Sublethal effects of DDT on some marine phytoplankton species

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Intern verslag

over

werkzaamheden verricht

in het tijdvak oktober 1970 - augustus 1971

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het NIOZ te Texel

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Contents

I. Abstract

The effect of very low concentrations of DDT on the metabolism of three marine phytoplankton species in batch cultures has been studied. Each species had a different response to the exposure. The carbon content was higher under DDT exposure in Dunaliella tertiolecta and Skeletonema costatum and did not change in Chlamydomonas sp. The nitrogen metabolism was affected in Chlamydomonas sp. and Skeletonema costatum: the excretion of dissolved organic nitrogen was higher than the controls in both species; assimilation of ammonia was stimulated in Chlamydomonas sp. and the nitrogen content was lower than the controls in Skeletonema costatum. Size and shape of the cells were not affected. Oxygen uptake was stimulated in Chlamydomonas; this is probably a side effect of the increase in cell division rate and the increased ammonia uptake. Dry weight biomass was lower in Chlamydomonas

sp. despite the fact that the number of cells was higher. Chlorophyll a and pigment diversity index (D430/D665) were not affected by DDT in the three species.

These findings suggest that the effect of DDT contamination in marine phytoplankton is unpredictable. Changes in the structure of phytoplankton communities may occur because of the different kind of response to DDT exposure in different species.

II. Introduction

DDT (p, p'-dichlorodiphenyltrichloroethane or 2, 2-bis (p-chlorophenyl)-1, 1, 1-trichloroethane) has a small water solubility. The best estimate appears to be 1.2 ppb at 25°C. Its large oil-water partition coefficient is the cause of its accumulation in biological systems (O'BRIEN, 1967).

DDT has long been used to control insect pests. Nevertheless, after decades of use and much research the mechanisms of the action of this insectide are far from understood. Its wide-spread use and its resistance to degradation are some of the causes of the present world-wide contamination with this synthetic chemical.

The contamination of the environment with chlorinated insecticides has caused much concern recently because the effects on nontarget organisms may cause a serious and unforeseen influence on the biosphere. In most of the pesticide literature the response of target organisms is documented, and only in part of it the effects on non-target animals are studied. Recent findings of "biological magnification" or pesticide concentration via food chains that start with algae (WOODWELL, 1967) and, moreover, the demonstration of the effects of DDT on photosynthesis (LAWLER & ROGERS, 1969), have stimulated

interest in the study of the possible consequence of the contamination of the marine environment with DDT for the primary productivity of marine phytoplankton.

SÖDERGREN (1968) showed that C^{14} -DDT is accumulated rapidly in the fresh water algae Chlorella sp. by passive absorption. COX (1970) found accumulation of DDT in cultures of three species of marine phytoplankton in terms of a partition coefficient. WURSTER (1968) showed (by the method of C^{14} uptake in cell material) that DDT reduces the photosynthesis of marine phytoplankton. MENZEL et al (1970) found that marine phytoplankton has different sensitivities to DDT inhibition of photosynthesis. Also they studied the effects of 100 ppb daily additions of DDT on cell division rate of different species and again obtained a variety of sensitivities. Like WURSTER (1968), they did the evaluation of photosynthesis by the method of C^{14} uptake in cell material without taking into consideration the extracellular products of photosynthesis.

Changes in the rate of release and quality of extracellular products caused by exposure to DDT may be expected. FOGG (1958) found that a portion of the carbon fixed into organic material by the photosynthesis of normal phytoplankton cells is released into the external environment. WATT (1966) found that productivity estimation methods, that measure carbon fixation into particulate matter only, may underestimate primary production by up to 30%. The importance of these extracellular products is at the present time a subject of concern in general marine ecology, especially in plankton ecology (FOGG, 1962).

It can safely be stated that the knowledge of the effects of DDT on marine phytoplankton is very poor. Without basic studies of these

effects it is very difficult to argue about authentic toxic or nontoxic levels. Moreover if we want to relate the experimental findings with natural communities, the subtle biochemical effects of DDT may be very important. This study was undertaken in order to find a general background of the effects of DDT in cultures of marine phytoplankton species.

The effect of DDT on the metabolism of three species was studied. A concentration of 0.9 ppb in water was chosen because this value lies below the water solubility of DDT and because it is probably representative of natural conditions of contamination. In order to include several metabolic pathways, the following parameters were measured: respiration, number of cells, dry weight, carbon content, nitrogen content, assimilation of ammonia, release of extracellular organic products, chlorophyll contents and pigment diversity index.

III. Material and methods

Inocula for monospecific cultures of two marine phytoflagellates, Chlamydomonas sp. and Dunaliella tertiolecta, and a marine centric diatom, Skeletonema costatum (hereafter referred to as Chlamydomonas, Dunaliella and Skeletonema) were obtained from the TNO Institute culture collection in Den Helder, Holland.

The algae were grown autotrophically in "Erd-Schreiber" culture solution sterilised by double membrane filtration, in 200 ml culture bottles, at 18 $\pm$ 0.5 ^{o}C. The cultures were illuminated with 12 hour periods of 3200-3500 lux provided by warm white tubes. Special care was taken to avoid the influence of external light conditions during all phases of the experiments. All cultures were agitated by hand for 2-3 minutes once a day. The sea water used in the culture solution was

obtained from the light-vessel "Texel" (53°01'30" N; 04°22'00" E).

The glass material used for the cultures was cleaned with laboratory powder soap, washed with tap water, placed in sulphuric acid for 5 hours and in acetone overnight, rinsed with distilled water and autoclaved for 30 minutes. To cover the mouth of the culture tubes, precleaned glass marbles were used in order to avoid the influence of rubber or cotton on the experiments.

The cultures were started in the following manner: 5 ml of the stock culture re (1×10^6 cells/ml) was added to 5 culture bottles with 25 ml of fresh culture medium (culture solution prepared the same day and filtered twice through a membrane filter before used); after 48-72 hours 25 ml more of fresh culture mediun were added to each bottle and 24-48 hours later another 50 mls were added. The content of the bottles was collected and mixed in a flask and then used as inoculum; 25 ml of this suspension was added to 5 culture bottles with the same amount of fresh culture medium. After 48 hours the cultures were mixed again and considered to be stock culture. This procedure was followed until the fastest growth was reached and the same number of cells in the five bottles and the smallest amounts of detritus were obtained. Then the stock culture was considered ready for the experiments.

Experiments were run as follows. Unless otherwise specified, 25 culture bottles with 25 ml of fresh culture medium each were inoculated with 25 ml of stock culture obtained as above. 48 hours later 50 ml of fresh culture medium was added, plus 40 µl of acetone to 12 culture bottles (control group) or 40 µl of DDT acetone solution to 12 culture bottles (experimental group) to give a concentration of .94 ppb of p, p' DDT in the experimental group. After 24 hours (during the next light period) all the culture bottles of the set were shaken by hand

and used to do the analyses.

Suspended carbon

The total contents of three bottles from the experimental group and 3 bottles from the control group were filtered through dried and weighed GF/C Whatman filters. The filters were dried at 50-60 °C during 24 hours and kept in a dessicator for 24 hours more, after which their weight was recorded.

All glassware was washed with distilled water between each filtration. Rinsing of the filters with distilled water was not done because rinsing with as little as 5 ml of distilled water resulted in a loss of 30-40% of cellular material.

The amount of carbon was measured with a Coleman carbon analyser, 800-850 °C combustion temperature.

Suspended nitrogen, ammonia and dissolved organic nitrogen

Three cultures from the experimental group and three from the control group were filtered and weighed as above. The filtrates were utilized immediately to do the analyses of ammonia and dissolved organic nitrogen, or frozen until analyzed. The filters were used to do the analysis of suspended nitrogen in a nitrogen analyser set up to 800-850 °C combustion temperature.

Ammonia

A solution of 5 ml of the filtered medium plus 5 ml of distilled water free of ammonia were employed to develop the blue color of indophenol formed by phenol and hypochlorite as reported by Koroleff in the ICES Interlaboratory Report No. 3 (1970).

Dissolved organic nitrogen

10 ml of filtered medium was collected in a Kjeldahl flask with 2 ml of sulphuric acid 50% v.v. The flasks were heated slowly until all the water was evaporated, then the temperature was increased; 4-5 minutes after white fumes started to form the flasks were taken from the units of the electric digester during 30 seconds. Next, some small drops of hydrogen peroxide (30%) were added (MARTIN, 1968). The flasks were replaced on the digester for 2-3 minutes and then two other additions of hydrogen peroxide were made following the same procedure. At the end of the last addition the digestion was continued for 8-10 minutes. The flasks were cooled at room temperature and 20 ml of distilled water free of ammonia were added to dissolve the precipitate. The pH was adjusted to 4.0 - 4.5 with sodium hydroxide solution and diluted to 100 ml; 10 ml of these solutions were used to make the determination of dissolved organic nitrogen as ammonia.

The recovery of organic nitrogen with this method was tested with alanine to be linear and constant; good sensitivity was obtained when blanks were run. Distilled water free of ammonia was used to prepare the reagents and to wash the glassware.

Some bacteria were observed in the cultures and in the filtrates. Their influence on the estimation of dissolved organic nitrogen was observed to be negligible. The influence of these bacteria on the dry weight was considered to be constant.

Number of cells, respiration, chlorophyll and pigment index

Samples of 5 ml of three culture bottles of the experimental group and three of the control group were diluted in 45 ml of fresh culture medium and preserved with lugol's solution. Subsamples of 5 ml

of these solutions were used to count cells with an inverted microscope (UTERMÖHL, 1958).

20 ml samples of the same culture were used to measure the respiration rate by the manometric method of oxygen consumption (UMBREIT, et al., 1964). For this method a Braun Warburg apparatus model VL85 was used. Warburg flasks were wrapped in aluminium foil and stored for about 10 hours before analysis. The center well of the Warburg flasks was not used since a CO_2 buffer was not necessary. The measurements were made during the "night" (12 hours), without any concentration procedure, and under constant agitation. The filtrates from the cultures were used as a blank and as a thermobarometer.

Samples of 50 ml of the same culture bottles were filtered onto glass fiber filter GF/C (Whatman) for the estimation of chlorophyll content and pigment index (MARGALEF, 1963). Determination of chlorophylls a, b, and c was done following the method described in the report of SCOR/UNESCO working group 17 (1966). Homogeneization was performed with an ultrasonic desintergrator and the calculations were done with the trichromatic equations.

Dry weight biomass

The records of the increase of weight of all the filters in both groups were used to obtain the value of dry weight biomass. Two kinds of values were obtained: those with 50% of the suspension and those with 100% of the suspension. The differences between these two kinds of values were caused by the 50% of the suspension only. The double of these differences was used as estimation of the dry weight biomass of the all suspension.

IV. Results

As is shown in Table I the three species had different responses to DDT exposure

Dunaliella, as the table shows, was not inhibited in any respect. The insensivity of Dunaliella agrees with the results of MENZEL et al (1970) who found complete insensitivity of photosynthesis (measured by the method of C^{14} uptake (STEEMANN & NIELSEN, 1952) and of cell division rate to exposures up to 1000 ppb of DDT.

The nitrogen metabolism was affected in different ways in two species. The assimilation of ammonia in Chlamydomonas was increased by 25%. The amount of dissolved organic nitrogen released into the medium increased about 40% in Chlamydomonas and 50% in Skeletonema. The nitrogen content of the suspension was almost unaffected in Chlamydomonas. However, it is interesting to note that the amount of nitrogen per cell was lower because the number of cells was higher than the controls. Skeletonema experienced a strong effect on its cell nitrogen content caused by DDT; its cell nitrogen was 40% less than the controls. Apparently this amount was released to the medium in the form of dissolved organic nitrogen products. In contrast, in Dunaliella, DDT had no influence on the nitrogen metabolism.

Oxygen consumption was stimulated in Chlamydomonas (157%): .05 ml of O_2 more were used by the cultures under exposure of DDT. Dunaliella and Skeletonema did not show any alteration in oxygen consumption. However, this does not mean that DDT has no influence on the respiration of these algae. Some reports in the literature from (O'BRIEN, 1967) show that the increase of oxygen uptake caused by DDT has a peak during the early hours of the exposure and after a while it returns to normal. The measurements of oxygen consumption reported

here were obtained 24 hours after the general experiments were finished. Therefore the nontoxicity of DDT in the respiration of these algae cannot be evaluated with this kind of information.

The growth of the cultures was stimulated in Chlamydomonas and partially inhibited in Skeletonema. In the latter the number of cells before the exposure was recorded (background in the table). The increase in cell number during the time of exposure was inhibited by 50% compared with the controls.

The dry weight of the biomass was diminished in Chlamydomonas by the effect of DDT. Skeletonema and Dunaliella did not show any effects.

The cell carbon content was affected clearly in Skeletonema; a stimulatory effect of 20% of carbon content by DDT was obtained. Dunaliella did not show a clear effect when carbon content is expressed (as in the table) and compared as total carbon content. However, when it is examined as increment of carbon from the initial content, or carbon uptake during the time of the experiments, an increase of 50% of carbon uptake on the cultures exposed to DDT compared with the controls is clear.

The chlorophyll content of the three species was not affected by DDT. The main pigment, chlorophyll a, was the same in cultures with and without DDT. Chlorophyll b and c concentrations were lower in the cultures of Chlamydomonas and Skeletonema under exposure of DDT. However, chlorophyllic pigments not existing in two of the algae of both groups (control and experimental) were obtained. This suggests that the method is not correct. Therefore, the results for chlorophyll b and c were taken out of consideration.

The pigment diversity index d430/d665 was not affected by DDT, which indicates that the proportion between yellow and green pigments

is the same under exposure of DDT.

V. Discussion

There are several reports in the literature about an increase of oxygen consumption by insects under exposure to DDT. One interpretation is that the oxidative phosphorilation is uncoupled and therefore more oxidation is necessary to produce the ATP required (O'BRIEN, 1967). Inhibition of oxidative phosphorilation has been reported for rat liver mitochondria (op. cit.). Inhibition of photophosphorilation was reported for susceptible barley by LAWLER & ROGERS (1968) at DDT concentrations of 200 µg of DDT/mg of chlorophyll.

In the present experiments an increase of oxygen consumption was observed only in Chlamydomonas. This fact may be considered as an indirect consequence of DDT effect on the metabolism of nitrogen and cell division rate. The cell nitrogen content was almost not affected despite the fact that an increase of dissolved organic nitrogen products in culture filtrates was recorded. Apparently the loss of organic nitrogen of the cells was counterbalanced by an increase in the rate of assimilation of ammonia. This process is well known to be related with the respiratory mechanism in algae (FOGG, 1953; SYRETT, 1962) as an energy consumption mechanism that can be supplied from high energy phosphate groups.

The nitrogen metabolism, especially of amino acids, is affected by DDT in insects (O'BRIEN, 1967), fishes (MEHRLE et al, 1971) and man (TOCCI et al, 1969). In his review O'BRIEN (1967) concluded that the decrease and changes in blood amino acids is not a simple reflection of a "demand of oxidable carbon" but perhaps a reflection of an elevated ability to excrete a variety of compounds including

toxicants, therefore a depression of the formate pool and its replenishment at the expense of proline and glutamine.

ALLEN (1956) reported the excretion of organic compounds by Chlamydomonas; she found almost no organic nitrogen in the culture filtrates. HELLEBUST (1965) found that considerable quantities of amino acids and peptides were excretory products of many of the algae; he generalized that healthy, rapidly growing marine phytoplankton excrete little or no protein (1967). In the present paper an increase of dissolved organic nitrogen in culture filtrates of Chlamydomonas and Skeletonema under exposure of DDT is reported. Apparently these products were released by the cells under exposure to DDT. Notice that the cell carbon content was higher rather than lower under DDT exposure. It is not probable that the cells lost protein by excretion, because they did not show any damage or changes in shape, which may be considered as an indication of apparent " healthy conditions" under which the loss of protein by excretion is negligible or absent. Excretion of amino acids could be increased or started following the explanation of O'BRIEN (1970) as a deintoxication mechanism. The results of this excretion could end, either, with a depression of the cell nitrogen content, as was the case in Skeletonema, or with the replenishment of the nitrogen pool through glutamine formation from an elevated ammonia assimilation (FOGG 1953; Fowden, 1962), as was the case in Chlamydomonas.

The increase of cell carbon in Dunaliella and Skeletonema after exposure to DDT suggests that the carbon uptake of these algae was stimulated. One possible explanation of this may be that the photosynthesis was stimulated. MENZEL et al (1970) and WURSTER (1968) found inhibitory effects of DDT on the C^{14} assimilation by photosynthesis

of marine phytoplankton, but some of their results suggest a stimulatory effect at the lowest concentrations that were used in the present experiment. However, another possibility to explain the increase of cell carbon may be by a nonphotosynthetic mechanism, as heterotrophic assimilation of organic carbon. SIKKA & PRAMER (1968) found that an inhibition of 50% of the growth of algal cultures caused by fluometuron (a herbicide recommended for weed control) was reversed by addition of an organic material in solution, as an exogenous source of energy. This second explanation may be more realistic.

The growth of the cultures of algae tested showed a wide range of responses: from 50% inhibition in Skeletonema, insensitivity in Dunaliella, to stimulation in Chlamydomonas. MENZEL et al (1970) found inhibition of cell division in Skeletonema under 100 ppb exposure to DDT. The cell division of Skeletonema is apparently very sensitive to DDT contamination in concentrations below the solubility of the pesticide. The stimulatory effect in Chlamydomonas found in the present experiments and the findings of MOORE (1970) of stimulatory effects of other pesticides at lower concentrations on the growth of Euglena gracilis suggest the possibility that pesticides in low concentrations stimulate growth in several species. However, additional information obtained by the author shows that this stimulation of growth of phytoplankton cultures could be a transient condition that changes to inhibition after some days of daily addition of .94 ppb of DDT.

The dry weight biomass of DDT-cultures of Chlamydomonas was less than in the control cultures despite the fact that the number of cells was higher. The cells were measured with an inverted microscope, no

differences in size and shape were found. This suggests that the decrease of dry weight biomass may be caused by a higher amount of water in the cells as a consequence of changes of the permeability of the membranes.

The results obtained suggest drastic effects on marine phytoplankton species exposed to low concentrations of DDT. To evaluate the meaning of these findings in natural communities is not simple. The apparent selective toxicity of DDT and the different effects or responses of the algae may alter the composition of natural phytoplankton communities. The consequence of these alterations on the structure and function of marine plankton communities may be considered unpredictable. The alteration of the composition of a community and the higher rate of excretion of extracellular products of phytoplankton, may produce a variety of ecologial effects.

The tendency of a diminution of the protein content of phytoplankton under influence of DDT may be considered as an effect of DDT on the whole ecosystem through the food web.

VI. Summary

The sublethal effects of DDT on the general metabolism of three species of marine phytoplankton was studied.

The respiration, as oxygen consumption, was increased in Chlamydomonas. This may be considered as an indirect effect of DDT; exposure to DDT causes an increase in amination through a higher assimilation of ammonia and a higher cell division rate.

The nitrogen metabolism of Chlamydomonas and Skeletonema was affected by DDT. The release of extracellular organic nitrogen products was increased in both. In Skeletonema there was almost no

cellular fixation of nitrogen.

Cell carbon content was higher in the cultures of Skeletonema and Dunaliella under exposure to DDT. It is argued that a stimulatory effect of DDT on the carbon assimilation results from an increase of heterotrophic assimilation rather than from a stimulation of photosynthesis.

The growth of the cultures was affected by DDT. Chlamydomonas has an increase in the number of cells but the growth of Skeletonema was inhibited 50% by DDT. No changes in size and shape of the cells were observed.

It is concluded that the apparent selective toxicity of DDT and the different responses of the algae may alter the composition and structure of natural phytoplankton communities.

VII. Acknowledgements

This study was carried out at the Nederlands Instituut voor Onderzoek der Zee (Netherlands Institute for Sea Research) during tenure of a research fellowship of UNESCO. I wish to express my gratitude to staff members and personnel of this Institute, especially to the director, Dr. H. Postma, and to Dr. W. Gieskes for much advice and comments. Thanks are due to the Universidad Nacional Autónoma de México for allowing me to accomplish this work.

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Table I

Results of the estimations and measurements obtained in the experiments of effects of DDT in three species of marine phytoplanton. Exposure: 0.94 ppb of p-p'DDT over 24 hours.

	CHLAMYDOMONAS			DUNALIELLA				SKELETONEMA			
	Group Control	Group Test	% of Control	Back-ground	Group Control	Group Test	% of Control	Back-ground	Group Control	Group Test	% of Control
Respiration ml O_2/12hrs/susp.	.084±.020	.132±.020	157	+	.114±.010	.120±.015	105	+	.163±.020	.177±.007	108
Number of cells cells/ml x 10^5	7.8±0.5	10.0±1.0	128	+	5.7±0.2	5.5±0.3	97	8.4	11.0±1.0	9.7±0.7	88
Dry weight biomass mg/suspension	8.0±1.3	6.9±0.9	87	+	2.5±0.2	2.3±0.2	92	+	5.64±0.7	5.64±0.7	100
Carbon content mg C/suspension	2.3±.3	2.2±.03	96	1.20±.03	1.54±.06	1.70±.15	110	+	.77±.12	.93±.07	120
Nitrogen content mgN/suspension	.582±.015	.533±.040	91	+	.244±.015	.257±.010	105	+	.208±.03	.128±.02	61
Ammonia in solution µg N/100 ml	13.8±2.0	10.2±2.0	73	29.4±0.7	16.4±0.8	16.3±.1	99	29.4	7.8±0.6	8.2±0.4	105
Dissolved organic nitrogen mg N/100 ml	.0468±.002	.0665±.001	142	.063±.01	.112±.02	.115±.01	102	+	.145±.01	.221±.04	152
Chlorophylls											
a) µg/suspension	125±20	133±06	106	+	105±006	103±006	98	+	17.3±2.5	17.1±0.2	98
b) µg/suspension	74±07	65±02	87	+	125±001	123±001	98	+	14.0±0.7	12.0±2.5	85*
c) µg/suspension	+	+	+	+	357±002	356±002	100 *	+	8.2±0.3	7.1±3.0	86
Pigments diversity D430/D665	2.02-0.1	1.88-0.05	93	+	.91±.05	.95±.03	104	+	2.15±0.0	2.19±0.06	101

*Chlorophylls not consigned in the literature for these algae.