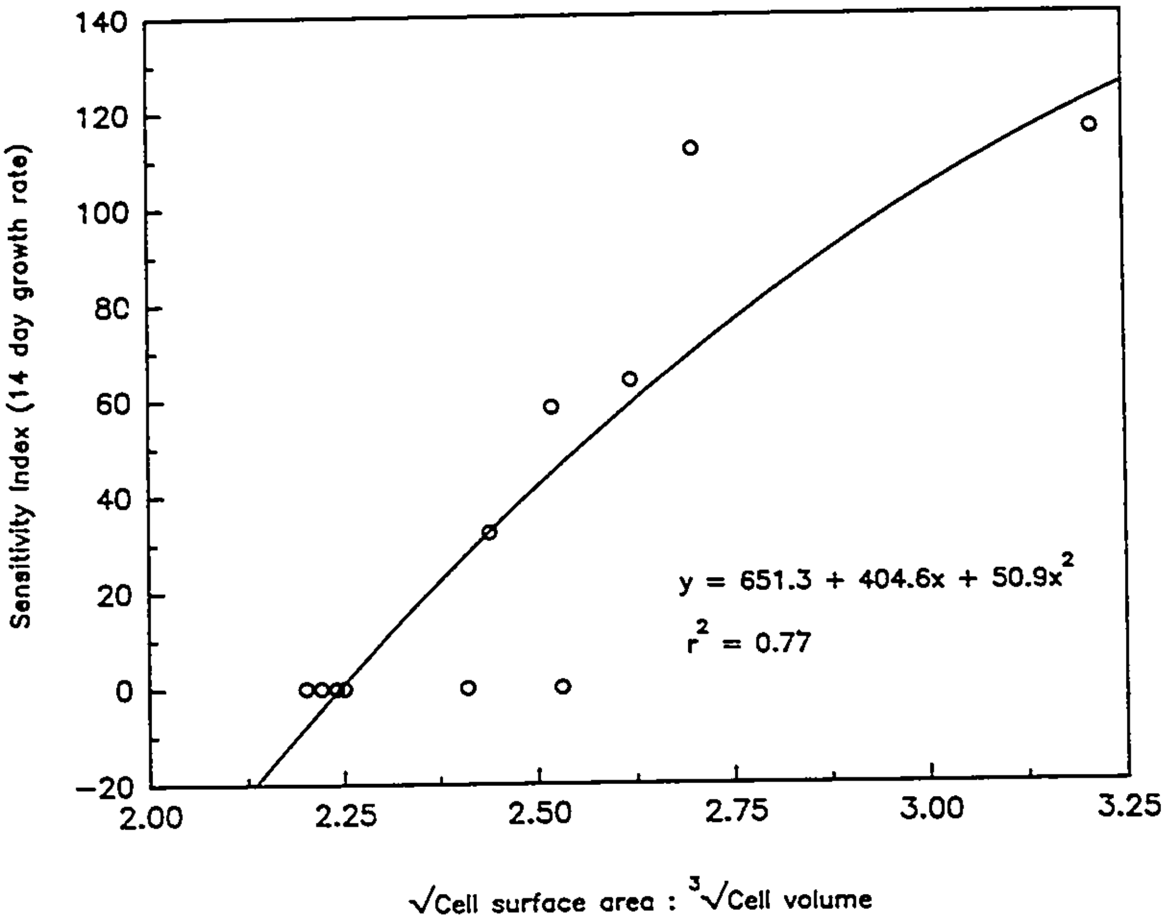


FIGURE 8. Relationship between transformed cell surface area : volume ratio of selected phytoplankton and their sensitivity to fenitrothion (sensitivity measured as significant differences in maximum growth rate from 14 day growth bioassays).

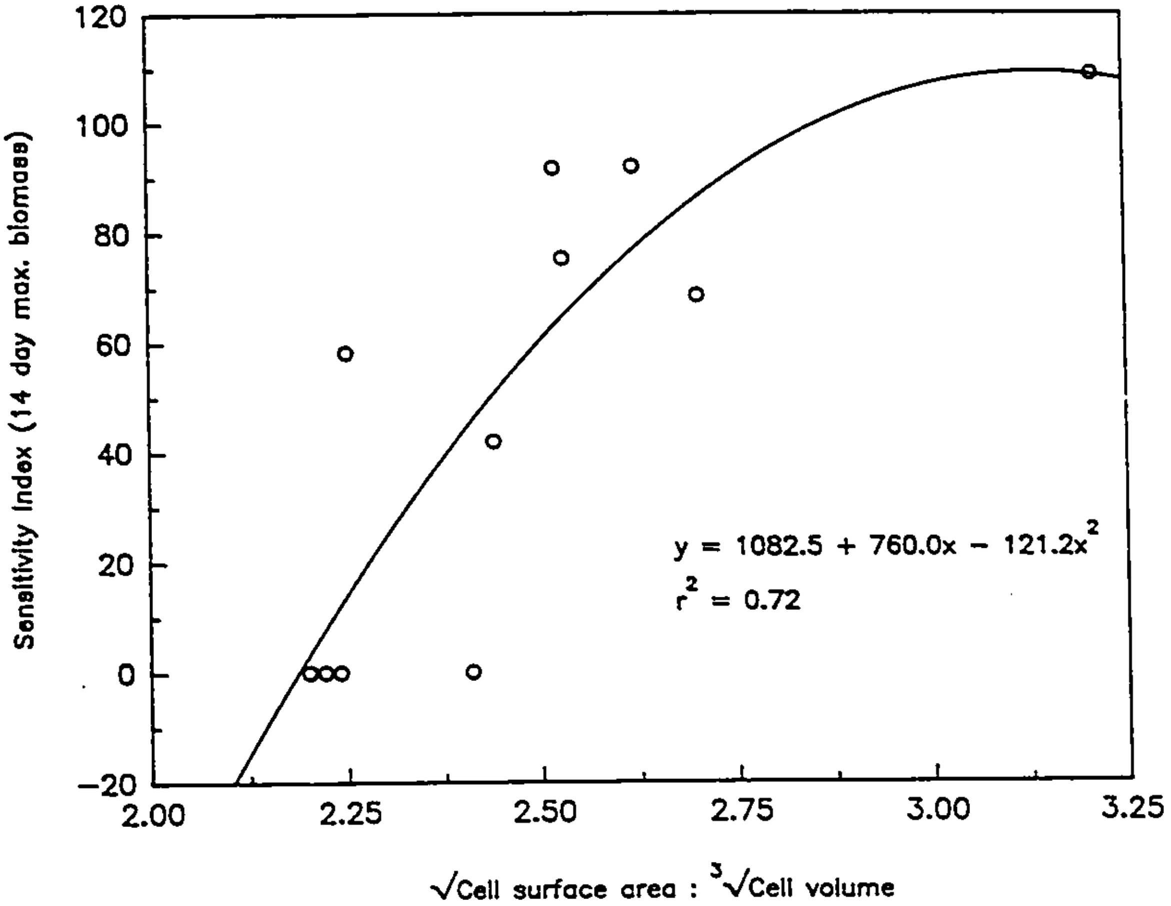


FIGURE 9. Relationship between transformed cell surface area : volume ratio of selected phytoplankton and their sensitivity to fenitrothion (sensitivity measured as significant differences in maximum attainable biomass from 14 day growth bioassays).

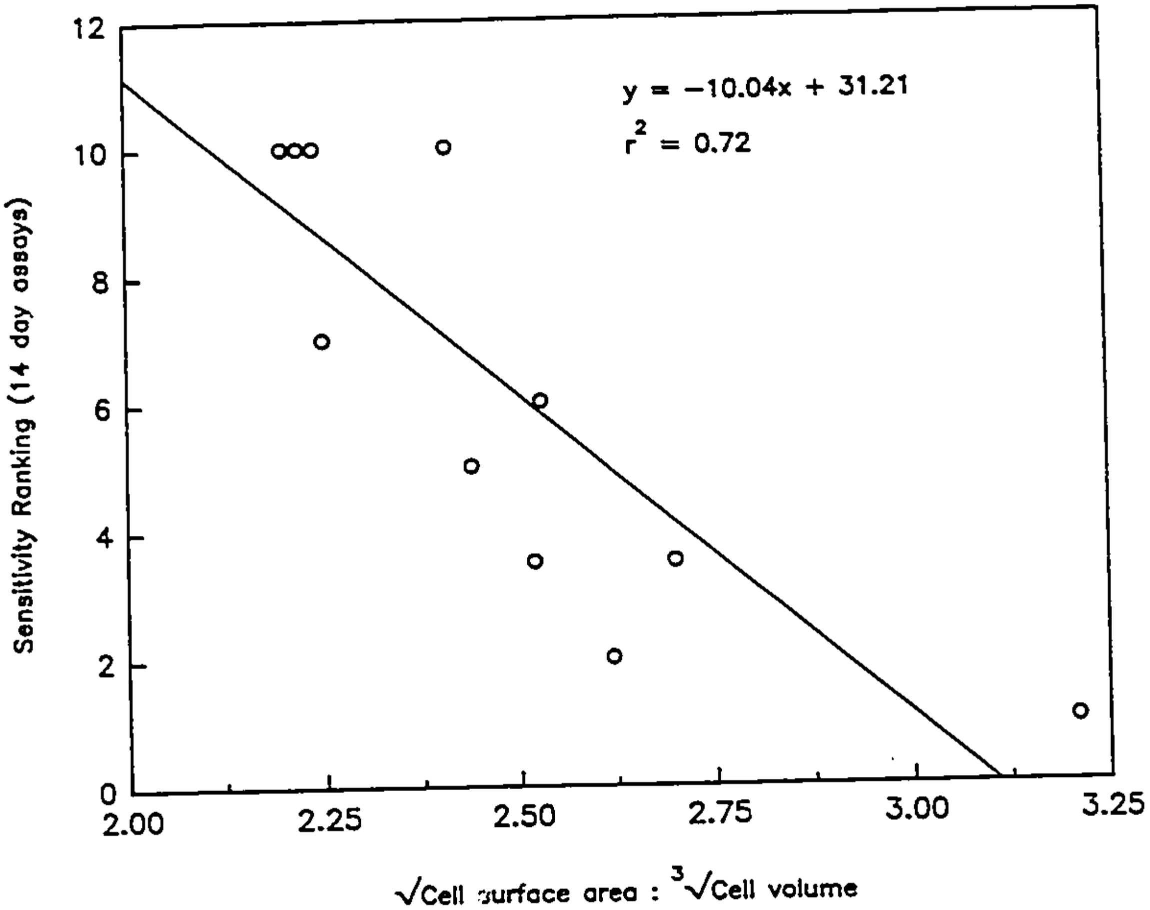


FIGURE 10. Relationship between transformed cell surface area : volume ratio of selected phytoplankton and their sensitivity to fenitrothion (sensitivity measured as overall ranking values from 14 day growth bioassays).

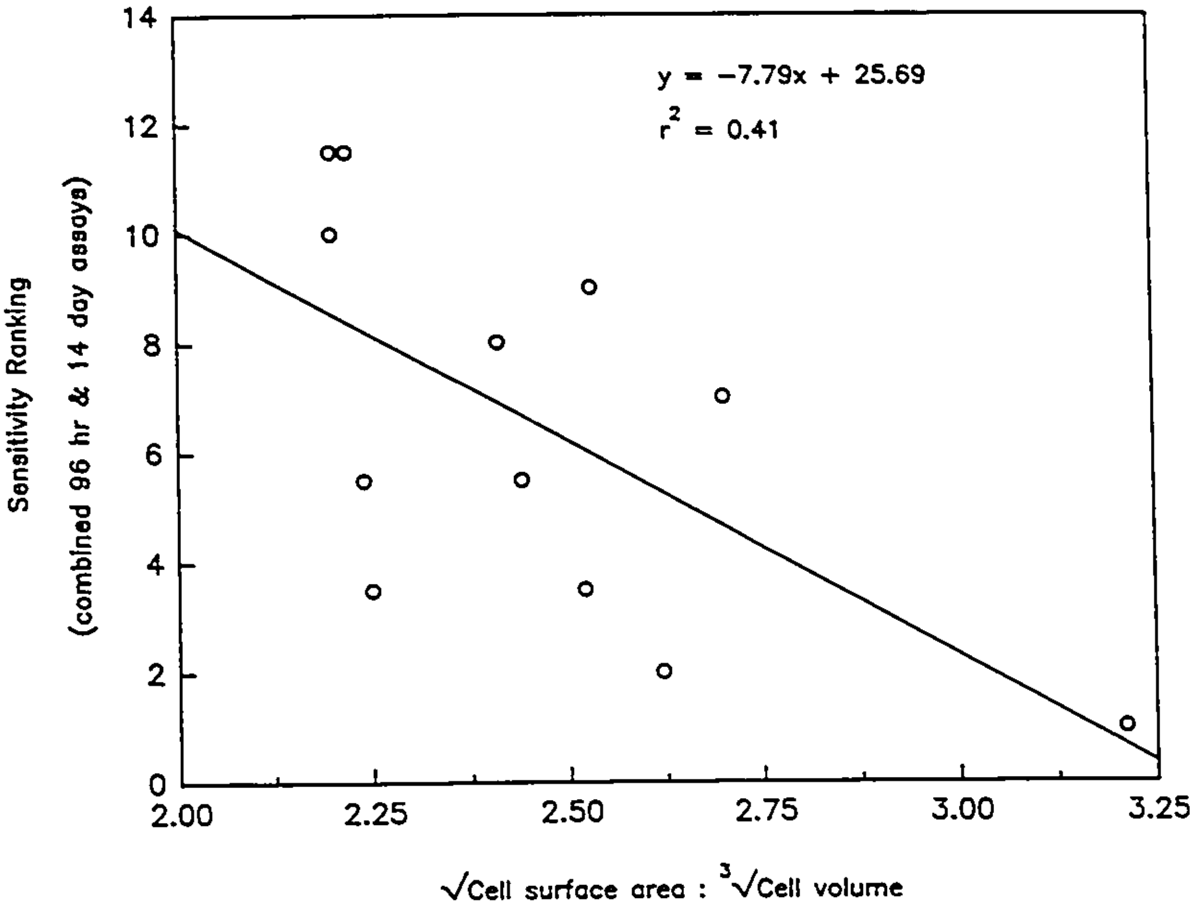


FIGURE 11. Relationship between transformed cell surface area : volume ratio of selected phytoplankton and their sensitivity to fenitrothion (sensitivity measured as combined overall ranking values based on 96 hour and 14 day growth bioassays).

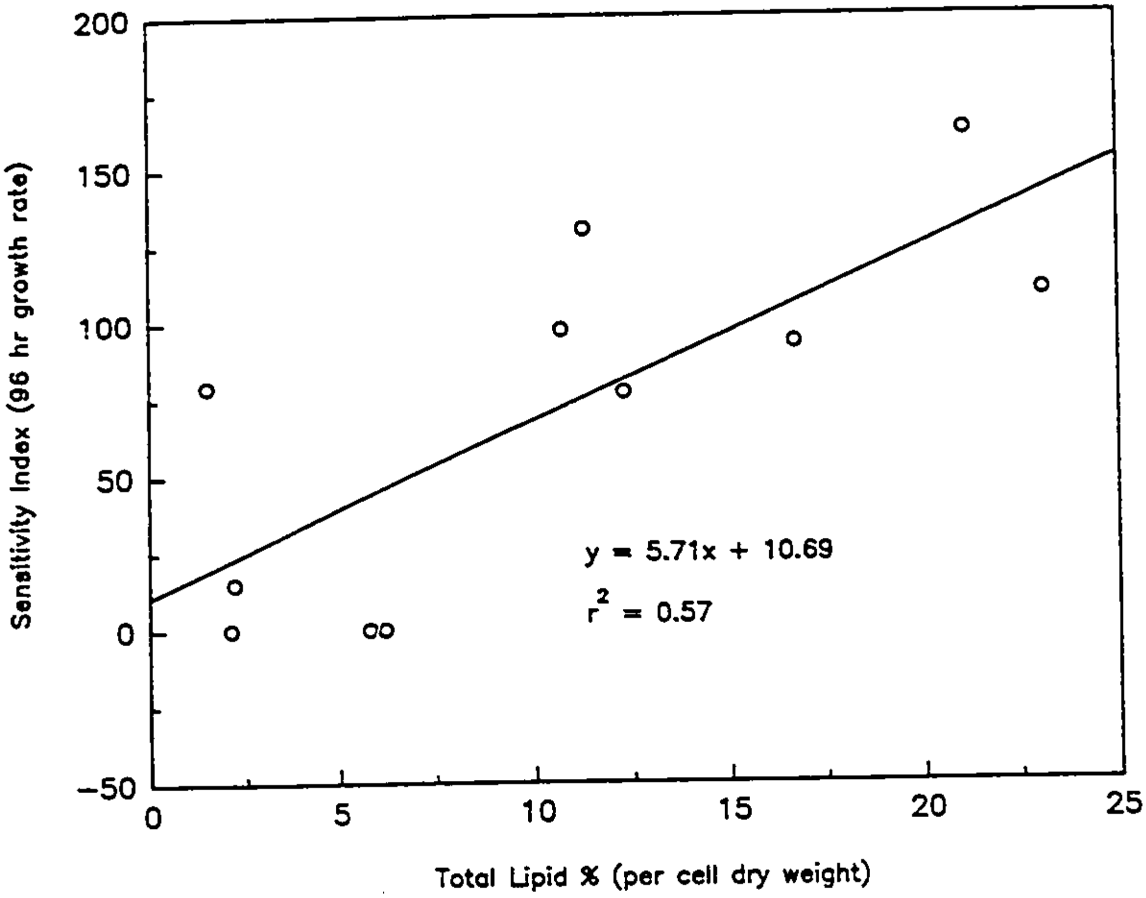


FIGURE 12. Relationship between total lipid content of selected phytoplankton and their sensitivity to fenitrothion (sensitivity measured as significant differences in maximum growth rates from 96 hour growth bioassays).

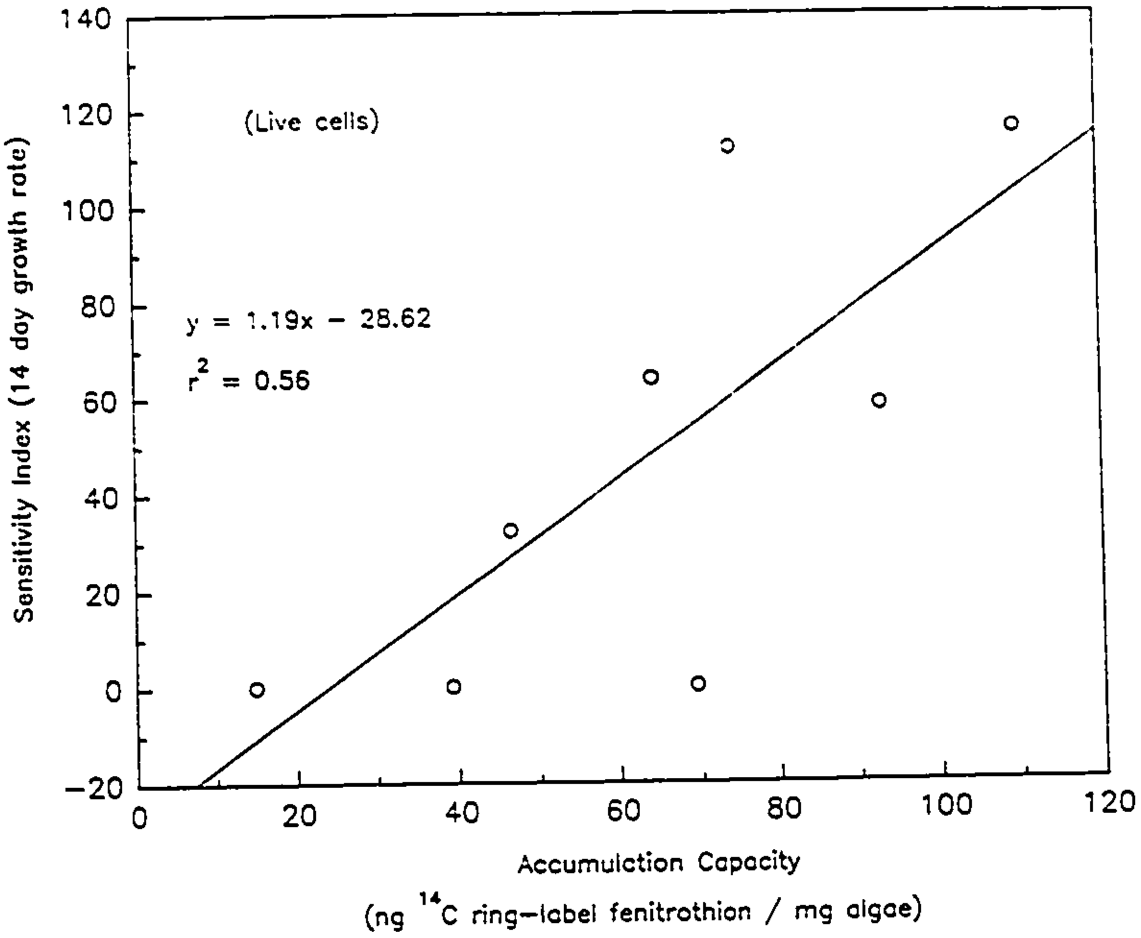


FIGURE 13. Relationship between capacity to accumulate fenitrothion in selected phytoplankton and their sensitivity to fenitrothion (sensitivity measured as significant differences in maximum growth rate from 14 day growth bioassays).

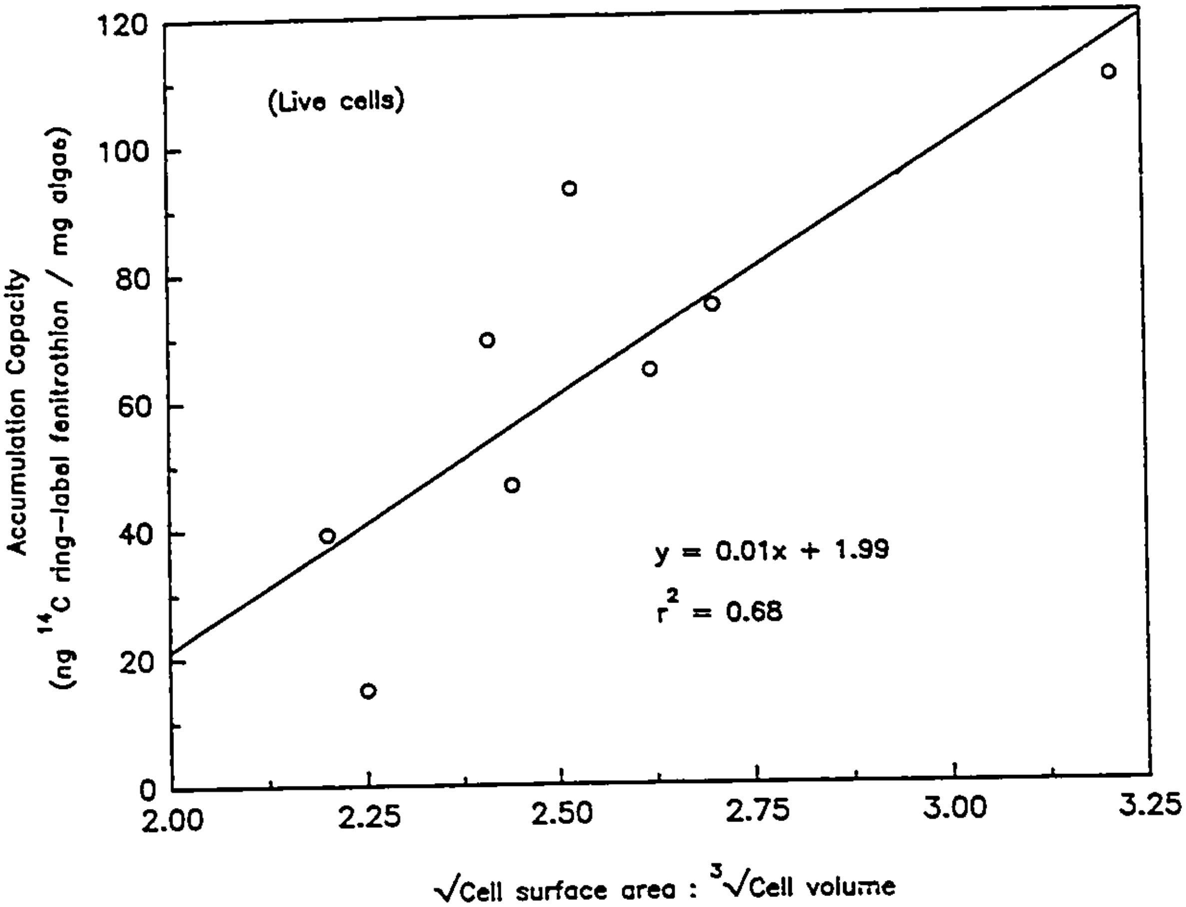
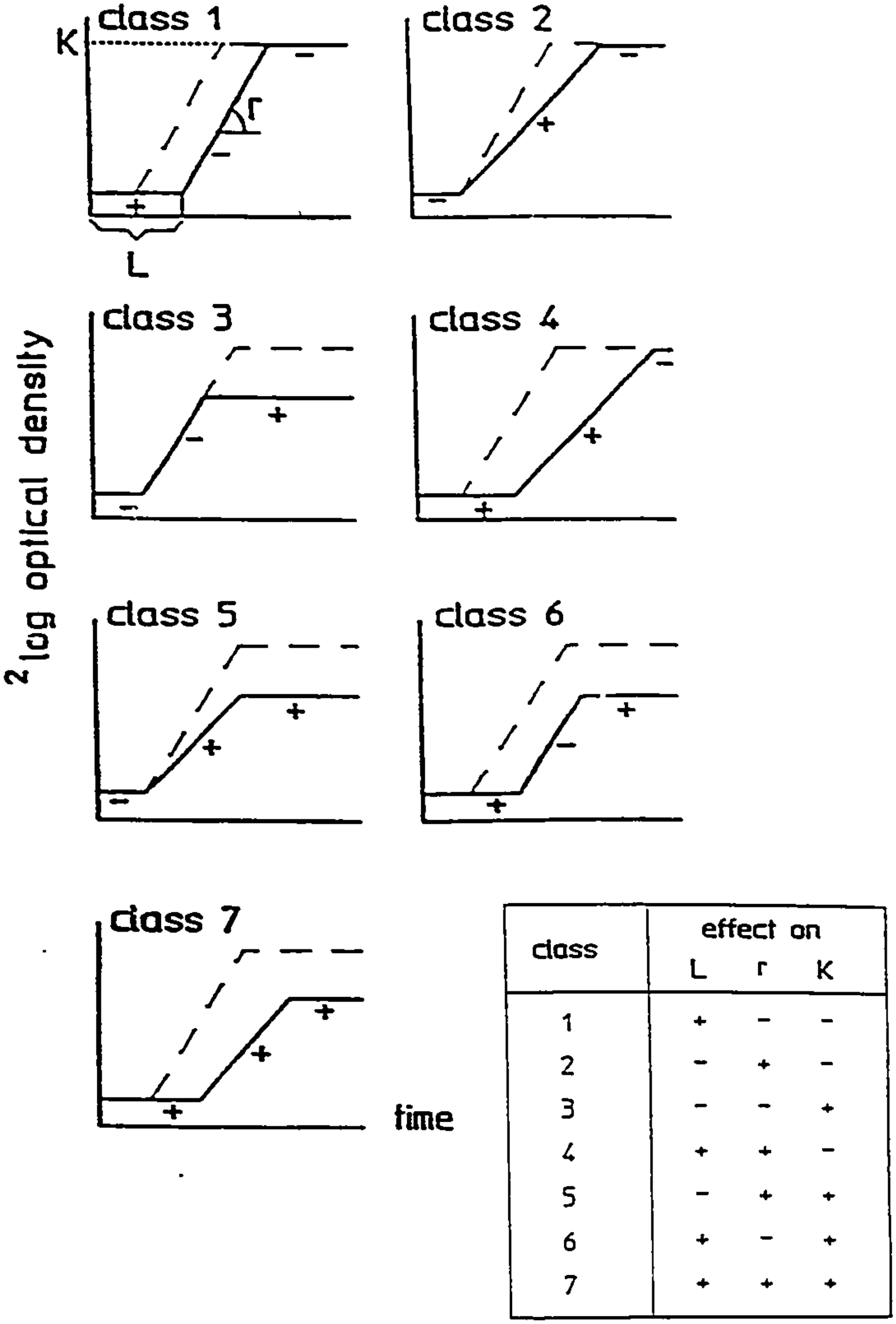


FIGURE 14. Relationship between transformed cell surface area : volume ratio of selected phytoplankton and their capacity to accumulate fenitrothion.



class	effect on L	effect on r	effect on K
1	+	−	−
2	−	+	−
3	−	−	+
4	+	+	−
5	−	+	+
6	+	−	+
7	+	+	+

FIGURE 15. Classification system of toxic effects of stressors on algal growth curves. Where L = length of lag-phase; r = maximum growth rate; K = maximum attainable biomass; + = negative effect (inhibition); - = no effect. Dashed line depicts growth curve of control.

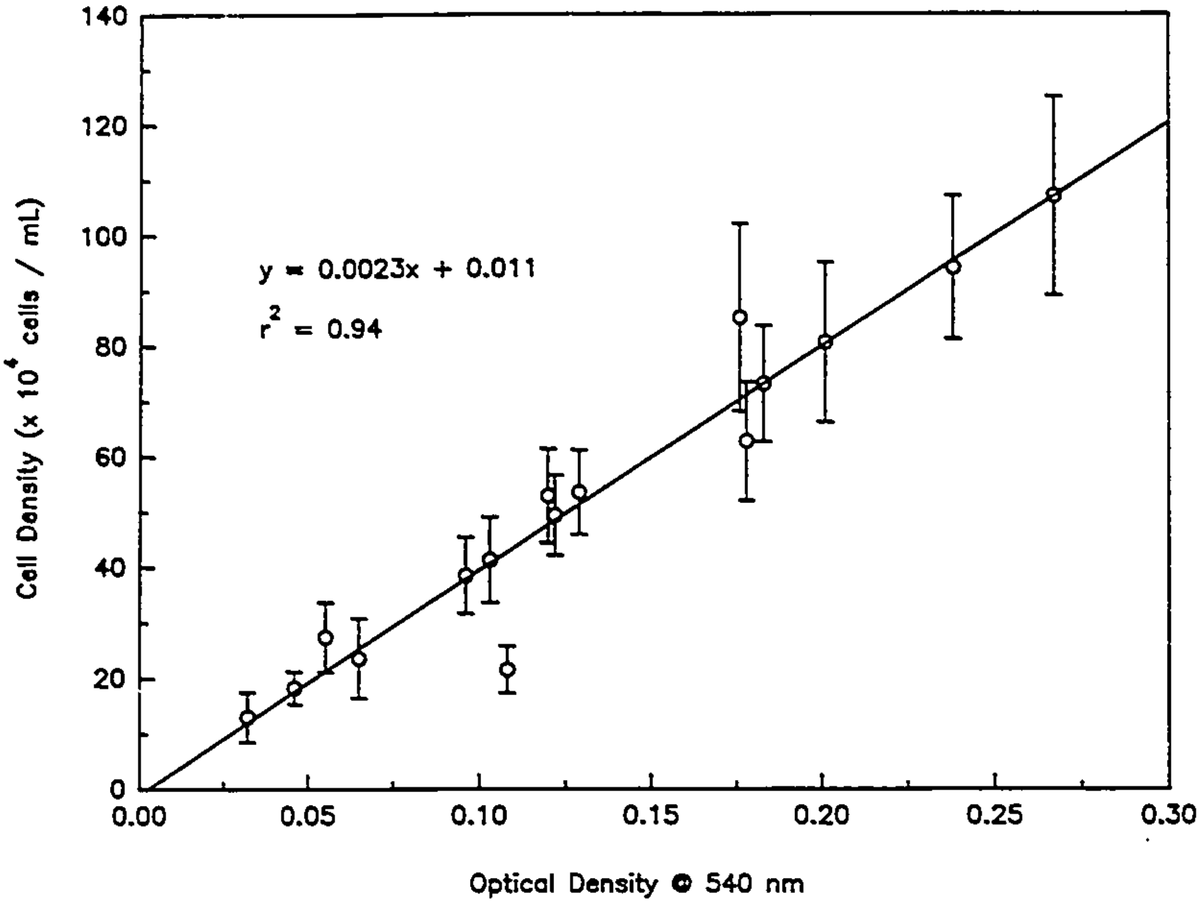


FIGURE 16. Relationship between cell density and O.D.$_{540}$, *Chlamydomonas segnis*. Data presented are means and standard deviations of four replicates. Grown at 20°C under cool-white/Grow-lux fluorescent lighting (75-95 μEinsteins m^{-2} sec^{-1} , 12:12 photoperiod).

TABLE 1. Lag-phase duration in batch cultures of selected phytoplankton exposed to 0.1, 1.0 and 10.0 mg/L fenitrothion.

SPECIES	TREATMENT(S) (mg/L)	DURATION (days)
Ankistrodesmus falcatus	10	14[1]
Chlamydomonas segnis	10	3
Navicula sp.	10	5
Scenedesmus obliquus	10	4 - 5
Selenastrum capricornutum	10	9 - 10
Staurastrum sp.	all treatments	4

[1] exponential growth was not observed over entire monitored growth period of 14 days.

TABLE 2. Summary results table of short-term (96 hour) algal growth tests including final standing crop (maximum biomass measured as O.D. at 540 nm) and growth rate of 96 hour static algal growth assays exposed to 0.1, 1.0 and 10.0 mg/L fenitrothion. Data presented are means and standard errors of three replicates, % difference from control means ($P \leq 0.05$) and total cummulative significant difference (all treatments) from control means.

SPECIES	TMT[1]	FINAL STANDING CROP				GROWTH RATE			
	(mg/L)	mean	s.e.	%[2]	cumm.%[3]	mean	s.e.	%[2]	cumm.%[4]
Anabaena sp.	control	0.029	0.003			0.427	0.040		
	0.1	0.028	0.002			0.389	0.040		
	1.0	0.018	0.002	37.9		0.150	0.059	64.9	
	10.0	0.052	0.004	79.3	117.2	0.059	0.031	86.2	151.1
Ankistrodesmus falcatus	control	0.156	0.007			0.691	0.017		
	0.1	0.176	0.007			0.685	0.012		
	1.0	0.124	0.009	20.5		0.630	0.020	8.8	
	10.0	0.017	0.001	89.1	109.6	0.080	0.031	88.4	97.2
Chlamydomonas reinhardtii	control	0.051	0.001			0.320	0.026		
	0.1	0.052	0.002			0.312	0.019		
	1.0	0.055	0.002			0.282	0.021		
	10.0	0.100	0.005	96.1	96.1	0.435	0.015	35.9	35.9
Chlamydomonas segnis	control	0.198	0.008			0.689	0.026		
	0.1	0.212	0.006			0.714	0.030		
	1.0	0.154	0.018	22.2		0.643	0.047		
	10.0	0.031	0.001	84.3	106.5	0.144	0.031	79.1	79.1
Chlorella pyrenoidosa	control	0.339	0.006			0.550	0.018		
	0.1	0.339	0.006			0.556	0.010		
	1.0	0.333	0.015			0.587	0.019		
	10.0	0.307	0.012			0.528	0.013		
Chlorella vulgaris	control	0.437	0.002			0.655	0.022		
	0.1	0.429	0.007			0.627	0.036		
	1.0	0.444	0.003			0.633	0.042		
	10.0	0.439	0.004			0.557	0.020	15.0	

[1] treatments

[2] significant difference from control mean % ($P \leq 0.05$)

[3] Sensitivity Index - 96 hour maximum biomass (*K*)

[4] Sensitivity Index - 96 hour maximum growth rate (*r*)

TABLE 2. cont. Summary results table of short-term (96 hour) algal growth tests including final standing crop (maximum biomass measured as O.D. at 540 nm) and growth rate of 96 hour static algal growth assays exposed to 0.1, 1.0 and 10.0 mg/L fenitrothion. Data presented are means and standard errors of three replicates, % difference from control means ($P \leq 0.05$) and total cummulative significant difference (all treatments) from control means.

SPECIES	TMT[1]	FINAL STANDING CROP				GROWTH RATE			
	(mg/L)	mean	s.e.	%[2]	cumm.%[3]	mean	s.e.	%[2]	cumm.%[4]
Cosmarium sp.	control	0.029	0.001			0.119	0.025		
	0.1	0.030	0.001			0.117	0.020		
	1.0	0.029	0.003			0.076	0.039		
	10.0	0.026	0.003			0.049	0.080		
Navicula sp.	control	0.043	0.003			0.583	0.048		
	0.1	0.046	0.006			0.516	0.032		
	1.0	0.033	0.001			0.588	0.055		
	10.0	0.004	0.002	90.7	90.7	-0.056	0.160	109.6	109.6
Pediastrum sp.	control	0.049	0.003			0.011	0.001		
	0.1	0.048	0.001			0.011	0.001		
	1.0	0.047	0.001			0.011	0.001		
	10.0	0.048	0.001			0.011	0.001		
Scenedesmus obliquus	control	0.067	0.005			0.298	0.041		
	0.1	0.057	0.007			0.250	0.039		
	1.0	0.050	0.003	25.4		0.253	0.029		
	10.0	0.030	0.001	55.2	80.6	0.069	0.039	76.8	76.8
Selenastrum capricornutum	control	0.288	0.025			0.824	0.028		
	0.1	0.299	0.021			0.848	0.036		
	1.0	0.239	0.033			0.785	0.045		
	10.0	0.016	0.001	94.4		0.058	0.021	93.0	
Staurastrum sp.	control	0.017	0.001			0.002	0.0004		
	0.1	0.016	0.003			0.0005	0.001		
	1.0	0.014	0.0002			0.0005	0.0002	77.5	
	10.0	0.017	0.002			0.0003	0.0002	84.51	62.0

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[1] treatments

[2] significant difference from control mean % ($P \leq 0.05$)

[3] Sensitivity Index - 96 hour maximum biomass (*K*)

[4] Sensitivity Index - 96 hour maximum growth rate (*r*)

TABLE 3. Summary results table of non-linear regression analyses of long-term (14 day) algal growth tests. Logistic model co-efficients *K* (maximum biomass) and *r* (maximum growth rate) of 14 day static algal growth assays exposed to 0.1, 1.0 and 10.0 mg/L fenitrothion. Data presented are means and standard errors of three replicates, % difference from control means ($P \leq 0.05$) and total cummulative significant difference (all treatments) from control means.

SPECIES	TMT[1]	FINAL STANDING CROP (*K*)				GROWTH RATE (*r*)			
	(mg/L)	mean	s.e.	%[2]	cumm.%[3]	mean	s.e.	%[2]	cumm.%[4]
Anabaena sp.	control	0.317	0.086			0.282	0.065		
	0.1	0.182	0.051			0.277	0.071		
	1.0	0.487	0.115			0.315	0.031		
	10.0	0.054	0.002			1.627	0.483		
Ankistrodesmus falcatus	control	0.710	0.009			0.717	0.037		
	0.1	0.707	0.007			0.731	0.029		
	1.0	0.632	0.013	11.0		0.643	0.050		
	10.0	0.015	0.001	97.9	108.9	-0.11	0.502	115.8	115.8
Chlamydomonas reinhardtii	control	0.874	0.018			0.459	0.015		
	0.1	0.838	0.017			0.445	0.014		
	1.0	0.701	0.036	19.8		0.441	0.035		
	10.0	0.682	0.043	22.0	41.8	0.311	0.021	32.2	32.2
Chlamydomonas segnis	control	0.527	0.007			0.566	0.028		
	0.1	0.507	0.007			0.574	0.035		
	1.0	0.546	0.020			0.468	0.049		
	10.0	0.221	0.010	58.1	58.1	0.518	0.064		
Chlorella pyrenoidosa	control	1.28	0.052			0.377	0.036		
	0.1	1.42	0.118			0.349	0.058		
	1.0	1.33	0.072			0.396	0.052		
	10.0	1.44	0.075			0.388	0.041		
Chlorella vulgaris	control	0.662	0.021			0.454	0.058		
	0.1	0.597	0.016			0.579	0.080		
	1.0	0.620	0.019			0.515	0.072		
	10.0	0.618	0.017			0.492	0.063		

[1] treatments

[2] significant difference from control mean % ($P \leq 0.05$)

[3] Sensitivity Index - 14 day maximum biomass (*K*)

[4] Sensitivity Index - 14 day maximum growth rate (*r*)

TABLE 3. cont. Summary results table of non-linear regression analyses of long-term (14 day) algal growth tests. Logistic model co-efficients *K* (maximum biomass) and *r* (maximum growth rate) of 14 day static algal growth assays exposed to 0.1, 1.0 and 10.0 mg/L fenitrothion. Data presented are means and standard errors of three replicates, % difference from control means ($P \leq 0.05$) and total cummulative significant difference (all treatments) from control means.

SPECIES	TMT[1]	FINAL STANDING CROP (*K*)				GROWTH RATE (*r*)			
	(mg/L)	mean	s.e.	%[2]	cumm.%[3]	mean	s.e.	%[2]	cumm.%[4]
Cosmarium sp.	control	0.312	0.008			0.490	0.036		
	0.1	0.331	0.011			0.457	0.037		
	1.0	0.309	0.013			0.470	0.050		
	10.0	0.077	0.003	75.3	75.3	0.420	0.066		
Navicula sp.	control	0.519	0.014			0.547	0.045		
	0.1	0.566	0.023			0.476	0.044		
	1.0	0.588	0.023			0.463	0.035		
	10.0	1.360	22.38			0.346	0.187		
Pediastrum sp.	control	0.410	0.019			0.340	0.017		
	0.1	0.445	0.036			0.309	0.020		
	1.0	0.411	0.013			0.334	0.010		
	10.0	0.385	0.018			0.346	0.019		
Scenedesmus obliquus	control	1.571	0.077			0.438	0.025		
	0.1	1.893	0.455			0.437	0.089		
	1.0	1.521	0.146			0.433	0.041		
	10.0	0.131	0.037	91.7	91.7	0.183	0.045	58.2	58.2
Selenastrum capricornutum	control	0.989	0.046			0.422	0.059		
	0.1	0.964	0.044			0.451	0.063		
	1.0	0.875	0.041			0.486	0.084		
	10.0	0.080	0.061	92.0	92.0	0.151	0.062	63.7	63.7
Staurastrum sp.	control	0.361	0.024			0.449	0.042		
	0.1	0.647	0.580			0.287	0.063		
	1.0	0.203	0.261			0.227	0.055	49.4	
	10.0	0.114	0.074	68.4	68.4	0.169	0.052	62.4	111.8

[1] treatments

[2] significant difference from control mean % ($P \leq 0.05$)

[3] Sensitivity Index - 14 day maximum biomass (*K*)

[4] Sensitivity Index - 14 day maximum growth rate (*r*)

TABLE 4. Estimated EC_{50} values for maximum growth rate (*r*) and standing crop (*K*) for short (96 hours) and long-term (14 days) exposures of freshwater phytoplankton to fenitrothion.

SPECIES	EC_{50}[1] (mg/L)			
	96 hour		14 day	
	r	K	r	K
Anabaena sp.	1.06	2.24	-[2]	-
Ankistrodesmus falcatus	3.39	2.53	3.52	2.38
Chlamydomonas reinhardtii	-	4.77	13.86	109.9
Chlamydomonas segnis	6.62	2.75	-	8.87
Chlorella pyrenoidosa	-	-	-	-
Chlorella vulgaris	24.39	-	-	-
Cosmarium sp.	-	-	-	6.99
Navicula sp.	3.52	5.46	-	-
Pediastrum sp.	-	-	-	-
Scenedesmus obliquus	6.85	6.40	8.86	5.35
Selenastrum capricornutum	-	5.02	8.20	5.32
Staurastrum sp.	0.84	-	2.81	7.69

[1] EC_{50} values, obtained using EPA Probit Analyses Program (Version 1.4), represent estimated concentration of fenitrothion producing a 50% difference from controls in a given response parameter.

[2] dash denotes that EC_{50} was unable to be estimated due to lack of significant growth effects observed over the exposure range tested.

TABLE 5. Sensitivity rankings of 12 algal species to fenitrothion. Ranking numbers are unitless values based on cummulative % differences ($P \leq 0.05$) from controls in growth rate (*r*) and maximum standing crop (*K*) measured in short (96 hours) and long-term (14 days) growth bioassays.

	SENSITIVITY RANKINGS[1]						
SPECIES	96 hour			14 day			COMB.[2]
	r	K	cumm.[3]	r	K	cumm.	
Anabaena sp.	1	1	1	9	10	10	5.5
Ankistrodesmus falcatus	3	2	2	1	1	1	1
Chlamydomonas reinhardtii	8	4	6	5	7	5	5.5
Chlamydomonas segnis	5	3	3.5	9	6	7	3.5
Chlorella pyrenoidosa	11	10	11	9	10	10	11.5
Chlorella vulgaris	9	10	9	9	10	10	10
Cosmarium sp.	11	10	11	9	4	6	9
Navicula sp.	2	6	3.5	9	10	10	8
Pediastrum sp.	11	10	11	9	10	10	11.5
Scenedesmus obliquus	6	7	7	4	3	3.5	3.5
Selenastrum capricornutum	4	5	5	3	2	2	2
Staurastrum sp.	7	10	8	2	5	3.5	7

[1] species are statistically neither more sensitive nor tolerant to fenitrothion than those with the same ranking value (based on the growth parameters measured)

[2] combined ranking (96hr and 14d rankings combined and ranked)

[3] cummulative ranking represents the ranked sum of r and K endpoints

TABLE 6. Total lipid content of selected algae. Values, expressed on a dry weight basis, are presented as the mean and standard deviation of three replicates.

SPECIES	pg / cell	% / cell
Ankistrodesmus falcatus	2.1 ± 0.25	10.7 ± 1.3
Chlamydomonas reinhardtii	3.5 ± 0.6	11.3 ± 0.7
Chlamydomonas segnis	0.52 ± 0.07	1.5 ± 0.5
Chlorella pyrenoidosa	0.84 ± 0.05	6.2 ± 0.5
Chlorella vulgaris	0.7 ± 0.03	2.2 ± 0.3
Cosmarium sp.	1.9 ± 0.21	5.8 ± 0.8
Pediastrum sp.	3.9 ± 0.8	2.1 ± 0.6
Navicula sp.	33.6 ± 1.8	23.1 ± 2.1
Scenedesmus obliquus	223.7 ± 0.07	12.3 ± 1.2
Selenastrum capricornutum	9.8 ± 0.3	16.7 ± 0.8
Staurastrum sp.	497.1 ± 23.8	21.1 ± 1.5

TABLE 7. The effects of 0.1, 1.0 and 10.0 mg/L of fenitrothion on the fatty acids of the total lipids of *Ankistrodesmus falcatus* cells following 48 hours exposure. Values are presented as the mean and standard deviation (of three replicates) of µmole fatty acid, fatty acid mole % of the total lipids, and µmole fatty acid per gram of cells.

FATTY ACID	UNITS	CONTROL	0.1	1.0	10.0
16:0[1] palmitic	µmole	1.66 (0.07)	1.61 (0.09)	1.71 (0.04)	1.74 (0.35)
	mole %[2]	22.23 a (2.01)	22.91 a (2.13)	23.08 a (1.1)	27.64 b (1.46)
	µmole.g^{-1} cells	64.70 (3.35)	64.06 (4.04)	64.01 (8.44)	57.32 (0.99)
16:1 palmitoleic	µmole	0.75 (0.07)	0.69 (0.03)	0.69 (0.05)	0.61 (0.14)
	mole %	10.06 a (0.75)	9.76 a (0.66)	9.35 a (0.57)	9.67 a (0.79)
	µmole.g^{-1} cells	29.49 (3.2)	27.26 (1.19)	26.16 (4.33)	20.09 (0.59)
18:1 oleic	µmole	1.90 (0.28)	1.80 (0.09)	1.97 (0.13)	1.84 (0.12)
	mole %	25.21 a (0.74)	25.50 a (0.2)	26.58 a (0.28)	29.46 b (2.43)
	µmole.g^{-1} cells	74.07 (10.53)	71.48 (3.85)	74.20 (13.9)	61.25 (8.55)
18:2 linoleic	µmole	0.51 (0.07)	0.44 (0.04)	0.39 (0.06)	0.32 (0.07)
	mole %	6.80 a (0.7)	6.27 ab (0.81)	5.24 ab (0.52)	5.08 b (0.01)
	µmole.g^{-1} cells	19.93 (3.24)	17.56 (2.31)	15.46 (5.97)	10.49 (0.12)
18:3 linolenic	µmole	2.70 (1.06)	2.51 (0.38)	2.65 (0.21)	1.77 (0.44)
	mole %	35.71 a (2.9)	35.55 a (3.4)	35.74 a (1.53)	28.16 b (0.08)
	µmole.g^{-1} cells	104.78 (16.8)	99.81 (12.9)	99.84 (19.27)	58.50 (3.36)

[1] Numerals indicate ratio of normal carbon chain length of fatty acid to number of double bonds

[2] The letters following each mole % value denote significant differences about the means using the Tukey's Multiple Range Test. Means marked with similar letters are not significantly different ($P \leq 0.05$).

TABLE 8. Fenitrothion incorporated by selected freshwater algae following 24 hours exposure to 10.0 mg/L ^{14}C ring-labelled fenitrothion. Values, expressed on a dry weight basis, are presented as the mean and standard deviation of three replicates.

SPECIES	ng ^{14}C / mg Algae	
	live cells	dead cells
Ankistrodesmus falcatus	109.9 ± 11.5	679.0 ± 46.7
Chlamydomonas reinhardtii	46.5 ± 15.9	146.0 ± 28.5
Chlamydomonas segnis	14.9 ± 1.6	160.9 ± 10.5
Chlorella pyrenoidosa	39.2 ± 8.9	198.9 ± 13.9
Navicula sp.	69.3 ± 9.2	725.4 ± 74.3
Scenedesmus obliquus	92.8 ± 5.6	270.3 ± 31.3
Selenastrum capricornutum	64.3 ± 2.9	178.9 ± 11.9
Staurastrum sp.	74.4 ± 9.3	123.4 ± 11.2

TABLE 9. : Summary table of mean cell surface areas, cell volumes, surface area:volume ratios of selected algae. Values presented for each species are means from a minimum of 50 cells.

SPECIES	S.A.[1] (μm^2)	Volume (μm^3)	S/V	Trans. S/V[2]
Anabaena sp.	44.8	26.5	1.7	2.24
Ankistrodesmus falcatus	201.9	87.9	2.30	3.21
Chlamydomonas reinhardtii	240.8	366.0	0.71	2.44
Chlamydomonas segnis	342.0	560.3	0.61	2.25
Chlorella pyrenoidosa	50.8	33.6	1.51	2.20
Chlorella vulgaris	98.5	92.0	1.07	2.20
Cosmarium sp.	73.3	40.7	1.80	2.53
Navicula sp.	292.3	358.1	0.82	2.41
Pediastrum sp.	285.6	433.2	0.66	2.22
Scenedesmus obliquus	119.8	93.5	1.28	2.52
Selenastrum capricornutum	115.9	70.8	1.64	2.62
Staurastrum sp.	568.6	708.3	0.80	2.70

[1] cell surface area

[2] transformed ratio (square root of cell surface area : cube root of cell volume)

CHAPTER 4.0

DISCUSSION

This study has demonstrated that: a) common freshwater phytoplankton exhibit significant differential sensitivity to fenitrothion under laboratory conditions; b) fenitrothion elicited significant phytotoxic effects in common freshwater phytoplankton under environmentally-relevant exposures; c) the effects of fenitrothion on phytoplankton can be observed and quantified over three levels of biological organisation; and d) the sensitivity of phytoplankton to fenitrothion can be related to algal cell characteristics (cell size and lipid content) and physiological capacities (bioaccumulation of fenitrothion).

4.1 Test Organisms

The selection of algal species for toxicological testing can be based on a number of criteria ranging from ease of experimental manipulation to ecological relevance. Commonly cited criteria include that the species used must be easily propagated or cultured under common laboratory conditions, be compatible to electronic particle enumeration, yield measurable growth endpoints in a short time period, be taxonomically distinct and well represented in natural ecosystems.

Species selection continues to be a major issue facing regulatory scientists in their efforts to standardize non-target plant testing guidelines for the registration of chemicals such as pesticides (Swanson and Peterson 1988). Currently, such guidelines are being developed in Canada. Other jurisdictions with established testing guidelines (U.S. Environmental Protection Agency, Organization for Economic Cooperation and Development, International Standards Organization) continue to rely upon the use of *Selenastrum capricornutum* as the preferred algal species primarily due to its historical use and resulting extensive database, ease of culture, amenability to electronic particle counting and international availability. This almost exclusive reliance on a single species however, has been globally recognized as an insensitive approach for predicting toxic effects in algae (Freemark *et al.*, 1990, Blanck *et al.*, 1984). North American regulatory scientists now endorse that a battery of test species is a more sensitive and effective way to gain a measure of the range in algal phytotoxicity of pesticides and other toxic chemicals. Adoption of a battery of test species from all major algal classes, not only optimizes the probability of obtaining results from a sensitive species but also from species from a wide range of aquatic habitats.

The twelve test species herein, were selected to represent a wide range in taxonomy, morphology and physiology. Their commercial availability in axenic form and ease of culture under similar laboratory conditions minimized the period to establish routine culture allowing fast start-up time for experimentation. These factors coupled with relatively fast growth rates (i.e. > 1 doubling / day) permitted good reproducibility of experiments. It was also important for the species to grow well in the same artificial growth medium under identical conditions of temperature, lighting and gas exchange to negate effects which may be attributable to these variables.

The twelve test species investigated in these studies represent three major classes of freshwater phytoplankton, the green algae (Chlorophyceae), the blue-green algae (Cyanophyceae) and the diatoms (Bacillariophyceae). All these species are typically (although not exclusively) planktonic and common in most Canadian freshwaters (Duthie and Socha 1976; Badour *et al.*, 1973). The test assemblage selected comprise seven different algal families within five separate orders (Table 1., Appendix I).

The green algae family Oöcystaceae is the most represented in these studies with four species (*Ankistrodesmus falcatus, Chlorella pyrenoidosa, Chlorella vulgaris and Selenastrum capricornutum*). Members of the Chlorococcales order are characterized by solitary or loosely aggregated cells, wide gelatinous sheaths, thin, resistant cell walls, and generally large surface:volume ratios. These Oöcystaceae genus' reproduce asexually solely by 2 or more autospores which are liberated from the parent-cell wall.

The other Chlorococcalean families examined in these studies include Hydrodictyaceae and Scenedesmaceae. The former, represented by *Pediastrum sp.* typically replicate sexually through fusion of biflagellate gametes. Members of this genus are coenobial, forming a stellate plate of 16 or more polygonal cells usually in concentric ring shape. *Scenedesmus obliquus* belonging to the latter family, reproduce asexually via autospores however, those new daughter cells always remain attached to the parent cells to form coenobial autocolonies of 4 cells.

The other green algal orders represented include Volvocales and Zygnematales. The Volvocales are characterized by cells which are flagellated and actively mobile during the vegetative phases of the life cycle. Commonly occurring representatives of the large Chlamydomonadaceae family of this order, *Chlamydomonas segnis* and *Chlamydomonas reinhardtii* are spherical biflagellate species which under the conditions provided, reproduced asexually by division of the parent protoplast into typically 2 or 4 daughter autospores. The latter order are represented by *Staurastrum sp.* and *Cosmarium sp.* from the family Desmidaceae. Typically unicellular, desmids are structurally distinct from other Chlorophyceaea, possessing a conspicuous median constriction or sinus which divides the cell into two semicells connected by an isthmus. Characterized by this bilateral symmetry, desmids vary considerably in shape, ranging from the simple spherical semicells of *Cosmarium sp.* to the complex geometric morphology of *Staurastrum sp.*. Reproduction is also distinctive as 2 to 4 daughter cells receive an unaltered half of the parent cell wall and regenerate a new half-wall having the same characteristic configuration and ornamentation.

A common blue-green algae, *Anabaena sp.* belongs to the order Oscillatoriales in which all genera are characterized by a thallus which is multicellular and filamentous in organization. Filaments or trichomes of *Anabaena sp.* are unbranched and may be straight or spiral. The sheaths surrounding the trichomes, an attribute of the order, are hyaline and virtually invisible microscopically. *Anabaena sp.* normally reproduces vegetatively by hormogonia but will also develop akinetes.

Lastly, a third division of algae used in these studies, Chrysophyta, was represented by the common diatom *Navicula sp.* Belonging to the Pennate order and the largest diatom family Naviculaceae, *Navicula sp.* has elongate valves which are bilaterally symmetrical to a median axis and attenuated towards the poles. Replication of diatoms largely occurs vegetatively where mitotic division of the cell contents is followed by cytoplasmic cleavage parallel to the valves. The resulting daughter protoplast assumes half the parent frustule (epitheca) and secretes the other needed half (hypotheca). Although less prevalent, sexual reproduction is also known in this family where two conjugating cells produce an auxospore.

4.2 Effects Studies:

Although the marginal environmental relevance of a laboratory degradation study of fenitrothion is acknowledged, the fate of this compound in the presence of aquatic microflora is more notable. These results indicate the significance of algal-mediated degradation mechanisms in the overall fate of this compound, supporting a hypothesis recently advanced in this same laboratory (Caunter and Weinberger 1988; Kent and Weinberger 1991). The lack of measurable levels of degradative products (both polar and non-polar) in the media or loosely associated with algal biomass support the conclusion that the algal effects observed are likely attributable to the toxic action of the parent compound.

4.2.1 Growth Effects

Optical density measurements were simple and effective biomass estimators in unialgal axenic cultures. The wavelength of 540 nm was utilized as this was well outside the absorption maxima of chlorophyll (440 and 680 nm). The biomass surrogate correlated well with cell number (Figure 1., Appendix III).

The effects of a chemical stressor on the growth of microalgae in laboratory batch cultures can be broadly categorized by an increase in lag-phase, a decrease in exponential growth, a decrease in the maximum attainable biomass or any combination of the these three. An expansion of a classification system introduced by Albertano *et al.*, (1980) to seven distinct population growth effect models has been used to categorize the types of toxic action of stressors on algae growth curves (Veer *et al.*, in press). An obvious deficiency in this system is the failure to acknowledge stimulatory responses as effects. It is important to realize that differential stimulation of selected species in multi-specific assemblages can also be ecologically deleterious, particularly when coupled with inhibition of others. The system proposed by Veer *et al.*, (in press) should be modified to reflect the three additional effects scenarios related to growth stimulation (i.e. decreased lag-time, increased growth rate and final biomass). For the purposes of the stressor and species examined in these studies, the classification system, depicted in Figure 15, can be used to interpret the resulting growth effects.

On examining the growth curves, the lag-phase extension data and the results from the 14 day non-linear regression growth analysis, the range of growth effects elicited by fenitrothion can be categorized under the following five classes:

Class #	Species	Effect
1.	*Anabaena sp.* *Chlorella vulgaris* *Chlorella pyrenoidosa* *Pediastrum sp.*	no effects
4.	*Cosmarium sp.*	decreased final biomass
6.	*Chlamydomonas reinhardtii* *Scenedesmus obliquus*	decreased growth rate decreased final biomass
7.	*Chlamydomonas segnis*	decreased growth rate
8.	*Ankistrodesmus falcatus* *Navicula sp.* *Staurastrum sp.* *Selenastrum capricornutum*	extended lag-phase, decreased growth rate, decreased final biomass

As indicated above, 50% of the growth curve effects include inhibition of both growth rate and maximum biomass. These results support the recent findings of Veer *et al.*, (in press) who examined 61 separate algal growth curves from toxicity tests and found that both growth rate and final biomass were affected in at least half of the cases. These results further indicate a weakness of current algal toxicity tests in the assessment of new and established chemicals. These standardized protocols still endorse the measurement of a single growth endpoint (usually final attainable biomass), disregarding other effects (e.g. lag-phase duration, maximum growth rate and biomass at stationary phase).

The lack of a consistent extended lag-phase in the species exhibiting class 6 effects may indicate inadequate time resolution associated with measurements. More frequent growth measurements could indicate the existence of a lag-phase where typical daily readings are unable (Adams *et al.*, 1985; Veer *et al.*, in press). A lag-phase extension attributed to fenitrothion stress, as operationally defined in these studies (Section 2.2.4), was observed in 50% of the test species. It is therefore possible that more frequent growth measurements might have indicated this growth phenomena was even more widespread.

The adoption of a non-linear regression analysis of growth curves enables simple quantitative examination of significant differences between treatments and species. This procedure allowed for a direct comparison to be made on the overall growth response fenitrothion had between species and thus the establishment of a sensitivity ranking. Veer *et al.*, (in press) and Bolier and Donze (1989) have also proposed that to obtain a more exact definition of the biological significance of toxicity assays using microorganisms such as algae, a complete parametric analysis of the entire growth curve is necessary. This method of growth analyses proved to be very effective as all twelve species fit the logistic model closely allowing for rapid, simultaneous quantification and statistical analysis of both growth rate and standing crop-related endpoints. A comprehensive survey of related literature suggests that this model-based approach has never been used to assess algal or microbial growth curves for routine toxicity measurements.

The duration of algal toxicity tests has received much attention in the scientific literature and in recent efforts to develop nontarget plant toxicity testing guidelines for pesticide registration (Walsh 1983; Nyholm and Kallqvist 1989; Freemark *et al.*, 1990; Swanson and Peterson 1988). The draft protocol currently endorsed by Canada specifies that test durations be no longer than 72 hours (constant illumination) in order to limit algal-induced alterations of the test conditions (i.e. pH, nutrient limitation, metabolite buildup etc.) (Keddy *et al.*, 1990). A further requirement is that initial cell numbers or inoculum must be as low as possible (i.e. 10^4 cells/mL). Preliminary experiments indicated that a long-term growth period of 14 days were required for the test species to reach stationary phase. Further, no species exhibited a declining growth rate by day 14. On the contrary, controls (and many treatments) in all species tested continued to show signs of positive growth right up to experiment termination. This supports that toxic metabolic products, pH shifts or nutrient limitation were not significant factors in inhibiting algal growth over these exposure durations. Subsequently, short-term growth analysis of 96 hours was chosen for comparison with 14 day results. The selection of 96 hours was arbitrary and at the time, the preferred short-term duration of most regulatory jurisdictions. The results of this research support the need for both short and long-term growth studies in the assessment of algal effects.

Initial studies were performed to identify the minimal starting cell densities for growth assays. The lowest inoculation densities which yielded measurable biomass in the shortest time period (shortest lag-phase) were determined to range from 2 - 10 X 10^4 cells/mL. These densities are within those recommended by OECD (1984) and draft Canadian testing guidelines (Freemark *et al.*, 1990). These low inoculum densities together with the alternating light-dark photoperiod minimized the pH changes normally associated with photosynthetic metabolism and nutrient uptake. Periodic measurements of pH over the 14 day growth period indicated a maximum pH drift of 7.2 - 7.8. It is therefore suggested that the theoretical and practical concerns over microorganism-induced changes in experimental conditions can be mitigated under properly controlled conditions of light, media chemistry and volume, and inoculum. Long-term growth assays incorporating the entire logistic growth pattern of successive phases: lag, exponential and stationary (Li 1984) can be employed to effectively characterize growth effects on multiple growth parameters.

By referring to Figures 2A - L, it is apparent that various growth phenomena (e.g. related to detoxification, delayed effects or recovery processes) which manifested well into the 14 day period would not have been observed if 72 hour exposure durations were exclusively used. What may be interpreted as delayed toxic responses in *Anabaena sp.*, *Ankistrodesmus falcatus* and *Cosmarium sp.*, or recovery in *Navicula sp.*, *Chlamydomonas segnis*, *Staurastrum sp.* and *Selenastrum capricornutum* were only detectable over longer (> 72 hours) exposures.

The selection of the appropriate endpoint for which to measure and compare growth effects in algal cultures has been subject for discussion (Nyholm 1985; Nyholm and Kallqvist 1989). In most algal toxicity testing to date, final biomass has been used to represent a growth effect relative to controls (Freemark *et al.*, 1990). Recent evidence indicates that appreciable differences may exist between EC (effective concentrations) estimates derived from the same experiments using growth rate and final biomass endpoints (Nyholm 1985). A comparison of EC_{50} estimates in Table 4 suggest that for both 96 hour and 14 day durations, EC figures derived from growth rate and those from biomass are generally comparable (EC_{50} r : K ratios ranging from 1.0 - 1.1). Further observations of growth results (Tables 2 and 3) support the same conclusions. The mean difference ($P \leq 0.05$) from control and 10.0 mg/L treatments as measured by biomass over 14 days was 72.2 ±.26.3% compared to 66.3 ± 30.1% when determined by growth rates. Similarly, for 96 hour exposures, the mean difference from control was 83.9 ± 13.9% based on biomass whereas the mean % difference using growth rate was 84.5 ± 31%. These results suggest that neither growth parameter was significantly more sensitive than the other in assessing the effects of fenitrothion over the twelve algal species. Rather than suggesting that scientists and regulators commit exclusively to one response over another, this work supports an equal reliance on all growth endpoints to gain a more complete understanding of the effects pesticides may have on the growth dynamics of microalgae. For instance, there may be cases like *Cosmarium sp.* (Figure 2G) when the yield is altered and inoculum (lag-phase) and reproductive rate remain unaffected. Frequent monitoring of biomass (in addition to final standing crops) will allow differentiation between short-time lag effects and cases of a permanently reduced growth rate (e.g. *Ankistrodesmus falcatus*).

The algistatic properties of fenitrothion levels ≥ 1.0 mg/L exhibited in this study resulted in delaying exponential growth and reducing growth rates and standing crop. Although previous studies reported algicidal effects of fenitrothion on marine diatoms at 50 mg/L (Butler 1977), its effects on the viability of freshwater algae has received less attention. Interference with normal growth in autotrophic microorganisms has been noted with the related organophosphorus insecticides parathion, chlorpyrifos, fenthion, and diazinon (Lal 1982).

The only published algal growth effects for fenitrothion (Couture *et al.*, 1982) are within the range determined in these studies. Although the EC_{50}s presented herein for *S. capricornutum* (14 day EC_{50}: 5.32 - 8.20 mg/L) were higher than the 1.2 mg/L value given by Couture *et al.*, (1982), the latter study reported no degradation of the parent compound over the 14 day growth period whereas, in the experiments presented herein, fenitrothion concentrations declined by approximately 60 - 80% by day 14. The study by Couture *et al.*, (1982) does not provide details on which growth parameter was monitored, however a number of interesting observations regarding factors influencing the phytotoxicity of fenitrothion were put forth. First, the presence of adjuvants produced an additive or synergistic toxicity as the EC_{50} concentrations for the formulated product were less than half of that for technical active ingredient. It was demonstrated that fenitrothion formulation of 14.9% fenitrothion / 50.1% Paratex /35.0% cyclo-sol 63 was twice as toxic to *Selenastrum capricornutum* over 14 days compared to the active ingredient alone. The predominant formulated product in use currently, Sumithion™, is an emulsifiable concentrate comprised of fenitrothion, a cosolvent Dowanol, an emulsifier Atlox 3409F and water in the volume ratios of 11:1.5:1.5:86 and 41:5:5:49 for large spray aircraft and smaller agricultural-style aircraft respectfully (pers. comm. W. Sexsmith, 1990). Dowanol has been shown to significantly increase algal uptake of the active ingredient several fold (Weinberger *et al.*, 1983). Couture and colleagues also observed that lower temperatures (5 and 10°C) decreased EC_{50} concentrations by half in *Selenastrum capricornutum* compared to 15 and 20°C. This result has significant ramifications since when operational spraying is typically initiated in May or June, surface water temperatures in northern temperate forests are normally $\leq$ 10°C. The threshold effect concentrations determined at 20°C could therefore be considerable overestimates of typical field scenarios.

4.2.2 Biochemical and Molecular Effects

Since growth is the culmination of many biochemical events, it is expected that biochemical changes occur before reductions in growth are observed. Related phosphorothioate pesticides, guthion, malathion (Lal and Saxena 1980), and methyl parathion (Saroja and Bose 1982) have been shown to affect macromolecular synthesis at levels as low as 10 mg/L in protists and unicellular chlorophytes. Most accounts report an inhibition of biosynthesis as a result of exposure. It is postulated that the macromolecular augmentations presented in this study are attributed to an inhibition of cell replication evident from growth assays, and subsequent concomitant accumulation of protein, carbohydrate and nucleic acid biomolecules.

The involvement of organophosphorus insecticides in enzymatic pathways of other microorganisms has been demonstrated (Clegg and Koevening 1974). Recognizing the intended design of fenitrothion as an enzyme inhibitor, enzyme interaction in aquatic microflora is a plausible mode of action. Methyl-parathion has been documented to interfere with enzyme induction involving the hormone gibberellic acid (Swamy and Veeresh 1987). Significant reductions in the activity of the key membrane enzyme alkaline phosphatase, in *Chlamydomonas reinhardtii* were measured following exposure to the phosphorodithioate insecticide phosalone (Netrawali and Gandhi 1990).

Many mechanisms may possibly account for fenitrothion's inhibition of algal cell division. It is known, for example, that macromolecules such as proteins rich in leucine and thiol-groups associated RNA are reorganized just prior to the onset of cell division, likely triggering the cells entry into mitosis (Mazia 1961). Fenitrothion-induced interference at this cell stage may have led to significant augmentations in protein and a trend in elevated nucleic acid levels. An unidentified "protein factor" is purported to be released and activated in algal cells at a specific time to initiate cell division (Mihara and Hase 1975). Fenitrothion may conceivably effect the enzymatic formation of this factor or alter its configuration in such a way as to prevent cytokinesis and the liberation of autospores.

Impeded cell division and inhibition of carbohydrate-metabolizing phosphorylation processes may also account for the elevated cellular carbohydrate levels. A plausible scenario which may be related to the mode of action of organophosphorus insecticides is that fenitrothion may have inhibited the activity of principle starch degrading enzymes such as polyglucan phosphorylase and &-amylase (Hirokawa *et al.*, 1982)

Accumulation of macromolecules as a result of inhibited eucaryotic replication and unaffected macromolecular synthesis has been demonstrated with organochlorine (Lal and Saxena 1980) and carbamate (De Chacin 1984) insecticides in green algae. Although fenitrothion is known to induce nuclei deformations and anomalous protein deposits in vascular plant cells (Weinberger *et al.*, 1981), this is the first study to report widespread cytological effects in autotrophic microorganisms from exposure to levels occurring in the field. Alterations in normal carbohydrate and protein levels in phytoplankton may have deleterious implications regarding specific nutritional requirements and energy acquisition efficiencies for zooplankton predators. Food quality and quantity may not only have significant effects of zooplankton fecundity but have been shown to significantly influence competitive interactions among invertebrate grazers (Geisy *et al.*, 1988).

Several primary sites of phytotoxic action have been proposed by researchers working with organophosphorus insecticides and algal receptors. This has included the photosynthetic system in microflora (Clegg and Koevening 1974; Torres and O'Flaherty 1976; Ambudurai *et al.*, 1981). Diazinon-induced (100 mg/L) photosynthetic inhibition in freshwater chlorophytes was suggested to result from interference with photophosphorylative reactions (Clegg and Koevening 1974). Malathion is known to significantly reduce algal pigment levels at exposures as low as 1 µg/L (Torres and O'Flaherty 1976). Methyl-parathion is also reported to induce cellular chlorophyll reductions in algae (Saroja and Bose 1982) and inhibit electron transport in vascular plant chloroplasts Ambudurai *et al.*, 1981). Experiments in our laboratory (Caunter and Weinberger 1988; Kent and Weinberger 1991) however, failed to show a significant influence on chlorophyll levels from fenitrothion exposure up to 10.0 mg/L. Fenitrothion has been reported to inhibit photosynthesis (oxygen evolution) in the freshwater diatoms *Navicula* and *Fragilaria* (Rama Rao *et al.*, 1979). Exposures of 2.0, 5.0 and 10.0 mg/L elicited reductions of 31, 57 and 62% respectively in primary production rates.

The biochemical indicators of fenitrothion stress together with cytological and morphotypic alterations may suggest a nuclear site of action. The augmentation of macromolecules together with elevated cellular dry weight and reduction in cell numbers was consistently observed in the algae monitored. We propose that as fenitrothion assumes critical internal levels in the nucleus, a resulting stress-induced inhibition of cytokinesis occurs, preventing normal mitotic divisional processes from occurring.

Lipids and fatty acids were also selected as clinical indicators of fenitrothion stress for several reasons. Firstly, the lipophilic nature of compounds such as fenitrothion ($\log K_{ow}$ = 3.4) indicates a high probability of exposure and subsequent toxic interaction with lipid-rich organisms such as algae. Secondly, although alterations in lipid levels and associated fatty acid composition of organisms exposed to a variety of stressors have been studied extensively in vertebrate and invertebrate fauna, plants, particularly aquatic plants have received little attention in this area (Geisy *et al.*, 1988). Lastly, other organophosphorus insecticides have been shown to interfere in lipid synthesis and structure in bacteria and terrestrial plants (Swamy and Veeresh 1987; De Storani *et al.*, 1986; Antunes-Medeira *et al.*, 1980).

Although lipid levels represent a non-specific indicator of the relative health of an organism, these macromolecules have particular significance when assessing hydrophobic, bioaccumulative contaminants. Lipids are an essential, readily available energy source particularly for many phytoplankton and are periodically mobilized from endogenous pools. As a result, bound lipophilic toxics can in turn be mobilized to exert adverse effects. Lipophilic membranes such as the plasmalemma and thylakoids are particularly sensitive to the effects of hydrocarbons and in view of the fact that toxicants can partition into membranes or absorb onto them, it is not surprising to observe effects that suggest alterations in membrane structure and permeability.

Recent evidence concerning photoperiodic changes in lipid content in phytoplankton demonstrates that lipid volume increases in the late part of the light period reaching a maximum in the dark (Sicko-Goad *et al.*, 1989b). Some phytoplankton classes (e.g. diatoms) in turn, are known to utilize lipids during the dark cycle for respiration and protein synthesis.

The contention therefore that cell cycle events may either mitigate or amplify effects of lipophilic stressors is well founded and has been empirically supported with work on the effects of chlorobenzenes on the diatom *Cyclotella meninghiniana* (Sicko-Goad *et al.*, 1989a) Clearly, timing of exposure and measurement are critical when assessing subtle, sub-lethal responses to lipophilic toxicants. In these experiments, synchronized cells of *Ankistrodesmus falcatus* were exposed for 48 hours (two full dark periods), however little is known regarding the metabolism of lipid reserves in chlorophytes. Even though total cellular lipid levels remained unchanged when normalized to cell weight, changes in lipid composition and structure were evident at the highest treatment level. In control untreated cultures sampled at the beginning of the light period, unsaturated fatty acids predominated (Table 7). Following 48 hours of fenitrothion exposure, a trend of increasing saturation was evident with significant decreases in the predominant 18:3 acid and less common 18:1 and an augmentation in saturated 16:0 fatty acid.

Saturation of fatty acids has been observed in plant tissues and algal cells exposed to other chemical stressors, in all cases leading to increased membrane permeability (Jackson and St. John. 1982; Nyberg and Koskimies-Soininen 1984). Netrawali and Gandhi (1990) attribute the significant leakage of cellular protein in *Chlamydomonas reinhardtii* cells to phosalone-induced destruction of the lipid bilayers. Although extracellular levels were not monitored, the observed augmentation in macromolecules suggests that significant losses of large biomolecules did not occur in *Ankistrodesmus falcatus* and *Chlamydomonas segnis* cells. This further implies that membrane integrity was sufficiently intact. Given this information and fenitrothion's effects on terrestrial plants (Weinberger *et al.*, 1981), it is likely that fenitrothion concentrations > 10 mg/L would lead to greater physical disruption of algal membranes since protein losses were only observed by Netrawali and Gandhi (1990) at high concentrations of phosalone (368 mg/L). At higher concentrations of fenitrothion therefore, one would expect a reversal in macromolecular responses observed (i.e. loss rather than augmentation).

Lipid reserves may serve to explain delayed or long-term effects. For example, it took more than 4 days at the highest treatment level for *Cosmarium sp.* and *Anabaena sp.* to elicit a decrease in growth rate. Similarly, the long-term growth inhibitions displayed by *Ankistrodesmus falcatus, Scenedesmus obliquus* and *Staurastrum sp.* may be a result of fenitrothion-induced mobilization of lipid reserves, in turn releasing pesticide to impact cell divisional processes.

Fenitrothion's destabilizing effect on membrane structure and permeability may also account for the apparent arrest of cell division in exposed cells. Membrane disruption would likely impact the transport and cytoplasmic balance of critical ions such as Ca^{++}, which is known to be directly linked to cell division (Wheatly 1982). Furthermore, this cation is also critical in ATPase activity thereby inferring that fenitrothion may indirectly influence energy metabolism in algal cells, preventing cell division (Marme 1982).

4.2.3 Cell Size and Population Size Spectra

Ecologists investigating relationships between plant associations and environmental stress and disturbances have long since recognized the significance of size and morphology (Grime 1977). Investigations into the effects of inorganic contaminants on phytoplankton have revealed significant alterations in cell dimensions (Davies 1974, Christianson 1979, Meisch and Schmitt-Beckman 1979, Gotsis 1982). Studies with organic pollutants in this area have been fewer and limited to chlorinated compounds (Powers *et al.*, 1977, Biggs *et al.*, 1978). Their results have also indicated significant effects in cell size distribution. More importantly, one investigation (Biggs *et al.*, 1978), indicated that the differential response to PCB exposure was size related. Significant alterations of phytoplankton cell size as a result of pesticide exposure has also been well demonstrated with organochlorine and organophosphorus pesticides (Saroja and Bose 1982; Powers *et al.*, 1977; Kikuchi *et al.*, 1984). Fenitrothion (5.0 - 10.0 mg/L) is reported to have exhibited this effect on ciliate protozoans (Lal *et al.*, 1987). Changes in cell size also suggest interference with cell division.

These findings are consistent with those of the present study where substantial departures from control population size distribution patterns were observed at concentrations as low as 1.0 mg/L in *Selenastrum capricornutum*. The cummulative long-term influence of fenitrothion on cell size is evident as an additional three days exposure nearly doubled the cell volume augmentation in Selenastrum capricornutum to 27% above control values. A sizable deviation from control size distribution also appeared with *Chlorella pyrenoidosa* (Figure 4B), a species identified as relatively insensitive to fenitrothion when comparing growth responses. This observation may reflect a function of the instrumentation used to determine size spectra. Given their intended design for mammalian cells, electronic particle counters yield higher resolution in measuring cell volume in spherical-shaped cells like *Chlorella*.

Because of the position that microflora occupy on the trophic pyramid, these responses have serious ecological implications. Firstly, prey selection by invertebrate grazers is principally size-dependent. Therefore, abnormally large or small cells may be rejected by consumers and thus alter the energy transfer in aquatic systems. In addition, algal cell dimensions and morphology directly influence its uptake of nutrients and light as well as its release of metabolic wastes. Phytoplankton size and geometry also directly influence sinking rates which play a vital role in algal population dynamics. Empirical evidence indicates that the range in surface area:volume ratios is remarkably conservative suggesting that competitive selection pressures influence phytoplankton cell dimension (Lewis 1976). Therefore, an alteration in algal cell size due to xenobiotic exposure not only implicates negative effects on organisms up the food chain but may also effect the competitive success of algae.

4.3 Differentail Sensitivity

The results from short and long-term growth effects analyses substantiate the existence of considerable differential sensitivity to fenitrothion among the twelve algal species. Some species such as *Navicula sp., Ankistrodesmus falcatus, Selenastrum capricornutum* and *Chlamydomonas segnis* displayed an immediate sensitivity with complete growth inhibition ranging from 3 - 14 days at 10.0 mg/L. A delayed toxic response starting from day 2 - 4 was evident in *Anabaena sp., Cosmarium sp.* and *Chlamydomonas reinhardtii*. Estimated concentrations eliciting 50% reductions in growth (EC_{50}) ranged over three orders of magnitude from 0.84 mg/L in *Staurastrum sp.* to 109.9 mg/L for *Chlamydomonas reinhardtii*. The threshold concentration for significant growth impairment was equally split between 1.0 - 10.0 mg/L for 50% of the test species battery and 0.1 - 1.0 mg/L for the more sensitive species (*Ankistrodesmus falcatus, Anabaena sp., Chlamydomonas segnis, Chlamydomonas reinhardtii, Scenedesmus obliquus*, and *Staurastrum sp.*).

A series of simple sensitivity rankings based on growth responses are presented (Table 5) to illustrate the relative sensitivity of phytoplankton exposed to fenitrothion. The disparity in rankings based on growth rate vs. biomass further emphasizes the importance both parameters when assessing algal growth effects. Furthermore, rankings based on short-term growth can be significantly different from those derived from long-term assays. A non-parametric correlation between 96 hour and 14 day cummulative rankings was not significant ($\alpha \leq 0.05$) and therefore supports this difference between short and long-term assay rankings. *Anabaena sp.* appears to be the most sensitive species over 96 hours but is relatively insensitive in 14 day tests. The opposite is apparent for *Selenastrum capricornutum, Scenedesmus obliquus* and *Staurastrum sp*. Conversely, several consistencies in rankings were also observed. *Ankistrodesmus falcatus* was consistently ranked the most sensitive whereas the two *Chlorella* species and *Pediastrum sp.* the least sensitive to fenitrothion.

The biochemical indicators of pesticide stress monitored in two species supports the sensitivity ranking with greater alterations in cellular macromolecules being observed in *Ankistrodesmus falcatus* than *Chlamydomonas segnis*. Further, the effects thresholds for these molecular-level responses are also consistent with those estimated for growth.

Phylogeny may also influence sensitivity patterns in microalgae (Wangberg and Blanck 1988). Taxonomically, no obvious trends in sensitivity were apparent apart from visible resistance demonstrated by the *Chlorella* genus. Interestingly, the universal algal species of choice in toxicity testing *Selenastrum capricornutum*, was relatively insensitive over the short exposure duration (96 hours) but had the most sensitive cumulative ranking over 14 days.

There is a body of empirical support on clonal variability that indicates that the response of a culture or population composed of one genotype is not always representative of species response (Mahoney and Haskin 1980; Kause 1987; Fisher 1977). Interspecific differential sensitivity therefore, does not necessarily predict intraspecific differential sensitivity in algal response to xenobiotic stress. Unfortunately, this presents yet another level of complexity when using laboratory data to predict natural phenomena.

This work contributes to dispelling the myth of a generally ***sensitive*** algal species. The relative degree of algal sensitivity to chemical stressors is more likely an interdependent function of the test species or strain, the chemical nature of the stressor, the experimental design (including exposure duration) and the response parameter selected. Obviously, to further justify generalizations in differential sensitivity between algal groups, a broad spectrum of stressors must be employed. Although other investigations incorporating multiple species and stressors have failed to identify a generally sensitive or insensitive species (Blanck *et al.*, 1984; Wangberg and Blanck 1988), this study suggest that algal species sensitive to fenitrothion are small (high $\sqrt{S}$ / $\sqrt[3]{V}$ ratios) and lipid rich.

4.4 Bioaccumulation Studies

Consistent with previous studies investigating fenitrothion uptake in aquatic plants (Moody 1982; Weinberger *et al.*, 1982), the test algae readily accumulated, or bioconcentrated this lipophilic compound to concentrations above those in ambient media. In observing the range of accumulated levels (14.9 - 109.9 ng ^{14}C / mg algae at 24 hours) of radiolabelled fenitrothion in viable cells, substantial differential uptake of fenitrothion exists within the range of species examined (Table 8). Although not as large (123.4 - 725.4 ng ^{14}C / mg algae at 24 hours), the range of bioaccumulation in dead cells is also highly variable. Bioconcentration ratios (BCF) determined for *Chlamydomonas segnis* and *C. reinhardtii* were comparable to BCF's (122 -177) determined in natural phytoplankton communities of shield lakes in New Brunswick (Lakshminarayana and Bourque 1980)

Not unlike related studies involving algae and fenitrothion (Caunter 1988; Kikuchi *et al.*, 1984), dead algal cells accumulated significantly more radiolabel than viable cultures. On an average, dead cells accrued 5.24 times more ^{14}C over 24 hours than live cells (ranged from 1.66 times for *Staurastrum sp.* to 10.47 times for *Navicula sp.*). This phenomena of preferential bioaccumulation in non-viable algal cells has been shown with other lipophilic pesticides (Rice and Sikka 1973). According to Kikuchi *et al.*, (1984), bioaccumulation ratios for fenitrothion in UV-killed algal cells were 2 - 17 times higher than those in living cells. This together with the present study indicates that passive partitioning processes are the principle mechanism involved in sequestering of fenitrothion in algal cells. This factor, together with ability of viable cells to actively depurate fenitrothion and mediate its enhanced photodegradation *in vivo* (Caunter and Weinberger 1988) accounts for the differences between live and dead burdens. This is consistent with other opinions (Chiou *et al.*, 1979) that "lipid-soluble compounds are absorbed primarily through passive saturable diffusion through the lipid portion of the membrane and secondarily through the aqueous pores".

The selection of a chemical treatment (carbonylcyanide m-chlorophenylhydrazone) to render cells non-viable was a novel approach for xenobiotic uptake studies. The predominant methods used to kill algal cells for this purpose are extreme heat or freeze treatments. These techniques however, often lead to membrane damage and cell lysis which prevents further fractionation. The complete deactivation of energy metabolism while keeping membranes and cellular wall components intact was the key criteria in designing a dead control in uptake experiments. CP, an inhibitor of phosphorylation, effectively deactivated biological uptake while maintaining cellular integrity.

The uptake dynamics of *Chlamydomonas segnis* were similar to those of *C. reinhardtii*. Uptake maxima were reached by 24 hours with considerably more label accumulated in dead cells. *C. reinhardtii* accumulated more than 3X the amount of radiolabel than *C. segnis* over a 24 hour period. As suggest by others (NRCC 1981), the differences in cellular lipid contents may account for this difference in burden. Examination of lipid data indicates that untreated *C. reinhardtii* cells (3.5 pg lipid / cell) have over 6X the total lipid content of *C. segnis* (0.52 pg lipid / cell).

Interestingly, a significant portion of radiolabelled fenitrothion was detected in the secondary supernatant, corresponding to the fraction of material loosely adsorbed to the cells. Some fenitrothion may be associated with exuded biomass or gelatinous matrix common in many of the test species.

4.5 Sensitivity Correlations

In the field of ecotoxicology over the last decade, substantial work has gone towards identifying various physical, chemical and environmental properties of xenobiotics which influence their toxicity to biota such as algae. Surprisingly, very little effort has been made in alternative approaches to predicting effects of toxicants, such as identifying properties of receptor biota which influence their susceptibility to xenobiotics. This latter "bottom up" approach may serve to explain the wide degree of sensitivity documented among organisms such as unicellular algae which are physiologically, morphologically and taxonomically analogous.

To examine whether certain physical, physiological or biochemical properties of algal receptors can predict their relative sensitivity to a lipophilic xenobiotic, relationships in the study data were investigated. Characteristics of the test species including functions of cell size, bioaccumulation capacity and cellular lipid content were correlated against sensitivity indices and rankings.

It is well known that algal cell size and morphology play a significant role in nutrient uptake, photosynthesis, respiration and regulation of waste products. It is not unreasonable to suggest that admission of toxins and the resultant consequences may also be related to some function(s) of cell size. Organism size (and surface:volume ratio) has been associated with pesticide tolerance in zooplankton (Evans and McNaught 1988). There is limited evidence, based on co-occurrence of pollutants and algae, to suggest differential responses to toxic contaminants to be cell size related in phytoplankton (Biggs *et al.*, 1978; Munawar *et al.*, 1987).

Sensitivity to fenitrothion was related to four functions of cell size including surface area, volume, surface area:volume ratio and the transformed ratio. It became apparent that cell volume or surface area in isolation was a poor predictor of sensitivity. The strongest predictor of fenitrothion sensitivity (related to algal size) was the transformed ratio with correlation coefficients ranging from 0.41 - 0.77.

In algae, as with most organisms, surface area increases with biomass volume. In unicellular algae, there is a theoretical minimum surface area for any given volume. This is the case for many simple spherical forms such as *Chlorella*. In order to differentiate cell shapes and better mathematically represent those species which depart significantly from simple spherical and ellipsoidal forms, the S/V ratio was transformed accordingly to better reflect the total reactive surface area over cytoplasmic volume. The transformation equation ($t = \sqrt{S} / \sqrt[3]{V}$) gave true spherical forms values of 2.20, which represents the minimum surface area for a given volume. Untransformed ratios did not correlate well with sensitivity as these ratios did not adequately reflect absolute departure from theoretical minimum surface area. The relationships with transformed ratios may be rationalized by recognizing that as absolute surface area of a biomass unit increases, so does the available bioactive surface membrane area. In addition, the smaller the cell volume, the lesser the internal stressor levels required to evoke adverse responses. One of the criteria used to select the test species was a wide range in morphology. Although the range in the 12 transformed ratios was 2.20 - 3.21, more attenuate forms like *Ankistrodesmus* may have better resolved some of the correlations recognized.

The transformed S.A/Vol. ratio is also strongly correlated with bioaccumulation capacity (r^2 = .68). Curiously however, a similar correlation with metabolically deactivated cells was not observed. This is unexpected, given the apparent non-exogenic bioaccumulation of lipophilic compounds in algae. The fact that bioaccumulation capacity also explained 56% of the variability in fenitrothion sensitivity implies that an increased sensitivity to fenitrothion may result from a greater toxic burden being accumulated coupled with the lack of adequate compensatory depuration or detoxification processes.

Total cellular lipid content explained nearly 60% of the variation in sensitivity ($r^2 = .57$) indicating that these macromolecules likely play a role in fenitrothion sensitivity. For hydrophobic compounds such as fenitrothion, total burden is a function of the partitioning of the chemical between internal lipid pools and the ambient environment (Clayton *et al.*, 1977). Lipid content is documented to be a significant factor in the bioconcentration and resulting toxic effects of lipophilic toxicants in fish (Sicko-Goad *et al.*, 1989b) however, its role as a contributing factor in algal toxicity studies is virtually unknown. It has been postulated that increased toxicity of lipophilic toxicants to unicellular algae may be attributed to the overall lipid content in algae and the greater ability of these compounds to penetrate and impact critical lipoprotein complexes in membranes (Hutchinson *et al.*, 1980). In addition to the magnitude of the lipid fractions, the specific composition and associated function (e.g. transport systems, sequestering potential) of membrane lipids and fatty acids may also influence an algal cell's ability to tolerate xenobiotics (Fisher 1977).

Several notable implications arise from the positive relationship between sensitivity and lipid content. It is possible that phytoplankton with typically large lipid fractions (e.g. diatoms) may be at greater risk to higher toxin burdens of lipophilic xenobiotics and subsequent phytotoxic consequences. Many phytoplankton species typically store photosynthetic energy reserves as lipids (Reynolds 1984). Natural demands such as nutrient or light deficiencies are known to enhance fat and oil storage (Shifrin and Chisholm 1981) thereby promoting maximum xenobiotic uptake and compounding anthropogenic stress factors.

Though there is general agreement that relative susceptibility to toxic stress is highly pollutant-specific, this work disputes the claim that it is not possible to predict responses of one species from results of experiments with another (Slooff *et al.*, 1983). The results herein suggest that algal morphological and chemical attributes may be used to predict relative sensitivities to lipophilic compounds in untested species. The logical validation of this hypothesis would be to test the algal battery with a variety of stressors. This would elucidate the existence of sensitivity patterns for a wide range of candidate test compounds. Knowledge of such patterns is a prerequisite for a rationale design of tests batteries and species selection (Wangberg and Blanck 1988).

Most toxicological tests with algae are done with a limited number of taxa (predominantly Chlorophyta and Cyanophyta). The planktonic flora of freshwaters normally contain a wide phylogenetic and physiological diversity of species. Considerable differences in the attributes tested herein (algal size, lipid content) could conceivably account for the expected wide response to chemical stressors. Fundamental differences in cellular physiology between prokaryotic cyanophytes and eucaryotic chlorophytes have helped explain the different sensitivity patterns in algae exposed to chemicals (Wangberg and Blanck 1988). This study also identified one particular order (Chlorococcales) as having a sensitivity pattern distinct from xanthophytes and other chlorophytes. This group, represented by the relatively sensitive *Ankistrodesmus, Selenastrum* and *Scenedesmus* and the insensitive *Chlorella*, did not appear to have a taxonomically distinct pattern response in the present studies. In addition, the growth responses of the lone prokaryote, *Anabaena sp.* were not any more distinct than other taxa.

Although these relationships are predicated on limited data sets, the resulting correlations (Figures 8 - 14) suggest that certain algal cell properties can predict relative sensitivity to fenitrothion stress.

CHAPTER 5.0

CONCLUSIONS

In conclusion, the present laboratory model study demonstrated that 1 - 10 mg/L fenitrothion exposure to freshwater phytoplankton lead to effects observed over three levels of biological organization, namely molecular/biochemical, cellular/organismal and population, and that these effects are temporally related in such order. It is suggested that although these deleterious growth inhibiting concentrations of fenitrothion may potentially occur in surface waters, under typical field practices, exposures would generally not be sufficient to evoke significant growth impairment. It is however conceivable that levels may persist long enough to induce subtle, biochemical alterations in sensitive algal species present.

These studies demonstrate that by selecting sensitive effects indicators over multiple levels of biological organization and increasing the number and taxonomic range of test species, the resolution of fenitrothion's phytotoxic potential is enhanced considerably beyond what is currently perceived. This phytotoxic potential is alarming given that the regulatory license for repeated, intensive use of this compound in the Canadian environment was provided without an assessment of non-target plant effects. This is not surprising considering plant toxicity data has represented less than 3% of all environmental effects testing information required for chemical manufacturing and use in North America over the last decade (Benenati 1990).

Whereas these studies demonstrate the phytotoxic potential of the active ingredient, it is important to keep in mind that formulations used may further enhance the potential for toxic effects. The probability of these chronic molecular effects occurring under field conditions is increased when one takes into account the additive or synergistic role that adjuvants play in the uptake and resulting toxic impact of formulated pesticides on non-target biota.

These investigations have a valuable predictive utility in providing a range of algal-related parameters such as surface area/volume ratios, lipid content and bioaccumulation capacity, which may be used to forecast which species are at higher risks than others to xenobiotics stress. Furthermore, relationships observed between cell S/V ratio and the bioaccumulation of fenitrothion further implicates algal cell size as a significant factor in algal/pollutant interaction and subsequent vulnerability to lipophilic xenobiotics. In this way, shifts in phytoplankton species profiles may be predicted following contamination. Similarly, alterations in normal algal assemblage hierarchies and major biomolecules may be useful indicators of anthropogenic stress in aquatic ecosystems.

In addition, these studies directly provide needed information to fill current data gaps in the environmental hazard assessment of a priority pesticides in Canada. The information produced has immediate practical applications in pesticide management and environmental protection programs in Canada such as regulatory re-evaluation of fenitrothion (Agriculture Canada 1990) and the development of water quality guidelines for the protection of aquatic life (Wong and Kent 1988).

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APPENDICES

APPENDIX I.

Table 1. Taxonomy of the 12 phytoplankton species.

GENUS/SPECIES	DIVISION	CLASS	ORDER	SUB-ORDER	FAMILY
Anabaena sp.	Cyanophyta	Cyanophyceae	Oscillatoriales	Nostochineae	Nostocaceae
Ankistrodesmus falcatus	Chlorophyta	Chlorophyceae	Chlorococcales		Oöcystaceae
Chlamydomonas reinhardtii	Chlorophyta	Chlorophyceae	Volvocales		Chlamydomonadaceae
Chlamydomonas segnis	Chlorophyta	Chlorophyceae	Volvocales		Chlamydomonadaceae
Chlorella pyrenoidosa	Chlorophyta	Chlorophyceae	Chlorococcales		Oöcystaceae
Chlorella vulgaris	Chlorophyta	Chlorophyceae	Chlorococcales		Oöcystaceae
Cosmarium sp.	Chlorophyta	Chlorophyceae	Zygnematales		Desmidiaceae
Navicula sp.	Chrysophyta	Bacillariophyceae	Pennales	Naviculineae	Naviculaceae
Pediastrum sp.	Chlorophyta	Chlorophyceae	Chlorococcales		Hydrodictyaceae
Scenedesmus obliquus	Chlorophyta	Chlorophyceae	Chlorococcales		Scenedesmaceae
Selenastrum capricornutum	Chlorophyta	Chlorophyceae	Chlorococcales		Oöcystaceae
Staurastrum sp.	Chlorophyta	Chlorophyceae	Zygnematales		Desmidiaceae

APPENDIX II.

Table 2. Kuhl's Inorganic Nutrient Algal Medium

Compound	Final Concentration (moles/L)
KNO_3	1×10^{-2}
$NaH_2PO_4.H_2O$	4.5×10^{-3}
$Na_2HPO_4.2H_2O$	0.5×10^{-3}
$MgSO_4.7H_2O$	1×10^{-3}
$CaCl_2.2H_2O$	1×10^{-4}
$FeSO_4.7H_2O$ Fe-EDTA complex[a]	2.5×10^{-5}
H_3BO_3	1×10^{-6}
$MnSO_4.H_2O$	1×10^{-6}
$ZnSO_4.7H_2O$	1×10^{-6}
$CuSO_4.5H_2O$	1×10^{-8}
$(NH_4)_6Mo_7O_{24}.4H_2O$	1×10^{-8}

[a] Refer to original citation (Kuhl and Lorenzen 1964) for preparation of this component.

APPENDIX III

TABLE 3A. Effects of fenitrothion (0.1, 1.0 and 10.0 mg/L) on cell number (X 10^5 cells/mL), cell dry weight (pg/cell) and mean cell volume (μm^3) of *Chlamydomonas segnis*. Data presented are means and standard deviations following 7 day exposure.

		Fenitrothion (mg/L)		
PARAMETER	CONTROL	0.1	1.0	10.0
Cell Number	33.4 ± 2.4	30.2 ± 2.8	29.9 ± 3.3	21.4* ± 3.5
Dry Weight	34.4 ± 7.8	35.8 ± 6.5	58.9* ± 15.5	120.2* ± 19.2
Cell Volume	560.0 ± 3.5	557.3 ± 3.7	564.8 ± 4.2	573.7* ± 4.3

* Denotes significant difference from control means ($P \leq 0.05$).

TABLE 3B. Effects of fenitrothion (0.1, 1.0 and 10.0 mg/L) on cell number (X 10^6 cells/mL), cell dry weight (pg/cell) and mean cell volume (μm^3) of *Chlorella pyrenoidosa*. Data presented are means and standard deviations following 7 day exposure.

		Fenitrothion (mg/L)		
PARAMETER	CONTROL	0.1	1.0	10.0
Cell Number	19.4 ± 1.4	18.6 ± 2.2	18.6 ± 1.2	15.2 ± 2.3
Dry Weight	13.4 ± 0.9	13.2 ± 3.3	13.9 ± 1.9	16.8 ± 1.6
Cell Volume	50.6 ± 1.8	52.3 ± 6.0	59.3 ± 2.0	65.9* ± 2.5

* Denotes significant difference from control means ($P \leq 0.05$).

TABLE 3C. Effects of fenitrothion (0.1, 1.0 and 10.0 mg/L) on cell number (X 10^5 cells/mL), cell dry weight (pg/cell) and mean cell volume (μm^3) of *Scenedesmus obliquus*. Data presented are means and standard deviations following 7 day exposure.

		Fenitrothion (mg/L)		
PARAMETER	CONTROL	0.1	1.0	10.0
Cell Number	70.5 ± 5.8	62.6 ± 10.8	54.8* ± 5.1	23.7* ± 9.4
Dry Weight	39.8 ± 0.8	42.7 ± 0.8	79.9* ± 14.2	91.2* ± 17.4
Cell Volume	91.2 ± 1.3	94.0 ± 2.0	89.4 ± 2.2	95.4 ± 1.9

* Denotes significant difference from control means ($P \leq 0.05$).

TABLE 3D. Effects of fenitrothion (0.1, 1.0 and 10.0 mg/L) on cell number (X 10^5 cells/mL), cell dry weight (pg/cell) and mean cell volume (μm^3) of *Selenastrum capricornutum*. Data presented are means and standard deviations following 7 day exposure.

		Fenitrothion (mg/L)		
PARAMETER	CONTROL	0.1	1.0	10.0
Cell Density	34.6 ± 1.6	42.8* ± 4.0	33.8 ± 2.8	9.9* ± 5.2
Dry Weight	43.1 ± 4.1	61.7 ± 23.3	55.7 ± 7.8	73.3 ± 11.9
Cell Volume	70.8 ± 2.4	68.0 ± 2.4	75.5* ± 2.5	83.7* ± 3.3

*Denotes significant difference from control means ($P \leq 0.05$).

TABLE 4A. Effects of fenitrothion (0.1, 1.0 and 10.0 mg/L) on cellular protein, carbohydrate, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) of *Chlamydomonas segnis* in pg/cell as observed following 48 hours treatment at the end of the dark period. Data presented are means and standard deviations.

		Fenitrothion (mg/L)		
PARAMETER (pg/cell)	CONTROL	0.1	1.0	10.0
Protein	10.9 ± 1.2	10.7 ± 3.7	9.6 ± 5.2	13.9 ± 5.2
Carbohydrate	8.7 ± 2.2	9.5 ± 3.0	11.9 ± 3.5	20.3* ± 3.5
RNA	4.0 ± 0.7	3.9 ± 1.9	4.3 ± 1.4	4.8 ± .7
DNA	0.37 ± 0.08	0.37 ± 0.10	0.44 ± 0.11	0.47 ± 0.10

* Denotes significant difference from control means ($P \leq 0.05$).

TABLE 4B. Effects of fenitrothion (0.1, 1.0 and 10.0 mg/L) on cellular protein, carbohydrate, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) of *Ankistrodesmus falcatus* in pg/cell as observed following 48 hours treatment at the end of the dark period. Data presented are means and standard deviations.

		Fenitrothion (mg/L)		
PARAMETER (pg/cell)	CONTROL	0.1	1.0	10.0
Protein	7.6 ± 1.6	7.5 ± 1.3	10.2* ± 1.3	11.5* ± 1.6
Carbohydrate	5.0 ± 0.7	5.2 ± 0.9	5.9 ± 1.4	7.6* ± 0.5
RNA	2.6 ± 0.6	2.4 ± 0.8	2.4 ± 1.0	3.7* ± 0.2
DNA	0.15 ± 0.05	0.16 ± 0.06	0.19 ± 0.06	0.22 ± 0.08

* Denotes significant difference from control means ($P \leq 0.05$).

TABLE 5. The effects of 0.1, 1.0 and 10.0 mg/L of fenitrothion on the total lipid content of *Ankistrodesmus falcatus* cells following 48 hours exposure. Values, expressed on a dry weight basis, are presented as the mean and standard deviation of three replicates.

		Fenitrothion (mg/L)		
PARAMETER	CONTROL	0.1	1.0	10.0
μg TL[1]/mg algae	31.3 ± 1.8	30.5 ± 2.2	36.3 ± 3.5	68.6 ± 7.3
pg TL/cell	2.1 ± 0.25	2.0 ± 0.38	2.4 ± 0.39	4.5 ± 1.1
% TL/cell	10.7 ± 1.3	9.7 ± 1.8	11.3 ± 2.4	11.4 ± 3.2

[1] Total Lipids