UPTAKE AND EFFECTS OF KEFONE® ON GROWTH,
RESPIRATION AND PHOTOSYNTHESIS OF
CHLORELLA SOROKINIANA AND CHLOROCOCCUM HYPNOSPORUM

by

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INTRODUCTION

The effect of pesticides on water quality, pesticide pollution, has become increasingly important as a determining factor of the quality of the world's environment. The increase in human population has resulted in greater use of water for industrial, municipal, and recreational activities. Often, without adequate monitoring, pollution problems develop to quite high levels before being realized. The growth of human population has led to increased industrial productivity and the creation of new industries. The pesticide industry is one of the most recent industries to develop during the last 30 years. Many pesticides have been produced and development is continuing, under stringent regulations, with the release of several new pesticides annually. Early growth of the pesticide industry resulted in the development of a number of various pesticides which were very effective for their particular use, but little was known about the persistence and long term effect of the pesticides on the environment. Scientific investigations have proven that a few of the early pesticides were not restricted from use unless an environmental hazard was predicted or actually occurred. Kepone, along with other pesticides, exists in aquatic environments and accumulates in aquatic organisms. In order for a pesticide pollutant to be removed from the environment it must be degraded; however, degradation is a slow process. Persistent pesticides which are accumulated can be distributed over areas away from the source of deposition. This accumulation of pesticides by aquatic organisms may alter the organisms' metabolism, result in degradation of the pesticide, cause death of the organisms, or a combination of all
these. Reports have indicated resistance and survival of some organisms (i.e. algae) to particular pesticides and that these organisms accumulate pesticides to levels much higher than that in the external environment (Menzel et al. 1970, Vance and Drummond 1969, and Wurster 1968).

Algae are common inhabitants of water, soil and surfaces exposed to air and light. Algae are important in regulating water quality by modifying pH, color, turbidity and quantity of inorganic and organic compounds. Although algae may be nuisances, they can be used to improve water quality. Algal populations vary and are dependent upon the amounts and kinds of dissolved and suspended materials which serve as nutrients. In recent years, attention has been devoted to algae because they accumulate and produce toxic organic substances which affect many kinds of wild and domestic animals (Palmer 1962).

Our society is becoming increasingly dependent upon water for domestic purposes; therefore, the effects of pesticides on non-target organisms in the aquatic food chain is being considered. Upon entry into the food chain, some pesticides are passed from one organism to another resulting in concentration of a toxic substance in the food chain. Algae are more resistant to certain organic compounds such as chlorinated insecticides and consequently, upon accumulation, cause severe effects on organisms higher in the food chain (Vance and Drummond 1969). Algae are, therefore, important in removing or redistributing residues from pesticide laden aquatic environments because they are in intimate contact with water, reproduce rapidly if the nutrient supply is sufficient, form a large surface to volume ratio, and accumulate pesticides.
The objectives of this research were to evaluate the level of uptake and accumulation of Kepone by two species of green algae and to determine the effect of this chemical on growth, photosynthesis and respiration of the algae.
Kepone® is the registered trade name for decachlorooctahydro 1,3,4-metheno-2H-cyclobuta cd pentalen-2-one, common name chlordecone. It is a member of the cyclodiene family of insecticides, others of which are chlordane, heptachlor, aldrin, dieldrin, endrin, Kelevan and Pentac (Kenaga and Allison 1971). Depending upon impurities, crystalline Kepone appears white to light gray or tan. The structure of the Kepone molecule appears cage-like, much like Mirex, except for a keto-oxygen replacing two chlorine atoms as shown below. Kepone is the ketone analog and degradation product of Mirex (USEPA 1978, Andrade and Wheeler 1975, Carson et al. 1976). It is made by reacting hexachlorocyclopentadiene with sulfur trioxide, sodium hydroxide and sulfuric acid in the presence of the catalyst antimony pentachloride. The above reaction yields approximately 81 percent Kepone (USEPA 1978). Kepone is soluble in acetic acid, acetone, lower aliphatic alcohols, other organic solvents and aqueous sodium hydroxide (Merck Index 1973, USEPA 1978).
Kepone is more water soluble than Mirex due to the oxygen atom forming hydrogen bonds. In water Kepone is soluble from 1.5 to 2.0 mg/liter over a 4.0 to 7.0 pH range. Solubility increases from 5 mg to 70 mg/liter when the pH is increased from 9 to 10. Anhydrous Kepone exposed to normal temperatures and humidities forms hydrates with water. This water of hydration has been specified as being 3.5 to 6.0% of the technical grade Kepone (USEPA 1978).

The insecticide was first formulated in 1952 by Allied Chemical Corporation. It was primarily manufactured in the United States to control ants and cockroaches. Much of the Kepone produced was exported to Central America and Africa to use on insect pests on bananas and potatoes and to Europe to be converted to other products. In the United States, use was limited to ant and roach traps with maximum levels set at 0.125% (USEPA 1978). Kepone also was recommended for use as a stomach poison for grasshoppers and housefly larvae (Metcalf et al. 1962).

The James River is the largest river in Virginia flowing more than 680 km from its source in West Virginia to the Chesapeake Bay. The lower portion of the James River, 180 km from Richmond to the Chesapeake Bay, is subject to diurnal tides. The river has very high turbidity throughout most of its length, with maximum turbidity occurring between Dancing Point, 129 km, and Hog Island, 56 km, from the Chesapeake Bay. The Dancing Point and Hog Island area is the saline-fresh water interface and where the greatest sedimentation occurs. Kepone was manufactured by Allied Chemical Corporation intermittently from 1966 to 1974 at their plant in Hopewell, Virginia. In November, 1973, the
Life Science Products Company, Inc. was contracted to produce Kepone. The city of Hopewell allowed discharge of its wastewater into the city sewerage system. In October, 1974, a Virginia State Water Control Board survey revealed that Kepone was being discharged into the city of Hopewell's sewerage system due to improper digestion equipment operation. By late July, 1975, the Life Science Products Company, Inc. agreed to terminate production due to evidence of air, soil, water and building contamination by Kepone and the compounds used for its manufacture. The insecticide was found in the blood of employees and their families and caused a neurological disorder. Subsequent investigation in the James River revealed high levels of Kepone in economically important shellfish and finfish. The result of these investigations caused the Food and Drug Administration to close the James River to commercial and sport fishing in December 1975 (USEPA 1978).

Sources of Kepone contaminated discharge were in the process of drying and bagging, atmospheric releases, water discharges, wastewater spills and bad batch dumping of solid and liquid wastes on terrestrial sites. The total amount of Kepone discharges were impossible to quantify due to the lack or unavailability of daily records. Estimations of total discharge were based on the material balance of raw hexachloropentadiene supplied by Allied Chemical to Life Science Products, the theoretical yield and sales to Allied Chemical. The total production, total sales to Allied Chemical gave an estimate of more than 91,000 kg potential loss from operations by Life Science Products. Between 1966 and 1974, the total production by Allied Chemical was 763,000 kg. Losses also occurred from Allied Chemical's plant; however, these
losses were even more difficult to estimate. Environmental samples taken in May, 1967, revealed Kepone levels from 0.02 to 0.04 µg/g in sediment samples at the Dancing Point-Jamestown area. Preserved samples of oysters and fish further confirmed the analysis (USEPA 1978). In 1976, Kepone levels in the top 9 cm of bottom sediment ranged from 0.02 to 1.0 µg/g throughout the James River from Hopewell to Newport News, Virginia. Bottom sediment levels in Bailey Creek were as high as 10 µg/g (USEPA 1978). Assays and estimates conducted by the Virginia Institute of Marine Science (VIMS) in 1977 revealed the total amount of Kepone to be from 11,000 to 18,000 kg in the bottom sediment of the James River. Surface waters in 1975 were found to contain 0.3 µg/l in the James River near Hopewell and from 1 to 4 µg/l in Bailey Creek. Recent studies have found that most of the levels of Kepone in the James River water are below detectable limits of 0.01 µg/l (Huggett et al. 1978); however, samples were found to contain 0.042 µg/l (VIMS 1977).

Much concern was emphasized on the movement of Kepone in the James River. Possible pathways of movement were through volatilization, sorption-desorption, bioconcentration and physical movement of Kepone-laden suspended solids (USEPA 1978). Theoretical calculations suggested that Kepone volatilizes from 1.34 to 4.92 x 10⁻⁵ g/hr/m² of water surface. In terms of Bailey Bay which comprises 3.2 x 10⁶ m² (800 acres), volatilization losses would be in the order of 80 g/hr. Garnas et al. (1977) found that Kepone volatilization did not occur at detectable levels in static and continuous flow experiments. Studies conducted at the Battelle Memorial Institute also confirmed that Kepone
did not volatilize when dry Kepone-contaminated sediments and sediments standing in water were exposed for 12 weeks to direct sunlight. Volatilization was probably insignificant due to the strong particulate affinity of Kepone. Water was considered as a sink for Kepone; however, due to the strong sorption of Kepone onto particulate matter, concentrations of Kepone dissolved in water were found below detectable limits. Sorption-desorption and laboratory elutriate tests found that Kepone partition coefficients (water to sediment concentrations) were in the order of $10^{-4}$, indicating a small fraction of Kepone would be present in water with high particulate levels (USEPA 1978).

Kepone sorption is similar to partitioning in an immiscible two solvent system. Non-polar to slightly polar solutes partition between the two solvent systems through their comparative affinity for each solvent. A slightly polar compound like Kepone accumulates in organic material because it has a higher affinity for organic material than water (USEPA 1978). According to Huggett et al. (1977), desorption is influenced by water quality parameters such as salinity, temperature, aeration and pH. Solution pH is the only parameter known to affect Kepone desorption. Leachate content of Kepone is increased from 0.03 to 6.84 µg/l when pH is increased from 7.2 to 12.0 (USEPA 1978 and Garnas et al. 1978).

Migration of Kepone is known to be a result of runoff and leachate solubilizing Kepone from contaminated sediments. Assays of runoff and leachates are estimated as being from a few grams per day during drought periods to 64 g per day during average rainfall from Hopewell to the James River. Mathematical modeling estimates Kepone movement
seaward at a rate of 76 to 170 kg/yr past Burwell Bay, 28 km from the Chesapeake Bay. As much as 65% of this movement is due to desorption of Kepone from sediments into the dissolved state. Transport of Kepone out of the James River by anadromous fish is estimated at 10 to 100 kg/yr (Huggett et al. 1977). Movement of Kepone into the Chesapeake Bay is of great concern. However, the low rates of movement and sorption onto uncontaminated sediments are believed too small to cause adverse affects on the Bay's environment. The above low rates of movement indicate that environmental and economical impacts will continue long into the future. Furthermore, laboratory information reveals that the effects of Kepone on aquatic life will continue until levels are reduced to 0.008 µg/l in water and 0.015 µg/g in sediment and food for biota (USEPA 1978).

The food chain begins with algae at the primary trophic level and ends with the tertiary consumers such as birds and mammals. Bioconcentration and bioaccumulation are two terms which are important in describing Kepone entry into the food chain. Bioconcentration is the process by which organisms of the same trophic level concentrate a given substance directly from water and/or sediments. Bioaccumulation is the process by which organisms acquire a given substance through water, sediments or food and continually increase the concentration within the body throughout the organism's active life (Kneip and Lauer 1973). Kepone contamination of the James River is of great concern to environmentalists because it poses a threat to aquatic and terrestrial biota. The concern is based on the toxicity, bioconcentration and bioaccumulation of Kepone in estuarine organisms found in laboratory studies.
The fate and accumulation of inorganic and organic compounds, especially the organochlorines, are important because these compounds degrade slowly in nature, and cause long-lasting effects on the environment. Aquatic organisms are important in the translocation and metabolic transformation of compounds which enter and exit bodies of water. Accumulation of organochlorine compounds altering metabolic processes could cause interference in competition among organisms; therefore, possibly causing the reduction of desirable organisms and an increase in less desirable organisms (Bourquin 1977).

Concentrations of Kepone approaching those in the James River sediment inhibit growth and oxygen uptake of aerobically grown microorganisms. Nontoxic effects of Kepone on anaerobic bacteria have suggested involvement of Kepone with electron transport and respiration (Bourquin 1977). Butler (1963) demonstrated that 1 mg Kepone/l reduced carbon fixation by 95%. Growth of four marine unicellular algae, Chlorococcum, sp., Dunaliella tertiolecta, Nitzchia sp. and Thalassiosira pseudonana was inhibited by Kepone (Walsh et al. 1977). In studies conducted by Bahner et al. (1977), Chlorococcum concentrated Kepone to near 6000 times that in solution within 48 hours in static tests.

Laboratory studies have shown that Kepone is taken up by algae (Walsh et al. 1977 and Bahner et al. 1977). According to Huggett et al. (1977), the average bioconcentration levels of Kepone in James River phytoplankton were 1.3 µg/g (wet weight or dry weight was not disclosed). Algae are located at the primary trophic level and pass Kepone to other organisms in the food chain. Bahner et al. (1977)
conducted food chain studies using Kepone contaminated algae, Chlorococcum, sp. fed to eastern oysters (Crassastrea virgínica) and Kepone contaminated plankton, brine shrimp (Artemia salina) fed to mysids (Mysidopsis bahia) which were in turn fed to spot (Leiostomus xanthurus). Bioconcentration of Kepone was demonstrated by the algae-oyster food chain and the plankton-mysid-fish food chain. Oysters were found to depurate the bioconcentrated Kepone within 10 days when fed Kepone-free algae. Kepone depuration by crustaceans and fish was slow with concentrations in tissues decreasing to 50% in 28 days.

According to Lowe et al. (1971), bluecrabs (Callinectes sapidus) fed Kepone contaminated eastern oysters bioaccumulated Kepone. When fed Kepone-free oysters, the bluecrabs contained detectable concentrations of Kepone after 90 days. The above food chain studies demonstrate Kepone is transferred. However, the major route of uptake in fish is from Kepone dissolved in water while the route of uptake by bluecrabs is from food material. Furthermore, Kepone detection in various bird species such as ducks, herons, gulls, geese and eagles reveals that Kepone is having an extensive impact on the environment of eastern Virginia (USEPA 1978).
MATERIALS AND METHODS

Kepone. Growth, respiration and photosynthesis studies were conducted with technical grade Kepone (89%). Uptake and efflux evaluations were conducted with uniformly labeled $^{14}$C-Kepone (specific activity 35.71 µCi/mg).

Algae culture. Chlorella sorokiniana Shihiya and Kraus and Chlorococcum hypnosporum Starr were obtained from Dr. J. A. Swader and Dr. D. M. Orcutt, respectively, Department of Plant Pathology and Physiology, Virginia Polytechnic Institute and State University, Blacksburg, VA. The algae were grown under aseptic conditions for four days in 250 ml Erlenmyer flasks containing 100 ml of $\frac{1}{2}$ strength Bold's Basic Medium (James 1974), adjusted to a pH 7 with 1 N NaOH, hereafter referred to as nutrient solution. Each flask was aerated with humidified compressed air. Aliquots from the stock cultures were sonicated (Bransonic Model 12 ultrasonicator, Branson Instruments) for 60 seconds and centrifuged at 3000 x g for 2 minutes. The algal pellets were resuspended in sterile nutrient solution by sonication. The centrifugation and re-suspension procedures were repeated 12 times (James 1974). Washed algal cells were then dispersed with an atomizer using filtered compressed air onto the following agar (15 g/l) nutrient media: Nutrient solution with 0.1 g proteose peptone/100 ml and nutrient solution with 0.3 g yeast extract/100 ml, each containing 30 µg of Penicillin "G" (46.8 units) and 50 µg of Streptomycin sulfate/100 ml. The antibiotic stock solution was filter sterilized with a 220 nm pore size milipore filter before addition to autoclaved media.
The algae were grown for several days over Westinghouse Cool White flourescent lamps which provided a light intensity of 85 µE/m²/sec at the surface of the petri plates. Algal colonies were transferred aseptically to the two groups of media described above with antibiotics deleted. Axenic algal cells, after several days of growth, were transferred aseptically to 250 ml Erlenmyer flasks containing 100 ml of sterilized nutrient solution per flask. The algae were grown under aseptic conditions, aerated with filtered, humidified, compressed air at room temperature under a light intensity of 90 µE/m²/sec provided by flourescent lamps. After four days, the cells were harvested by centrifugation, resuspended in sterile nutrient solution, pH 8.0, and counted with an improved Neubrauer counting chamber. The cells were transferred aseptically to a 6 liter Erlenmyer flask containing 3 liters of sterile nutrient solution, pH 8.0. Initial cell concentrations were $1 \times 10^5$ Chlorella cells/ml and $4 \times 10^4$ Chlorococcum cells/ml. Algae were treated with 0.0, 0.5, 1.0, 1.5 and 2.0 mg Kepone/l and 50 ml aliquots dispensed aseptically into four 125 ml Erlenmyer flasks. Kepone stock solutions were prepared with acetone and 50 µl/l of solution was added to the treatments and the same amount acetone was added to the controls. The incubation flasks were placed on a rotary shaker set at 80 excursions/min., under flourescent lamps providing 85 µE/m²/sec at the surface of the treatment solution. The algae were incubated under aseptic conditions at 25°C and aerated with 200 cm³/min humidified, compressed air. Cell populations were measured spectrophotometrically at 520 nm at 12 hr., 24 hr., 48 hr., and 96 hr. time intervals. The absorbance of the algal cells plotted as a function of cell density was
calibrated with the counting chamber (Fig. I and II). Averages of four replications were tabulated as cells/ml for each treatment at each time interval. The data was tested for significance at the 5% level of confidence according to the Duncan's Multiple Range Test (Sokal and Rohlf 1969). The experiments were repeated with results similar to those recorded.

Effect of Kepone on respiration. Chlorella and Chlorococcum grown for four days in the growth studies were used to study the effect of Kepone on respiration. Algal cell concentrations were $1 \times 10^8$ cells/ml and $6 \times 10^6$ cells/ml for Chlorella and Chlorococcum, respectively. Respiration was measured by oxygen uptake from solution with an oxygraph (Model K-ICC, Gilson Medical Electronics) equipped with a Clark electrode and a 2 ml reaction chamber. Five replicates of the control, control plus acetone and 1.0 mg/l Kepone treatment were examined under dark conditions for five minutes at 25°C. Averages of five replications were tabulated as $\mu$mol $O_2$ consumed/mg dry wt/hr. The data was tested for significance at the 5% level of confidence according to the Duncan's Multiple Range Test (Sokal and Rohlf 1969). The experiments were repeated with results similar to those reported.

Effect of Kepone on Photosynthesis. Photosynthesis was measured with aliquots of Chlorella and Chlorococcum from the growth study. The cells were centrifuged and diluted with fresh nutrient solution to obtain $1 \times 10^7$ cells/ml and $6 \times 10^6$ cells/ml for Chlorella and Chlorococcum, respectively. The 2 ml reaction vessel was illuminated with two 300W, 120v Ken-Rad reflector lamps placed on each side of the reaction cell,
Fig. 1. Absorbance of *Chlorella* at 520nm as a function of cell concentration.

Fig. 2. Absorbance of *Chlorococcum* at 520nm as a function of cell concentration.
one lamp per side. Each lamp provided 2000 µE/m²/sec at the surface of the reaction vessel. Tap water flowing through a Pryex container between each lamp and the reaction cell served as a heat filter. Photosynthesis was measured by oxygen evolution with the oxygraph. Averages of five replications were tabulated as µmol O₂ evolved/mg chlorophyll/hr. Amount of chlorophyll was determined according to the method described by Arnon (1949). The data was tested for significance at the 5% level of confidence according to the Duncan's Multiple Range Test (Sokal and Rohlf 1969). The experiments were repeated with results similar to those reported.

Removal of Kepone from solution. Chlorella and Chlorococcum were grown in nutrient solution for four days, after which the cells were harvested by centrifugation and resuspended in fresh nutrient solution. The algae suspension was then transferred to a 6 liter Erlenmyer flask containing 3 liters of sterile nutrient solution, aerated with 500 cm³/minute of cotton filtered, humidified air. After 10 days in a growth chamber at 25°C and 85 µE/m²/sec of light, the algae were counted and volume determined to obtain 8 x 10⁹ cells of Chlorella and 8 x 10⁸ cells of Chlorococcum. After centrifugation, each of the following alga concentrations, 1 x 10⁷, 5 x 10⁶ and 1 x 10⁶ cells/ml for Chlorella and 1 x 10⁶, 5 x 10⁵, and 1 x 10⁵ cells/ml for Chlorococcum, were suspended in 500 ml of radiolabeled nutrient solution, pH 7.0. Chlorella and Chlorococcum were exposed to ¹⁴C-Kepone concentrations of 60.9 µg/l (2.17 µCi/ml) and 59.2 µg/l (2.12 µCi/ml), respectively. Each cell concentration was replicated five times in 125 ml Erlenmyer flasks.
containing 100 ml of algae suspension. The cells were incubated at 25°C at a light intensity of 15 µE/m²/sec on a reciprocal shaker set at 80 excursions per minute. The incubation period was for 48 hours. A complete set of flasks without algae were included to serve as a control. At each sampling interval, the cells were shaken by hand and 4 ml samples were centrifuged at 3000 x g for two minutes. At the termination of study, a portion of the algal cells were counted and percent increase in cell number was calculated. Aliquots of 0.5 ml of the supernatant were added to 3 ml of Aquasol (New England Nuclear) and radioactivity measured with a liquid scintillation counter (Beckman Model LS-250). All samples were corrected for quenching. The data was expressed as disintegrations per minute (DPM)/ml remaining in solution for each cell concentration at each sampling interval and tested for significance at the 5% level of confidence according to the Duncan's Multiple Range Test (Sokal and Rohlf 1969).

Efflux of Kepone from Chlorella and Chlorococcum. Algal cells were obtained after the 48 hour incubation period of the uptake study and utilized to measure efflux of ¹⁴C-Kepone. Four milliliters of incubation medium for each of five replicates of each alga cell concentration were centrifuged at 3000 x g for 2 minutes. The algal cells were resuspended in fresh sterile medium without labeled material. After three washings the cells were suspended in 2 ml of nutrient solution, allowed to incubate for 20 minutes under the same conditions as described for the uptake study, centrifuged and radioactivity of 0.5 ml of supernatant was measured by liquid scintillation counting. All
samples were corrected for quenching. The data was expressed as DPM/ml for each alga cell concentration and was tested for significance at the 5% level of confidence according to the Duncan's Multiple Range Test (Sokal and Rohlf 1969).
RESULTS AND DISCUSSION

Influence of Kepone on growth. The growth of Chlorella and Chlorococcum was inhibited by 1.5 and 2.0 mg/l of Kepone after 12 hr. of incubation (Tables I and II). All treatments of Kepone after 48 hr. inhibited the growth of Chlorococcum, whereas, Chlorella was inhibited by Kepone concentrations greater than 0.5 mg/l. No effect on growth was evident on both algal species by Kepone concentrations up to 1.5 mg/l 96 hr. after treatment, indicating recovery from the effect of Kepone. Chlorococcum appeared to be more sensitive to Kepone than Chlorella, but the initial cell concentration of Chlorella was 2.5 times higher than Chlorococcum.

These results show that Kepone inhibited algal growth, but not completely. The concentrations of Kepone and algal cells used in these experiments were greater than those in the James River (USEPA 1978).

Lower concentrations of various pesticides, than those used in this study were toxic to algae (de la Cruz et al. 1973, Vance and Drummond 1963, Wurster, 1968, Menzel et al. 1970 and Butler 1963), however, Hollister et al. (1975) found that Mirex, a compound similar to Kepone, did not affect growth when various species of algae were exposed to 0.2 µg/l. Populations of algae may, therefore, be affected depending upon the concentrations of Kepone to which they are exposed in the natural environment.

Effect of Kepone on respiration. Respiration of Chlorella and Chlorococcum was reduced by 47 and 34%, respectively when exposed to 1 mg/l of Kepone for 96 hr. (Table III). These results support observations
TABLE I

Effect of Kepone on Growth of Chlorella

<table>
<thead>
<tr>
<th>Kepone (mg/l)</th>
<th>Cells x 10^-5/ml^a/</th>
<th>Hours of incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>0.0</td>
<td>2.02 a</td>
<td>5.75 a</td>
</tr>
<tr>
<td>0.0 + Acetone^b/</td>
<td>1.89 a</td>
<td>5.35 ab</td>
</tr>
<tr>
<td>0.5</td>
<td>1.89 a</td>
<td>5.75 a</td>
</tr>
<tr>
<td>1.0</td>
<td>2.02 a</td>
<td>4.24 ab</td>
</tr>
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<td>1.5</td>
<td>1.89 a</td>
<td>3.84 bc</td>
</tr>
<tr>
<td>2.0</td>
<td>1.89 a</td>
<td>2.65 c</td>
</tr>
</tbody>
</table>

^a/ Values in each column are the means of four replicates and those followed by the same letter within each column are not significantly different at the 5\% level of confidence according to the Duncan's Multiple Range Test.

^b/ Stock solutions of Kepone were prepared with Acetone and the same volume of Acetone, 0.5 µl/ml, was added as in the Kepone treatments.
### TABLE II

Effect of Kepone on growth of Chlorococcum

<table>
<thead>
<tr>
<th>Kepone (mg/l)</th>
<th>Cells x 10^{-5}/ml(^a/^)</th>
<th>Hours of incubation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12</td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>0.0</td>
<td>0.625 a</td>
<td>1.016 a</td>
<td>2.600 a</td>
</tr>
<tr>
<td>0.0 + Acetone(^b/^)</td>
<td>0.673 a</td>
<td>0.858 b</td>
<td>2.520 a</td>
</tr>
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<td>0.5</td>
<td>0.600 a</td>
<td>0.800 b</td>
<td>1.830 b</td>
</tr>
<tr>
<td>1.0</td>
<td>0.653 a</td>
<td>0.754 b</td>
<td>1.620 b</td>
</tr>
<tr>
<td>1.5</td>
<td>0.600 a</td>
<td>0.600 c</td>
<td>1.070 c</td>
</tr>
<tr>
<td>2.0</td>
<td>0.600 a</td>
<td>0.600 c</td>
<td>0.650 c</td>
</tr>
</tbody>
</table>

\(^a/^) Values in each column are the means of four replicates and those followed by the same letter within each column are not significantly different at the 5% level of confidence according to the Duncan's Multiple Range Test.

\(^b/^) Stock solutions of Kepone were prepared with Acetone and the same volume of Acetone, 0.5 μl/ml, was added as in the Kepone treatments.
TABLE III
Effect of Kepone on Respiration

<table>
<thead>
<tr>
<th>Kepone (mg/l)</th>
<th>Chlorella</th>
<th>Chlorococcum</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>3.16 a</td>
<td>9.92 a</td>
</tr>
<tr>
<td>0.0 + Acetone b</td>
<td>3.58 a</td>
<td>9.22 a</td>
</tr>
<tr>
<td>1.0</td>
<td>1.89 b</td>
<td>5.83 b</td>
</tr>
</tbody>
</table>

a/ Values in each column are the means of five replicates and those followed by the same letter within each column are not significantly different at the 5% level of confidence according to the Duncan's Multiple Range Test.

b/ Stock solutions of Kepone were prepared with Acetone and the same volume of Acetone, 0.5 \( \mu l/ml \), was added as in the Kepone treatments.
of de la Cruz et al. (1973) that 1 ppm of Mirex inhibited respiration of Chlamydomonas.

Mitochondria have many important roles in plant and animal cells, such as a source of energy for metabolic functions. Acute, or chronic influences of various substances may cause interference with cellular functions (Pardini et al. 1971). Mitochondrial membranes, as well as other cellular membranes, have a high content of phospholipids. Since organochlorine pesticides are lipid soluble, it is expected that cell membranes would contain high concentrations of the pesticides which might interfere with mitochondrial function (Pardini et al. 1971). Insecticides which inhibit respiration are heptachlor, chlordane, toxaphene, rotenene, Mirex and Kepone (Pardini et al. 1971, Byard et al. 1975, Anderson et al. 1977, Anderson et al. 1978, Desaiah et al. 1975 and Desaiah et al. 1977).


**Effect of Kepone on photosynthesis.** Kepone at 1.0 mg/l reduced rates of photosynthesis of *Chlorella* and *Chlorococcum* 17 and 10%, respectively, after 96 hr. of incubation (Table IV). Effects of chlorinated hydrocarbons on photosynthesis of algae have been documented; however, the mechanism is not understood (Menzel et al. 1970 and de la Cruz et al. 1973). Some reports suggest that chlorinated hydrocarbons inhibit
<table>
<thead>
<tr>
<th>Kepone (mg/l)</th>
<th>µmol O₂ Evolved (µmol chlorophyll⁻¹/hr⁻¹)</th>
<th>Chlorella</th>
<th>Chlorococccum</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>66.0 b</td>
<td>76.4 a</td>
<td>33.8 ab</td>
</tr>
<tr>
<td>0.0 + Acetone</td>
<td>63.4 b</td>
<td>34.1 a</td>
<td>30.8 b</td>
</tr>
</tbody>
</table>

a/ Values in each column are the means of five replicates and those followed by the same letter within each column are not significantly different at the 5% level of confidence according to the Duncan's Multiple Range Test.

b/ Stock solutions of Kepone were prepared with 0.5 µl/ml, was added as in the Kepone treatments.
the Hill reaction (Butler 1963, Wurster 1968 and Södergren 1968).

Chlorella at a concentration of $2.54 \times 10^7$ cells/ml exposed to 1 mg Kepone/l in these experiments gave a ratio of $4 \times 10^{-5}$ µg Kepone/cell. While no actual information was found before completion of this study on populations of algae throughout the James River, information was available on cell populations in the Hampton Roads area and chlorophyll a concentrations in the James River. Marshall (1967) found that populations of algae in the Hampton Roads area were approximately 200 cells/ml during the summer months. Chlorophyll a content in the James River ranged from less than 10 µg/l near Hampton Roads to more than 40 µg/l near Hopewell, Virginia (USEPA 1978). Assuming cell concentration as a direct function of chlorophyll content, cell populations ranged from 200 to 800 cells/ml. Sampling of the James River water revealed Kepone concentrations from as high as 0.042 µg/l to less than 0.006 µg/l (USEPA 1978). Calculations of the above information revealed the amount of Kepone/cell ranged from $2.1 \times 10^{-4}$ µg to $7.5 \times 10^{-6}$ µg Kepone/cell in the James River. The $4 \times 10^{-5}$ µg Kepone/cell is within the range found under natural conditions and it appears Kepone may affect photosynthesis of algae in the James River.

Amount of $^{14}$C-Kepone removed from solution. Chlorella, at concentrations of $1 \times 10^6$, $5 \times 10^6$ and $1 \times 10^7$ cells/ml removed 25, 40 and 57% of Kepone from solution, respectively, after 30 min. of incubation (Fig. 3), indicating the removal of Kepone was dependent upon cell concentration. Chlorella cells showed a significant efflux of Kepone during longer incubations. The rates of efflux peaked at 1 and 3 hr. incubations at the lower cell concentrations but continued during the
Fig. 3- Amount of $^{14}$C-Kepone remaining in solution after uptake by Chlorella over a 48 hr. sampling period.
a/All bars with the same letter are not significantly different at the 5% level of confidence according to the Duncan's Multiple Range Test.
entire 48 hr. period at the highest cell concentration. The lower cell concentrations of *Chlorella* removed Kepone from solution at a steady rate after 3 hr. of incubation until the experiments were terminated, which may have been the result of higher cell populations (Fig. 5). The amount of Kepone removed in 48 hr. by each of the cell concentrations, $1 \times 10^6$, $5 \times 10^6$ and $1 \times 10^7$ cells/ml was 16.6, 17.3 and 9.9%, respectively.

*Chlorococcum* cells did not demonstrate the efflux of Kepone observed with *Chlorella* except at the $1 \times 10^6$ cells/ml (Fig. 4). It appears that an equilibrium of Kepone in solution and with the algal cells was reached rapidly. A trend in an increased removal of Kepone was evident in the later sampling interval, however, this may have been due to increased cell number (Fig. 6). *Chlorococcum* at $1 \times 10^5$, $5 \times 10^5$ and $1 \times 10^6$ cells/ml removed 7.8, 40.4 and 56.8%, respectively, of the Kepone from solution.

The reason for Kepone efflux was not completely resolved, however, equilibriums between adsorption/desorption and absorption are probably involved. Many organochlorine insecticides have adsorptive properties (Acree 1963) and Kepone has a high affinity for organic material (USEPA 1978). Adsorbed Kepone may be desorbed when the pH of the medium becomes more alkaline (USEPA 1978). It is not known if the pH of the medium changed during the incubations; however, algal cells increase the pH of the medium during photosynthesis (Swader 1978).

Absorption may occur actively or passively and is a physical and complex physiological process in which photosynthesis and respiration play an important role (Hill et al. 1967). Lipid soluble materials
Fig. 4. Amount of $^{14}$C-Kepone remaining in solution after uptake by Chlorococcum over a 48 hr. sampling period.

a/All bars with the same letter are not significantly different at the 5% level of confidence according to the Duncan's Multiple Range test.
Chlorella
Cells/ml
A. $1 \times 10^6$
B. $5 \times 10^6$
C. $1 \times 10^7$

Fig. 5. Percent increase of Chlorella cell number during uptake study over 48 hr.

Chlorococcum
Cells/ml
A. $1 \times 10^5$
B. $5 \times 10^5$
C. $1 \times 10^6$

Fig. 6. Percent increase of Chlorococcum cell number during uptake study over 48 hr.
move through lipid containing cell walls and membranes. This penetration is due to lipid and water solubility of the substance with the lipid solubility determining the extent of penetration. A substance which has a higher lipid than water solubility will move from the water phase to the lipid phase (Södergren 1968). The solubility of Kepone in water is approximately 1.5 to 2.0 mg/liter at pH 7. Its solubility in lipids is quite high, but the actual quantity is not known (USEPA 1978). Algae are approximately 20% or less lipid on a dry weight basis (Orcutt 1978), therefore, the rapid initial uptake plus the high lipid solubility of Kepone suggests absorption was the primary means of Kepone uptake. Kepone inhibits photosynthesis, respiration and various enzymes and an alteration of the metabolism of Chlorella and Chlorococcum by Kepone may have caused the efflux of Kepone.

Efflux of Kepone. The data (Fig. 7 and Fig. 8) illustrate a portion of the activity associated with algal cells was not bound and was released into solution, indicating that Kepone was both adsorbed and absorbed by algal cells. If absorption was the only mechanism of uptake, the data would have indicated more Kepone returning to solution. Adsorption was probably the primary mechanism of Kepone uptake, however, absorption did occur. Algae can accumulate Kepone to toxic levels from non-toxic concentrations (Walsh 1977); however, the data reported here indicate that desorption occurred. Movement of Kepone contaminated algae by flowing water may release Kepone in uncontaminated areas allowing Kepone to become available to clean sediments and accumulation by aquatic organisms.
Fig. 7. Efflux of $^{14}$C-Kepone after 48 hr. from Chlorera cells. Cell concentrations, A, B, C, refer to final cell counts, A($6 \times 10^5$), B($2.85 \times 10^7$), and C($5.69 \times 10^7$) centrifuged from 4 ml of treatment solution and placed in 2 ml of nutrient solution without labeled material.

$^a$/Bars with the same letter are not significantly different at the 5% level of confidence.
Fig. 8. Efflux of $^{14}$C-Kepone after 48 hr. from Chlorococcum cells. Cell concentrations, A, B, C, refer to final cell counts, A($9.9 \times 10^5$), B($4.0 \times 10^6$), and C($6.8 \times 10^6$) centrifuged from 4 ml of treatment solution and placed in 2 ml of nutrient solution without labeled material.

$\text{a/Bars with the same letter are not significantly different at the 5% level of confidence.}$
SUMMARY

Kepone affected the growth of Chlorella and Chlorococcum in studies with concentrations of Kepone approaching the solubility limit in water. Growth of Chlorella was more tolerant than the growth of Chlorococcum to 2 mg/l of Kepone. Effects on growth were significant after 24 hours of incubation.

Photosynthesis and respiration were inhibited in both algae by 1 mg Kepone/l. Photosynthesis of Chlorella and Chlorococcum was inhibited by 17 and 10%, respectively. Respiration of Chlorella and Chlorococcum was inhibited by 47 and 34%, respectively.

The initial uptake of Kepone was proportional to the cell concentrations of both algae. Kepone was released back into the medium by algal cells after the initial uptake, which may have been the result of Kepone inhibiting the algal metabolism. Uptake of Kepone was proportional to Chlorococcum cell concentration at the end of the 48 hours of study, but not for Chlorella cells. Both algae at all cellular concentrations showed an efflux of Kepone when washed, suspended in untreated nutrient solution and incubated for 20 minutes.
LITERATURE CITED


Huggett, R.J. 1977. The role of sediments in the storage, movement, and biological uptake of Kepone in estuarine environments. In: Appendix C to EPA Mitigation Project Report, USEPA, Environmental Research Laboratory, Gulf Breeze, FL.


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UPTAKE AND EFFECTS OF KEPONE on growth, respiration, and photosynthesis of 
CHLORELLA SOROKINIANA AND CHLOROCOCCUM HYPNOSPORUM 

by 
Gary R. Young 

(ABSTRACT) 

Kepone®, the registered trade name for decachlorooctahydro-1,3,4-metheno-2H-cyclobuta-c pentalen-2-one, inhibited growth and reduced rates of photosynthesis and respiration of Chlorella sorokiniana Shihira and Kraus and Chlorococcum hypnosporum Starr. The insecticide reduced rates of respiration more than photosynthesis. Uptake of ¹⁴C-Kepone by the algae was proportional to cellular concentration. A net efflux of Kepone was exhibited by Chlorella cells after 30 minutes of incubation, whereas, equilibrium occurred within 15 minutes of incubation for Chlorococcum. Desorption of Kepone was evident when both algal species were removed from Kepone treated solutions and incubated in untreated nutrient solutions.