the test of *S. dehiscens* has an isotopic composition intermediate between that of its crust and that of *G. sacculifer-trilobus* from the same sample. Visual estimates indicate that the crust comprises roughly 70 ± 20 percent of the mass of *S. dehiscens*. Thus, within the uncertainty limits of the visual estimation of the relative masses of the two parts, the measured isotopic composition of the bulk is not far from the calculated isotopic composition of a mixture of 70 percent crust and 30 percent *G. sacculifer-trilobus*. Although the isotope data do not rule out the possibility that *S. dehiscens* from this location is simply encrusted *G. sacculifer-trilobus*, encrustation could not possibly have taken place at the depths suggested by Bé. The sum of the isotope data argues very strongly for the consideration of *S. dehiscens* as a separate species.

In summary, phenotypes of a single species and test parts of individual phenotypes sometimes record different isotopic temperatures. Where test parts record different temperatures, as in the case of *S. dehiscens*, conclusions may be drawn concerning the temperatures at which the animal lived during different stages of growth. The occurrence of diminutive final chambers is correlated with temperature for the shallow-water species, *G. ruber*. This is particularly important for paleotemperature studies, since if our model is correct, temperatures determined on entire populations of shallow-water species may be colder than those determined when only the "normal" phenotype is used.

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$$\delta O^{18} = \frac{(O^{18}/O^{16})_{sample} - (O^{18}/O^{16})_{standard}}{(O^{18}/O^{16})_{standard}} \times 1000$$

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12. The outer crust was separated from the rest of the test by breaking the test and removing as much inner material as possible.
13. Supported by research grants from the GSA and Sigma Xi to A.D.H. and by NSF grant GA-1693. R. Cifelli, W. Ruddiman, and R. G. Douglas provided samples for analysis. One sample was collected by A.D.H. while on board the oceanographic research vessel *Eastward* of Duke University. We thank A. W. H. Bé, R. G. Douglas, J. Hower, and F. G. Stehli for helpful discussions. Contribution No. 63 of the Case Western Reserve University Department of Geology.
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DDT Residues in Marine Phytoplankton: Increase from 1955 to 1969

Abstract. *Phytoplankton samples collected in Monterey Bay, California, from 1955 to 1969 contained compounds identified as* p,p'-*DDT,* p,p'-*DDD, and* p,p'-*DDE. Total concentrations of these compounds were approximately three times greater in the later samples. Lower concentrations throughout the period were associated with higher densities of standing crop.*

Annual use of DDT in the United States has declined in the past decade (*1*), yet there is recent evidence of abnormally high DDT residues in marine fish from U.S. coastal waters, and such contamination in these areas may exceed that in freshwater habitats (*2*). This could indicate either (i) that environmental DDT residues are increasing or (ii) that these recent analyses simply reflect current DDT input and that DDT concentrations have in fact been even higher in the past. Although DDT residues in estuarine shellfish (*3*) have shown no consistent upward or downward trends, the time has been too short and the estuarine system too responsive to weather conditions and local sources of pesticides to provide any measure of the trends in the coastal environment. Declining reproductive success in species of marine pelagic birds, attributable to DDT residues (*4*), does suggest that residues of DDT are increasing in the coastal pelagic food chains of which these birds are high-order consumers.

A decision between the alternatives could be made if historical collections of marine organisms were available. At the Hopkins Marine Station, samples (composed primarily of phytoplankton) collected with a fine-mesh net from Monterey Bay, California, have been collected from 1955 to 1969 (*5*). Phytoplankton samples are particularly suited for analysis because they represent the first link in pelagic food chains. Trends in their concentrations of DDT residues are relevant to all higher-order consumers on the food chain. Also, DDT uptake by phytoplankton is rapid and essentially irreversible (*6*); thus, it can be assumed that the content of DDT residues of phytoplankton reflect prevailing amounts of environmental DDT. To examine the change in content of DDT residues over the collection period, 23 samples from the collection were analyzed. All the samples had been preserved in a 3 percent solution of formalin in seawater. The estimated concentrations of DDT residues (*7*) for the samples were based on their carbon content as determined by wet combustion (*8*) of replicate portions. Formalin induces error in carbon determinations of marine planktonic material (*9*), but the errors in this instance were small (< 10 percent). Treatment of freshly collected material from the same station with formalin had no apparent effect on estimates of the DDT content when compared to that of frozen controls.

Samples were filtered onto combusted GFC glass-fiber filters (Whatman) after filtration through 0.33-mm netting to remove larger zooplankton. The sample and filter pad were ground together in three successive rinses of high-purity *n*-hexane. The pooled rinses were concentrated and chromatographed on silica-gel microcolumns (*10*). Eluates from the columns were concentrated at 37°C under a stream of nitrogen and analyzed by gas-liquid chromatography (GLC). All glassware used in the procedure was combusted

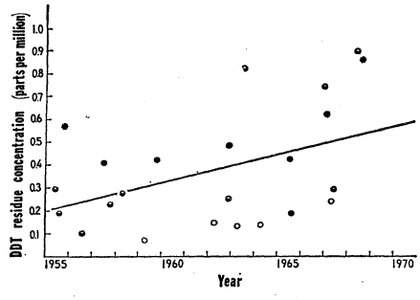

Fig. 1. Concentrations of DDT residues (*7*) in samples of phytoplankton collected by towed nets from Monterey Bay, California, 1955 to 1969. Concentrations are expressed as weight of estimated DDT residues per unit wet weight of phytoplankton as converted from measurements of oxidizable organic carbon content of the samples (*16*). Solid circles indicate smallest samples (<0. mg of carbon); half-solid circles indicate samples with 0.2 to 0.4 mg of carbon; open circles indicate samples with greater than 0.4 mg of carbon.

at 350°C overnight prior to use; this treatment reduced background contamination nearly to zero for the GLC analyses. Recovery from a variety of samples of known content of DDT exceeded 95 percent in all cases.

The extracts were injected into a Beckman GC-4 gas chromatograph equipped with two columns and two electron-capture detectors (*11*). Each sample was chromatographed on at least two columns of different composition (*12*). Peaks were identified by standard injection retention times, fortified injections, and disappearance of presumptive DDT and DDD peaks caused by treatment of the extracts with KOH in alcohol.

There were higher concentrations of DDT residues in more recent samples (Fig. 1). Inasmuch as sample storage may have affected this apparent temporal trend, experiments were performed to test the effect of decomposition on the relative proportions of the three DDT constituents found in the samples. Ring-labeled ^{14}C-DDT was added to sealed ampules that contained portions of a phytoplankton sample preserved in formalin. The amounts of labeled compound added were comparable to those amounts found in the 23 analyzed samples. Because the samples had been stored in the dark, it was assumed that any possible breakdown would be thermochemical, not photochemical. Elevated temperatures for short periods of time were used to recreate longer periods at room temperature. The contents were heated at 30°C, 60°C, and 75°C for 6 days and then removed from the ampules, extracted, and analyzed by thin-layer chromatography (*13*). Narrow (0.5-cm) zones were scraped from the chromatoplates into scintillation vials for measurements of ^{14}C activity. The degree to which DDT was broken down to polar compounds affected the relative proportions of the remaining nonpolar constituents: *p,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDE. No breakdown of the ^{14}C-DDT occurred in the samples heated at 30°C. In the samples heated at 60°C, 28 percent of the ^{14}C-DDT broke down to polar compounds, but about half of the remaining nonpolar material was *p,p'*-DDT. In the sample heated at 75°C, 38 percent of the ^{14}C-DDT broke down to the polar compounds, but *p,p'*-DDT comprised only 15 percent of the nonpolar material, whereas *p,p'*-DDD and *p,p'*-DDE comprised 83 percent. On the basis of these experiments, a change in the relative proportions of the DDT residues in a sample would be expected if any net decomposition to nonpolar products had occurred during storage. In fact, the relative proportions of the DDT constituents found in the samples were quite constant (*14*); percent regression analysis showed the slope of each percentage versus time function to be not significantly different from zero. Therefore, the trend indicated in Fig. 1 represents an actual increase in the DDT residues in the phytoplankton rather than a loss of analyzable residues during sample storage.

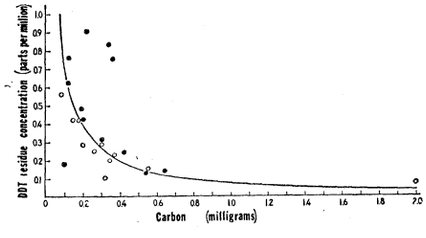

Fig. 2. The effect of size of relative standing crop (milligrams of carbon) on the estimated concentration of DDT residue (*7*). The theoretical curve was computed according to the relationship $C \times D = k$, where C = carbon content, D = DDT residue concentration and k = weight of the mean amount of DDT residues in the samples. Solid circles indicate samples taken from the later half of the sampling period; open circles indicate samples from the earlier half. Values on the vertical axis were derived as in Fig. 1.

Part of the variability in the values in Fig. 1 is attributable to sample size. Due to the nature of the collection technique, the sample sizes were directly related to the density of the phytoplankton in the water at the time of collection. The estimates of carbon content in the 23 samples were based on standard portions of the samples, which in turn contained the entire contents of a vertical ¼-meter net tow from a depth of 15 meters to the surface. The carbon values are thus indices of standing crop density (Fig. 2). The assumption behind the theoretical curve (Fig. 2) is that a fixed amount of pesticide residue becomes incorporated in the algal material present in a given volume of water, regardless of the density of the standing crop. However, density of the standing crop affects the final concentration of acquired residues according to the relationship in Fig. 2. This suggests that the partition coefficient of DDT residues for phytoplankton and similar material (*6*, *15*) diminishes as the density of the phytoplankton increases. A comparison of the points falling above or below the theoretical curve in Fig. 2 shows that the preponderance of later points have higher concentration values, despite the effect of the size of standing crop. The same conclusion may be reached by examining the points in Fig. 1 by size classes.

The residues of DDT may be increasing in the primary stages of coastal pelagic food chains. If the processes of decomposition and dispersal of these residues in succeeding steps are not sufficiently rapid to counteract this apparent increase, a delay may be expected before the decline of domestic usage of DDT begins to be reflected in the components of these food chains.

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11. All GLC parameters were those suggested in *Pesticide Analytical Manual* (U.S. Dept. of Health, Education, and Welfare, Food and Drug Administration, revised, 1968), vol. 2.
12. Coatings used on the columns were 5 percent DC-200, 5 percent QF-1, 5 percent mixed bed of DC-200 and QF-1, and 3 percent SE-30 with 6 percent QF-1 in a mixed bed. All coatings were made on DMCS Chromosorb W.
13. Silica gel G was used as the adsorbent. Chromatoplates were developed in *n*-heptane, compounds were identified by cochromatography with pure standards.
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Pulmonary Hemorrhage in Hamsters after Exposure to Proteolytic Enzymes of Bacillus subtilis

Abstract. *Single exposures of Syrian hamsters by aerosol inhalation or intratracheal instillation to proteolytic enzymes of* Bacillus subtilis *produced massive pulmonary hemorrhage within the first week. Of 46 animals exposed, eight died as a result of extensive hemorrhage. The remainder made uneventful recovery with no apparent residual pulmonary disease 6 weeks after exposure.*

Recent reports in the literature indicate that some workers who handle proteolytic enzymes derived from *Bacillus subtilis* suffer pulmonary reactions. Asthmatic manifestations, as well as symptoms suggestive of a more peripheral pulmonary reaction, have been reported (*1*). Allergic reactions including nasal irritation, coughing, wheezing, and difficult breathing have also been documented (*2*). Severe respiratory symptoms in a number of workers handling enzyme preparations for washing apparently resulted from complex allergic reactions in bronchi and in peripheral lung tissue (*3*). The primary respiratory reactions included bronchospasm, chest pain, and hemoptysis. The Food and Drug Administration and the Federal Trade Commission have received notices of skin rash and other types of allergic reactions among housewives using stain-removing products (*4*). Contact dermatitis has also been reported (*5*). Respiratory disorders resembling asthma and influenza are considered serious problems among workers in detergent plants (*3*).

The use of enzymes, of both plant and bacterial origin, has increased in recent years and now involves large numbers of persons, both in the manufacture and in the application of such enzymes. More recently the use of bacterial enzymes has reached the level of the household user and they have thus been introduced into millions of homes. Their possible health hazards have not been adequately investigated (*6*).

Experimentally, enzymes of plant origin were employed as inhalants and also applied by intratracheal instillation to determine the pathological effects on the tracheobronchial tree and pulmonary parenchyma of laboratory animals. Papain, a plant protease, as well as ficin and bromelain, has been shown to produce emphysema in hamsters when it was inhaled (*7, 8*).

Table 1. Treatment of animals and time of killing after treatment. Abbreviations: A, aerosol; I, intratracheal. Results are given as the number of animals in each category.

Time of death after treatment	Experimental		Control	
	Alcalase (A)	Maxatase (I)	Saline (A)	Saline (I)
24 hours	4	4	2	1
48 hours	3	4	2	1
72 hours	1	4		1
7 days	4	2	2	1
10 days		3		
14 days	3	2	2	1
20 days		1		
28 days	4	3	2	
42 days	3	1	2	1

In the present studies, similar experiments with bacterial enzymes were undertaken to explore the possible pathological changes in the lungs of laboratory animals. The enzymes used were Alcalase (*9*) and Maxatase (*10*), two proteolytic enzyme products derived from *Bacillus subtilis,* which are utilized in the manufacture of a variety of household enzyme detergent agents. Alcalase is a finely divided powder containing approximately 60 percent sodium sulfate, 5 percent sodium chloride, and 35 percent organic material of which 5 to 10 percent is the enzyme (*2*). Maxatase is a fine powder, the major part of whose enzyme activity is proteolytic, but according to the manufacturer it also contains a small amount of α-amylase.

The animals used in the current experiments were Syrian golden hamsters of both sexes; age range was 37 to 165 days.

The solubility of the two enzymes in water determined the route of administration. Alcalase was administered as an aerosol and Maxatase by intratracheal instillation. Three percent solutions of the enzymes were administered by methods previously described (*7, 11*). A total of 46 animals were treated with the enzymes and an additional 18 animals served as controls; these received physiological saline. The disposition of the animals is indicated in Table 1.

A slight loss of weight was exhibited by a number of the experimental animals during the first few days after exposure. Some animals developed severe respiratory symptoms characterized by dyspnea, rales, and cough. A bloody nasal discharge was observed in a few. Eight of the 46 experimental animals died; six within the first 24 to 48 hours after treatment, one after 7 days, and one after 20 days. In each case, massive lung hemorrhage was observed. The remainder were killed at prescribed intervals of 24, 48, and 72 hours, as well as 7, 14, 28, and 42 days after exposure. Complete necropsies were performed on all animals, special attention being given to the respiratory organs. The method of fixation of the lungs and the histologic staining techniques employed have been described earlier (*12*).

The tracheas of the experimental animals failed to show significant pathologic alteration. Loss of cilia, usually the first evidence of damage, was ex-