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TOXICITY OF POLYCHLORINATED BIPHENYLS TO 'EUGLENA GRACILIS': CE--ETC(U)
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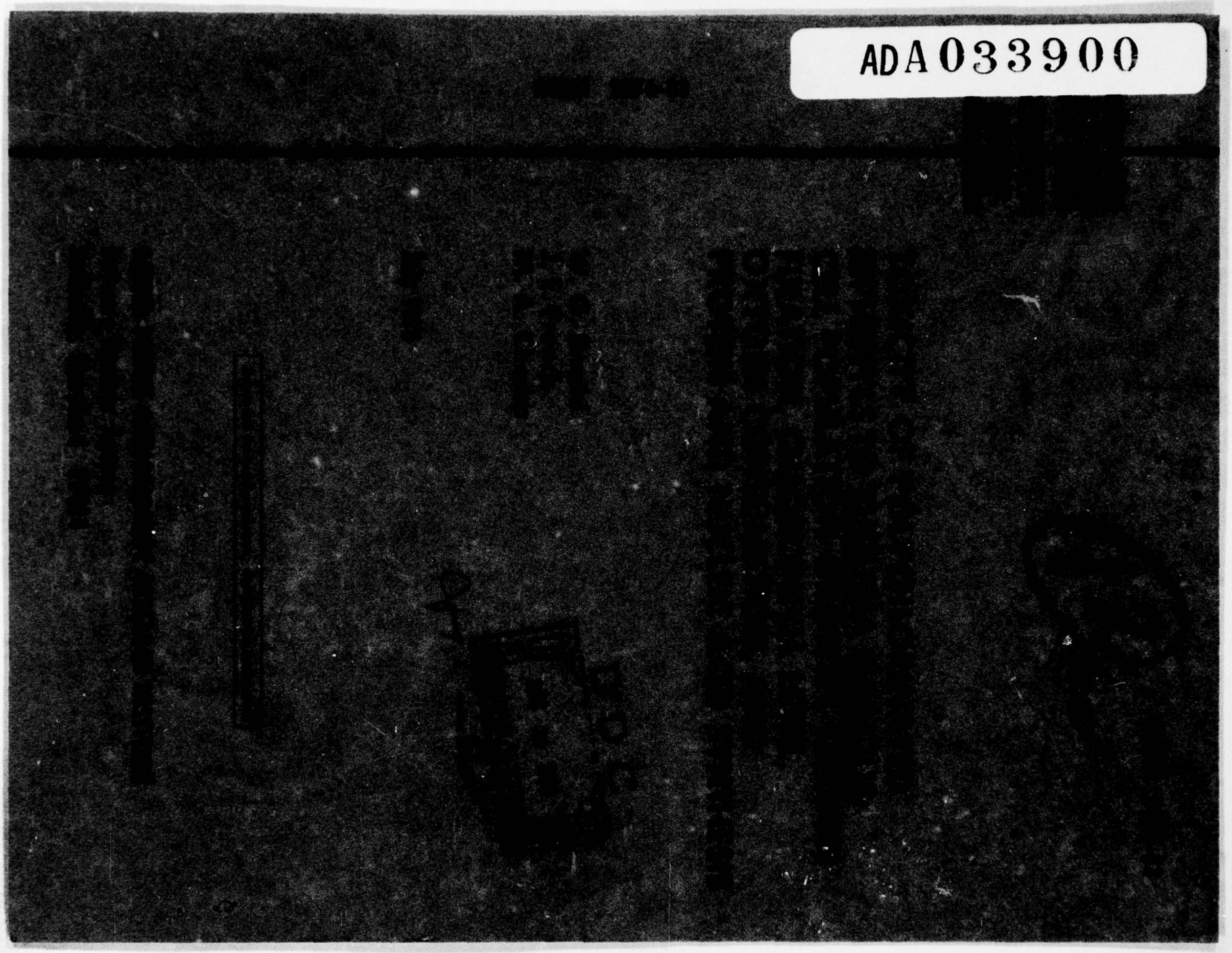

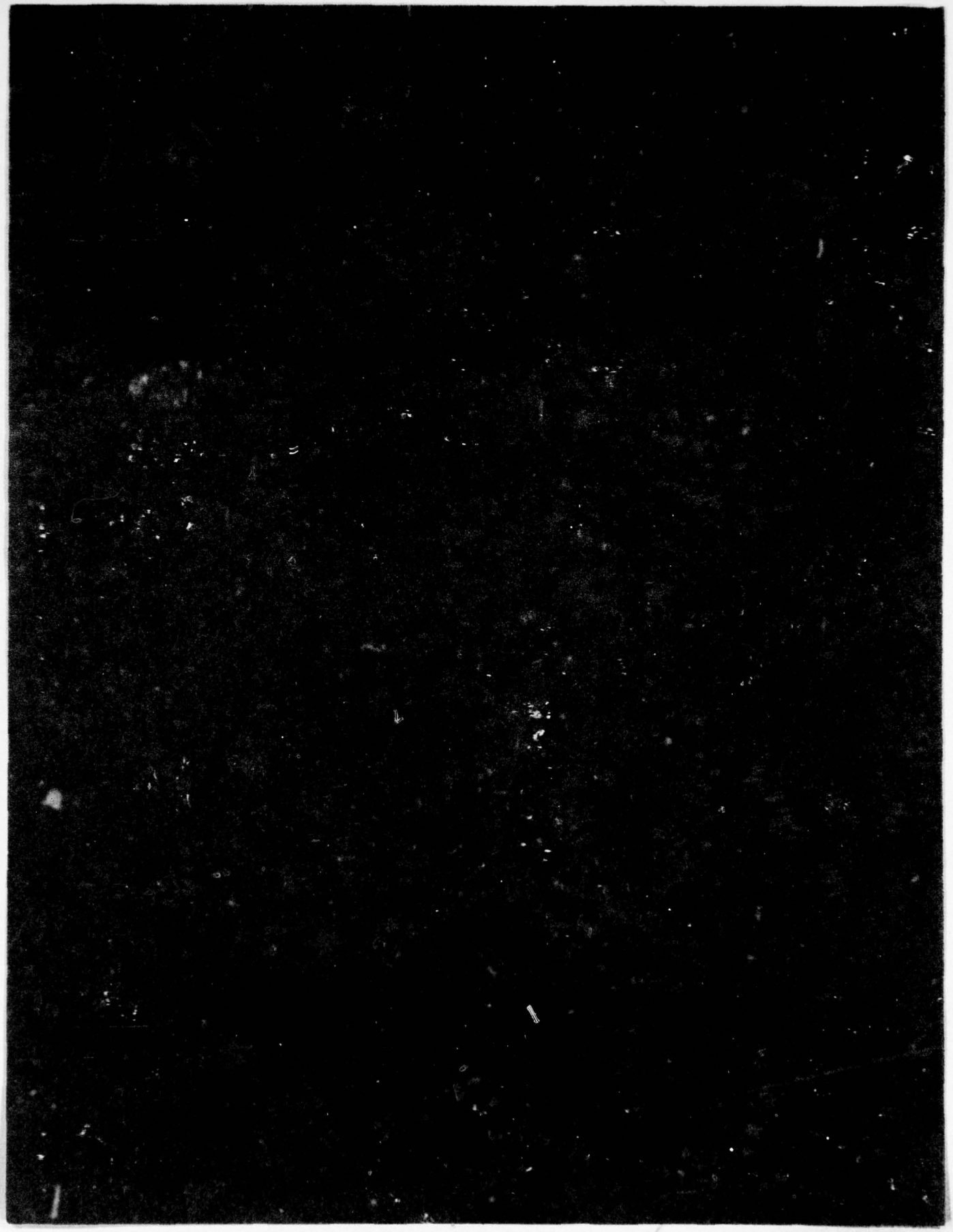

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Populations of Euglena gracilis in exponential growth under light were exposed to 2.5, 5.0, 7.5, and 10 ppm of Aroclor 1221. The $ID_{50/48}$ of Aroclor 1221 was estimated to be 4.4 ppm, while Aroclor 1232 tested at 20, 35, 50, and 100 ppm resulted in an $ID_{50/48}$ of 55 ppm. With Aroclor 1242, no inhibition of growth was observed with up to 100 ppm exposure. Cell cultures exposed to 4.4 ppm of Aroclor 1221 for 48 hours had a significantly reduced rate of carbon fixation and reduced

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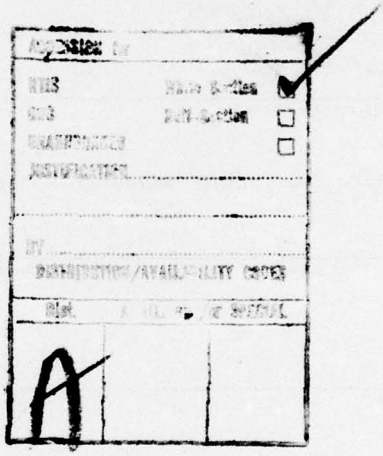
levels of chlorophyll after correction for cell density. Oxygen consumption was not affected at the ID_{50} level of the Aroclor. Uptake of [^{3}H]-leucine in treated cultures was twice that of controls, and [^{3}H]-uridine uptake was significantly lower. Uptake of [^{3}H]-thymidine, and incorporation of [^{3}H]-leucine, [^{3}H]-thymidine, and [^{3}H]-uridine were not significantly different in treated and control cultures. These results suggest that at the ID_{50} level, polychlorinated biphenyls (PCBs) reduce cell population growth in Euglena gracilis by inhibition of photosynthesis and/or chlorophyll production. An inexpensive and rapid screening method for potential toxic environmental chemical compounds used by military organizations is of great importance in case such compounds are accidently introduced into the environment. To meet these requirements, such a method was developed using the aquatic protozoan Euglena gracilis as a sensitive indicator.



PREFACE

Aroclors 1221 and 1242 were provided by W. B. Papageorge of Monsanto Industrial Chemical Company. This paper was taken in part from a thesis submitted to American University by W.G.E. in partial fulfillment of the requirements for the Master of Science degree.

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INTRODUCTION

Polychlorinated biphenyls (PCBs) are toxic, persistent, and widespread in the environment.[12] PCBs and DDE (1,1-dichloro-2,2-bis(p-chlorophenyl)-ethane) together may be the most abundant of the chlorinated hydrocarbons in the global ecosystem.[13]

Commercially manufactured mixtures of PCBs are known as Aroclors (Aroclor is a trademark of Monsanto Industrial Chemical Company) and are classified according to percent chlorine by weight. Aroclor designation consists of four digits (e.g., Aroclor 1221). The first two digits denote the biphenyl molecule, the last two the percent chlorine (e.g., 21 percent). These compounds are relatively inert, have low water solubilities, and are lipophilic.[4]

Recent investigations indicate the need for more information on the effects of PCBs on the lower trophic levels of freshwater and marine ecosystems. A brief summary of these investigations is as follows. In a heterogeneous marine phytoplankton community the total radiocarbon uptake was reduced by 50 percent at 6.5 ppb of Aroclor 1242, 15 ppb of Aroclor 1254, or 35 ppb of DDT after 24 hours exposure.[9] However, the rate of photosynthesis per cell, as measured by radiocarbon uptake, was shown to be unaffected in marine phytoplankton at 10 ppb of Aroclor 1254 and 50 ppb of DDT.[5] The growth rates of two species of marine diatoms were reduced by exposure to Aroclor 1254 at 10 ppb for 1 week.[10] These investigators also found PCBs to be more toxic than DDT to diatoms. Also, Aroclor 1254 in concentrations up to 100 ppb did not inhibit growth of Euglena gracilis, a freshwater flagellate, which suggests that E. gracilis is more resistant to PCBs than marine phytoplankton species.

Aroclor 1254 or DDT in concentrations up to 100 ppb added to mixed cultures of a marine diatom and a marine alga resulted in the loss of dominance by the diatom,[11] suggesting that natural species relationships can be disrupted by these chlorinated hydrocarbons. Aroclors 1242 and 1260 reduced growth rate and cell population size after 96 hours in cultures of an estuarine ciliate at 1 ppm.[1]

Aroclor 1254 caused a reduction in these parameters at 1 ppb. Accumulation of the Aroclors in the ciliate was greater with increased chlorination of the Aroclor.

RNA levels were reduced by 63 percent and chlorophyll index was reduced by 80 percent in a marine diatom after 2 weeks exposure to 0.1 ppm of Aroclor 1242.[7] Accumulation of this Aroclor was up to 1000 times the level in the culture medium.

The objectives of this study were to determine levels of Aroclor toxicity to Euglena gracilis, a representative freshwater phytoplankton species. Cell population growth, carbon fixation, chlorophyll level, oxygen consumption, and protein and nucleic acid synthesis were examined in order to define the metabolic site of toxicity of Aroclor 1221.

METHODS AND MATERIALS

Cell culture. Cultures of Euglena gracilis Z (#12716, The American Type Culture Collection, Rockville, Maryland[15]) were axenically maintained in Hutner's medium (ATCC 1974) with 1 percent glucose at $25^{o} \pm 1^{o}C$ under fluorescent light of approximately 1000 foot-candles with a light-dark cycle of 14-10 hours. Thirty-milliliter volumes of medium were cultured in 125 ml nephelometer flasks. Side arms of the flasks were inserted into a B&L Spectronic 20 (Bausch and Lomb, Rochester, New York) to read the optical density at 750 nm (OD_{750}) as a measure of cell population density.[3]

Cell population growth inhibition. Cultures were adjusted to 10^4 cells/ml and after 24 hours incubation as described above were exposed to initial dose levels in the medium of 0 (solvent control), 2.5, 5.0, 7.5, and 10 ppm of Aroclor 1221 (Monsanto Industrial Chemical Company, St. Louis, Missouri). Dimethylsulfoxide (DMSO) (Fisher Chemical Company, Fairlawn, New Jersey) was used as the solvent for dilution and delivery of the Aroclor, at less than 0.1 percent (v/v) in all experiments. Controls without DMSO were also compared for growth with solvent controls. After 48 hours exposure to the Aroclor, OD_{750}

readings were taken and the percent inhibition of growth when compared with solvent controls was determined for each dose level. The same procedure was used for Aroclor 1232 (Analabs, North Haven, Connecticut) at dose levels of 0, 20, 35, 50, and 100 ppm. Three separate replications for each Aroclor were carried out with three flasks per replicate at each dose level. The $ID_{50/48}$ (that dose level which results in a 50 percent inhibition of cell population growth after 48 hours exposure) was estimated for each Aroclor from the log-probit dose-response curve.[8]

Carbon fixation. Cultures grown for 24 hours from 10^4 cells/ml were exposed to 4.4 ppm (the $ID_{50/48}$) of Aroclor 1221 in DMSO. Control cultures received DMSO alone. After 48 hours, OD_{750} readings were taken of control and treated cultures, and [^{14}C]-bicarbonate (New England Nuclear Corporation, Boston, Massachusetts) was added to each flask at 17 nCi/ml and incubated for 1 hour under light at 25°C. Cells from 5 ml of each culture were collected by vacuum filtration (#GA-6, Metricel 0.45 μm membrane filter, Gelman Instrument Company, Ann Arbor, Michigan). Each filter was washed with 10 ml of fresh culture medium and placed in a scintillation vial. After drying the filter overnight at room temperature, 5 ml of Concentrol scintillation fluor (Yorktown Research, Hyde Park, New York) were added, and radioactivity measured with a Nuclear-Chicago Mark-II liquid scintillation spectrometer. Results were expressed as counts per minute (CPM) divided by OD_{750}.

Oxygen consumption. After 48 hours PCB exposure, 30 ml volumes of control and treated cultures were centrifuged at 600 x g for 10 min, and resuspended in 3 ml of culture medium. Cell density of each sample was determined with a Model B Coulter counter (Coulter Electronics, Hialeah, Florida). The rate of oxygen consumption was determined after 5 min equilibration with air at 25°C with a YSI Model 53 oxygen polarograph (Yellow Springs Instrument Company, Yellow Springs, Ohio). Values were expressed as μ-atoms $0_2/10^6$ cells/h.

Chlorophyll level. Five-milliliter aliquots of cell suspension from cultures exposed for 48 hours were taken from treated (4.4 ppm Aroclor 1221) and control cultures and cells collected on filters as described above. Chlorophyll was extracted for 24 hours in stoppered test tubes with 10 ml Fisher Spectranalyzed acetone. After centrifuging, the absorbance of the acetone extract was read at 665 nm in a B&L Spectronic 20. Chlorophyll absorbance (Ch A_{665}) values were divided by the OD_{750} of the 48-hour cultures in order to correct for population density.

Protein and nucleic acid synthesis. After 48 hours PCB exposure, 5-ml aliquots were taken from treated and control cultures and placed in 10-ml Erlenmeyer flasks. One-tenth milliliter [^{3}H]-L-leucine (New England Nuclear Corporation) was added to each flask at 0.2 μCi/ml and incubated for 1 hour at 25°C. Uptake of [^{3}H]-leucine was measured by collecting cells on filters and washing with an equal volume of culture medium before scintillation counting. To measure [^{3}H]-leucine incorporation after 2 hours incubation, 5 ml of 10 percent trichloroacetic acid (TCA) (Fisher Chemical Company) were added to the flasks. After 5 min the protein precipitate was collected on filters and washed with 10 ml of 5 percent TCA. Filters were dried and radioactivity determined as described above. CPM values were divided by the population density (cells/ml). The same procedure was followed with [^{3}H]-thymidine and [^{3}H]-uridine at 0.2 μCi/ml in order to measure uptake and incorporation into DNA and RNA respectively.

RESULTS

Cell population growth inhibition. After 48 hours exposure, DMSO in the concentrations used ($\leq$ 0.1 percent) had a minimal effect on cell population growth of E. gracilis. The $ID_{50/48}$ for Aroclor 1221 was estimated to be 4.4 ppm (Figure 1) and 55 ppm for Aroclor 1232 (Figure 2).

There was no inhibition of cell population growth when using Aroclor 1242 in concentrations up to 100 ppm. It was not possible to solubilize Aroclor 1242

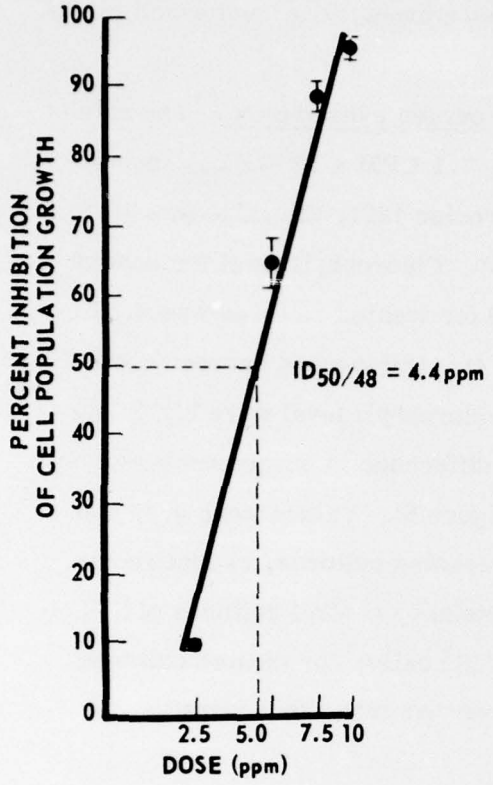

Figure 1.
Estimation of $ID_{50/48}$ to Euglena gracilis of Aroclor 1221

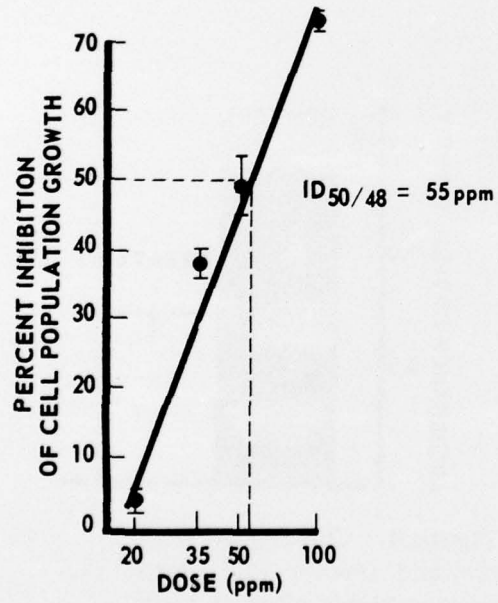

Figure 2.
Estimation of $ID_{50/48}$ to Euglena gracilis of Aroclor 1232

in the aqueous culture medium, using DMSO as solvent, to a level which would reduce growth by 50 percent.

Carbon fixation, chlorophyll level, and oxygen consumption. The rate of carbon fixation in control cultures was 71.7 ± 7.1 CPM x $10^3/OD_{750}$ (mean ± S.E.). For cultures treated with 4.4 ppm Aroclor 1221, the value was 45.9 ± 4.3, an inhibition of 36.1 percent (Figure 3). Chlorophyll level for control cultures was 1.38 ± 0.05 Ch A_{665}/OD_{750} and for treated cultures was 0.72 ± 0.01, an inhibition of 43.2 percent (Figure 4). Differences between control and treated cultures for carbon fixation and chlorophyll level were highly significant ($p \leq 0.01$). There was no significant difference in oxygen consumption between control and Aroclor treated cells (Figure 5). Values were 0.89 and 0.90 μ-atoms $O_2/10^6$ cells/h for control and treated cultures, respectively.

Protein and nucleic acid synthesis. Uptake by control cultures of [^{3}H]-leucine after 48 hours was 48.2 ± 12.3 CPM/10^5 cells; for treated cultures 99.4 ± 6.8. This was a twofold increase over control. Incorporation of

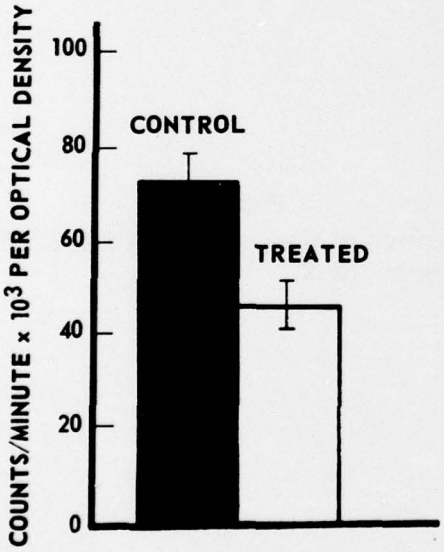

Figure 3. Carbon fixation in control and Aroclor 1221 treated (4.4 ppm) cultures after 48 hours

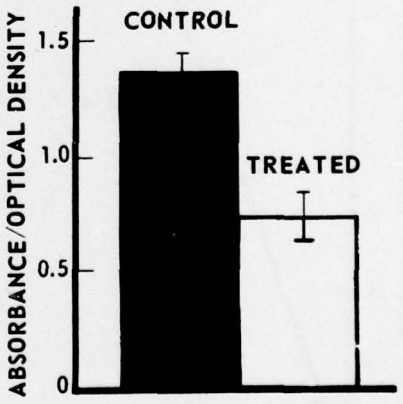

Figure 4. Chlorophyll levels in control and Aroclor 1221 treated (4.4 ppm) cultures after 48 hours

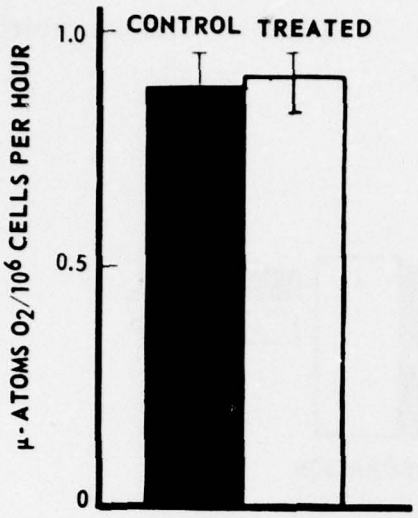

Figure 5.
Oxygen consumption in control and Aroclor 1221 treated (4.4 ppm) cultures after 48 hours

[^{3}H]-leucine showed no difference between control (75.3 ± 3.2) and treated (78.3 ± 4.8) cultures (Figure 6).

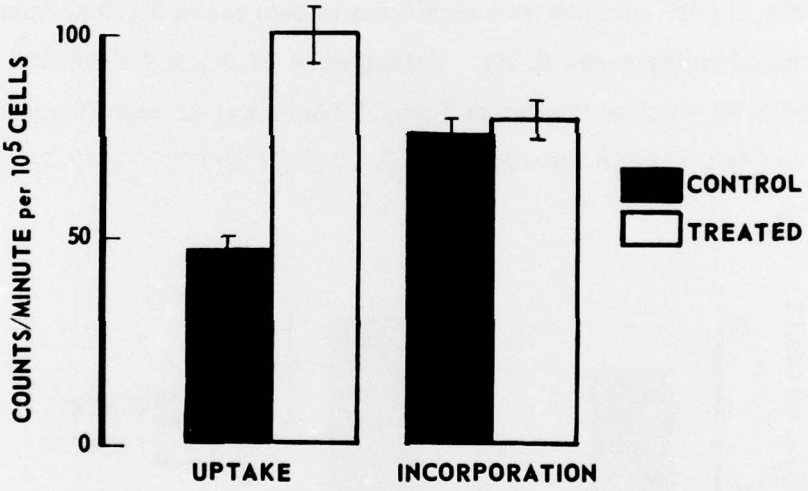

Figure 6. Uptake and incorporation of [^{3}H]-leucine in control and Aroclor 1221 treated (4.4 ppm) cultures after 48 hours

Uptake of [^{3}H]-thymidine in treated cultures was reduced by 27.4 percent to 19.3 ± 1.9 CPM/10^5 cells, relative to that of control (26.6 ± 3.0). This difference was not significant ($p \geq 0.05$). There was no difference in [^{3}H]-

thymidine incorporation (20.1 ± 1.4 and 20.8 ± 1.7 CPM/10^5 cells for controls and treated, respectively) as shown in Figure 7.

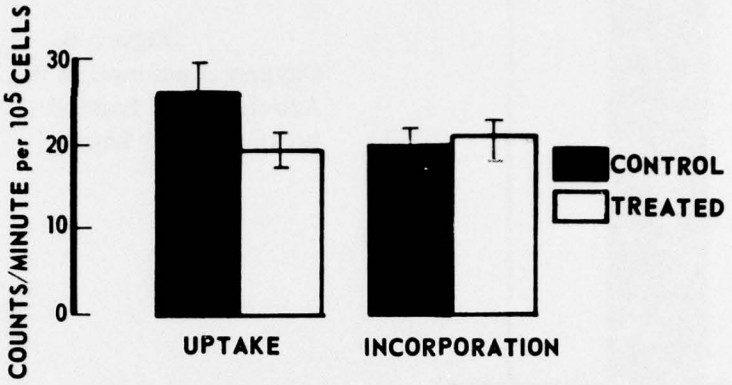

Figure 7. Uptake and incorporation of [^{3}H]-thymidine in control and Aroclor 1221 treated (4.4 ppm) cultures after 48 hours

Uptake of [^{3}H]-uridine was significantly decreased (51.3 percent inhibition) in treated cultures ($p \leq 0.01$). Values were 17.3 ± 2.7 CPM/10^5 cells in control and 8.42 ± 1.0 in treated cultures. There was no significant difference ($p \geq 0.05$) in [^{3}H]-uridine incorporation (81.0 ± 6.1 and 71.0 ± 12.7) as shown in Figure 8.

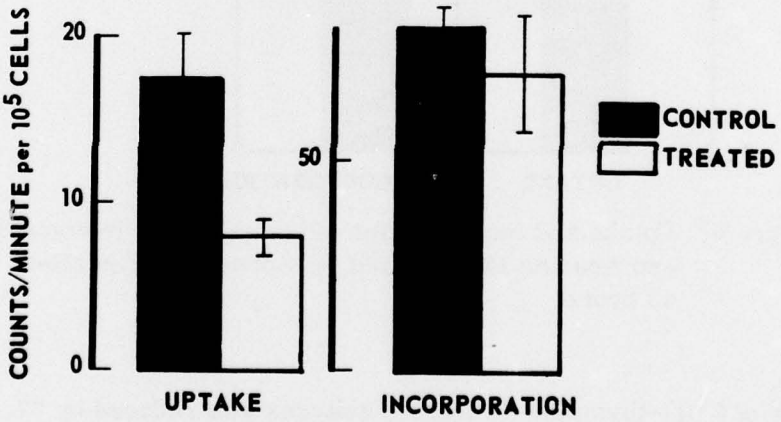

Figure 8. Uptake and incorporation of [^{3}H]-uridine in control and Aroclor 1221 treated (4.4 ppm) cultures after 48 hours

DISCUSSION

We have determined that the $ID_{50/48}$ for Aroclor 1221 (4.4 ppm) was less than that for Aroclor 1232 (55 ppm), and Aroclor 1242 showed no inhibition of cell population growth with concentrations up to 100 ppm over 48 hours exposure. Aroclor 1221 at the ID_{50} level was found to depress significantly carbon fixation and chlorophyll levels, but not to affect oxygen consumption. Uptake of L-leucine was increased twofold, but incorporation was not affected. Only uridine uptake was significantly decreased by Aroclor 1221 treatment, whereas uridine incorporation and thymidine uptake and incorporation were not different from control values.

With Aroclors 1221, 1232, and 1242, toxicity to E. gracilis decreased with increasing percent chlorination of the Aroclors. In marine phytoplankton, a lower toxicity was found with 1254 than with 1242.[9] Aroclor 1254, however, was found to be more toxic to the ciliate Tetrahymena pyriformis than either 1242 or 1260.[1] In most cases toxicity appears to be inversely related to percent chlorination.

DDT reduced photosynthesis as well as population growth in cultures of marine phytoplankton.[16] PCBs were shown to be toxic to communities of marine phytoplankton, with toxicity measured as inhibition of radiocarbon uptake.[9] A more recent study determined the rate of photosynthesis per cell in phytoplankton exposed to PCBs and to DDT, and showed no difference between control and treated cells.[5]

It has been suggested that DDT taken up by phytoplankton becomes associated with the chloroplasts because of the lipophilic properties of the DDT molecule, and because the chloroplast contains most of the cell lipid during active cell growth.[2] Aroclor 1242 reduced chlorophyll index in a diatom.[7] In this study, Aroclor 1221 significantly inhibited carbon fixation and reduced chlorophyll levels in E. gracilis, after correction for cell population density. Our results, and the similarity in structure and properties of the DDT and PCB molecules, suggest that at the ID_{50} level, depression of photosynthesis and/or

chlorophyll production is the mechanism of population growth inhibition in E. gracilis.

The twofold increase in [^{3}H]-leucine uptake in PCB treated cultures over that of controls may be due to an alteration of the cell membrane permeability to the amino acid. PCBs have been shown to bind to membranes and subsequently to alter membrane organization at these binding sites.[14]

RNA levels in a diatom were reduced after 2 weeks exposure to Aroclor 1242.[7] In this study, [^{3}H]-uridine uptake, but not incorporation, was inhibited by the ID_{50} level of Aroclor 1221 after 48 hours. Incorporation may be depressed as well after a longer exposure period than 48 hours.

E. gracilis is more resistant to PCB toxicity than are marine phytoplankton species, yet it is difficult to compare the relative toxicity of PCBs to E. gracilis and other phytoplankton because of the varying times of exposure, initial density of cell cultures at exposure, different Aroclors and different solvents used in the various studies in the literature. These factors should be standardized for better correlation between investigations. Also, it has been observed that there is a geographic difference in phytoplankton sensitivity to PCBs.[6]

In conclusion, we have shown that PCBs inhibit cell population growth in cultures of Euglena gracilis. At 4.4 ppm, the Aroclor 1221 $ID_{50/48}$ level, reduction in cell population growth results from inhibition of photosynthesis and/or chlorophyll production.

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