Loss of Five Pesticides from Cultures of Twenty-one Planktonic Algae

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In an earlier paper the authors utilized thirty-six isolates of planktonic algae to study the effect of atrazine, carbaryl, methoxychlor, diazinon and 2,4-D (butoxy ethanol ester) on growth (BUTLER et al. 1974). At the end of the growth period the cultures were also analyzed by gas chromatography to collect evidence that certain strains might metabolize the pesticides at a significant rate. The evidence sought in this case was the disappearance of the pesticides from cultures of actively growing algae. This report concerns the loss of the five pesticides from actively growing cultures.

MATERIALS AND METHODS

The pesticides studied were atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) (99.0%) supplied by Aldrich Chemical Co., Inc., methoxychlor (2,2-bis(p-methoxyphenyl)-1,1,1-trichloroethane) (88.0% p-methoxyphenyl ester, 12.0% other isomers) supplied by E. I. DuPont, diazinon (0,0-diethyl-0-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate) (99.9%) supplied by CIBA-GEIGY Corporation, carbaryl (1-naphthyl methylcarbamate) (99.7%) supplied by Union Carbide Corporation and 2,4-D, BOEE (2,3-dichlorophenoxyacetic acid, butoxy ethanol ester) (98.2%) supplied by Amchem Products, Inc.

The cultures for the uptake and metabolism studies were prepared using the procedure described for the growth experiments (BUTLER et al. 1974). Control tubes contained culture medium and pesticide but no algae. The concentration of pesticide used was 1 ppm for atrazine, carbaryl and diazinon and 0.01 ppm for methoxychlor and 2,4-D. At the end of a two week growth period the cultures were extracted and the extracts were analyzed for loss of pesticide and the appearance of any possible metabolite(s) using gas-liquid chromatography.

Each 10 ml culture was extracted twice with 5 ml of a 80:20 v/v mixture of pesticide grade benzene and

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hexane (Burdick & Jackson Laboratories, Inc.). Any water present in the solvent extracts was removed with anhydrous sodium sulphate and the volume was reduced to less than 10 ml using a gentle stream of air. The extracts were analyzed for pesticide content using a Tracor MT-220 Gas-Liquid Chromatograph equipped with a Nickel-63 electron capture detector.

RESULTS

Morphological observations and results of the growth studies (BUTLER et al. 1974) indicated that several of the isolates were identical or closely related strains (Table 1). Therefore, only twenty-one isolates were used for the uptake and metabolism studies. Each of these twenty-one isolates appeared to be a separate taxonomic or physiological entity.

Table 2 shows the percent of pesticides recovered (controls taken as 100%) at the end of the two week growth period. Percentages are averages of four samples. Preliminary work on the photolysis and hydrolysis of carbaryl showed that 80% of the parent compound was broken down before the end of the first week. Because of this rapid, non-biological breakdown, uptake of carbaryl in this study was dropped.

Essentially all of the atrazine was recovered from the 12 isolates capable of limited growth at 1 ppm. Only 62% of the diazinon was recovered from isolate No. 6 and from 75% to 96% was recovered from the other 20 isolates. Substantially less methoxychlor was recovered than either atrazine or diazinon. The amount recovered ranged from a high of 79% for isolate No. 20 to a low of 20% for isolate No. 32. The cultures exposed to 0.01 ppm 2,4-D had the greatest loss, with the most recovered being 64% for isolate No. 11. Less than 20% of the total 2,4-D added was recovered from seven of the isolates.

DISCUSSION

The algae do not appear capable of removing atrazine from the medium, as indicated by the fact all of the atrazine added to the cultures was recovered (Table 2), but several of the isolates were capable of

TABLE 1

Strain	Isolate Number
Chlorella sp.	1,5,8,27
Chlorella sp.	2,26
Golinkiniopsis sp.	3
Chlorella sp.	4
Chlorella sp.	6,24,25
Chlorella sp.	7
Chlorella sp.	9,10,13
Monoraphidium sp.	11
Actinastrum sp.	12
Koliella sp.	14,23
Chlorella sp.	15
Chlorella sp.	16
Carteria sp.	17,19
Chlorella sp.	18
Chlorella sp.	20
Scenedesmus sp.	21
Chlorella sp.	22
Scenedesmus sp.	28,29,30
Nitzschia sp.	31,35,36
Nitzschia sp.	32,34
Nitzschia sp.	33

Different Morphological and Physiological Strains Represented by the 36 Isolates

TABLE 2

Isolate number	Pesticide			
	ATRAZINE	2,4-D	DIAZINON	METHOXYCHLOR
1.	91	33	96	34
2.		17	94	37
3.		35	82	63
4.	99	13	90	65
6.		24	62	42
7.	99	27	82	63
10.	98	41	80	65
11.	98	64	78	40
12.		20	78	40
15.	104	18	88	63
16.	104	24	89	76
17.		13	78	55
18.	104	53	94	67
20.	102	44	91	79
21.		25	83	37
22.	104	18	86	69
23.	98	51	87	61
28.	107	25	80	42
31.		17	77	57
32.		14	75	29
33.		22	85	44

Pesticides Recovered From Cultures After Two Week Growth Period as Percentage of Pesticide in Controls

removing significant amounts of either diazinon, 2,4-D or methoxychlor. It is known that 2,4-D acid will enter algal cells (SWETS and WEDDING 1964; WEDDING and ERICKSON 1957) and several metabolites were found in cultures of Scenedesmus quadricauda (VALENTINE and BINGHAM 1973). However, little is known about the uptake and metabolism of methoxychlor, diazinon or the butoxy ethanol ester of 2,4-D by algae.

The disappearance of pesticides from actively growing cultures of certain algal strains may not conclusively demonstrate metabolism of the pesticides by these strains. However, these experiments do identify strains which can profitably be investigated using labelled pesticides to determine whether uptake occurs by adsorption or absorption, and thin-layer chromatography to determine whether metabolites are formed and the identity of any metabolites.

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