depositional solution than those during the later warm stages. The rate of terrigenous sedimentation must have been particularly low. In contrast, higher rates of sedimentation led to excellent preservation of the smaller foraminiferal shells during all cold stages. During catathermal 3-2 and cold stage 2, even shells of pteropods were preserved in abundance (*9*).

Oxygen isotopic analysis was performed on samples of the carbonate sediment fraction between 62 and 250 μm at the usual 10-cm intervals (Fig. 2). The curves thus obtained reproduce the general trend of curves previously obtained using *Globigerinoides sacculifera* [figures 3 and 4 in (*2*)]. This is not surprising because it was shown (*10*) that the early stages of all pelagic foraminiferal shells are deposited in shallow water where *G. sacculifera* spends its entire life cycle. The strongest deviations from the *G. sacculifera* curves occur in correspondence with the sediment layers where postdepositional solution is heaviest. In these layers (core P6304-8, 1000 cm below the top, and core P6304-9, 910 to 920, 1060, and 1210 cm below the top) ^{18}O maxima occur while *G. sacculifera* shows minima. This is so because the layers with heavy solution are largely composed of shell fragments of the least soluble, deeper-living species. It is apparent that not only faunal analysis, but also isotopic analysis of bulk faunal samples [see (*5*)], is biased by postdepositional solution (*1, 8*). The need to limit isotopic analysis to monospecific samples is emphasized, although bulk faunal analysis of well-preserved juveniles is capable of producing meaningful results and may be used if larger specimens are not available.

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8 July 1977; revised 30 September 1977

Polychlorobornane Components of Toxaphene: Structure-Toxicity Relations and Metabolic Reductive Dechlorination

Abstract. *2,2,5-*endo,*6-*exo,*8,9,10-Heptachlorobornane and four derivatives of this heptachlorobornane, with an additional chlorine atom at position 3-*exo,*8, 9, or 10, account for a major portion of the acute toxicity of toxaphene and for up to 23 percent of toxaphene composition as analyzed by open tubular column gas-liquid chromatography with an electron capture detector. Both in several organisms and model environmental systems and on photolysis, this heptachlorobornane undergoes facile reductive dechlorination at the geminal-dichloro group and sometimes dehydrochlorination.*

Toxaphene is a complex insecticidal mixture containing heptachlorobornane **1** (*1, 2*), octachlorobornanes **2** and **3** (*3, 4*) (Fig. 1) and > 170 other $C_{10}H_{18-n}Cl_n$ and $C_{10}H_{16-n}Cl_n$ (n = 6 to 10) components (*5–7*). Compounds **2** and **3** are major contributors to the acute toxicity of toxaphene to mice, goldfish, and houseflies (*1, 3, 5*). Toxaphene is metabolized in rats (*5, 8*) and degraded in iron(II) protoporphyrin systems (*9*) by cleavage of about half of the carbon-chlorine bonds. It is important to define more precisely the relations between structure and toxicity for polychlorobornanes and the chemical nature of their initial metabolic products, since in the United States the amount of toxaphene used continues to be larger than that of any other insecticide. We find that compound **2** from chlorination of **1** is the most toxic of a series of newly prepared hepta-, octa-, and nonachlorobornanes. Further, we report that **1** undergoes metabolism and photolysis, in part, by reductive dechlorination at the geminal-dichloro substituent to form **4** and **5** and sometimes by dehydrochlorination to give **6** (Fig. 1).

Heptachlorobornane **1** was prepared (*10*) in gram quantities by photochlorination of 2-*exo*,10-dichlorobornane (*11*) in carbon tetrachloride solution to a chlorine content of 66.7 percent. It was isolated in 2 to 3 percent overall yield by chromatography on a silicic acid column with hexane (*1*), with bifluorenylidene (*7*) as a marker dye for the elution position of **1**. Further chlorination of **1** under the same conditions to an overall composition of $\sim C_{10}H_{10}Cl_8$ yields a mixture of unreacted heptachlorobornane (22 percent), **2** (13 percent), **3** (27 percent), and the 3-*exo*-chloro- (12 percent) and 10-chloro- (13 percent) derivatives of **1**. These hepta- and octachlorobornanes account for up to 23 percent of the composition of toxaphene as determined by open tubular column gas-liquid chromatography with an electron capture detector (*7, 10*). Additional minor products from the chlorination of **1** are its 5-*exo*-chloro-, 3-*exo*,10-dichloro-, and 8,10-dichloro-derivatives (*10*). These eight hepta-, octa-, and nonachlorobornanes were isolated by the same column chromatographic procedure used to obtain **1**. Their structures were assigned by proton magnetic resonance spectroscopy and mass spectrometry (*10*).

The structure-toxicity relations of polychlorobornanes and polychlorobornenes were examined with male albino mice treated intraperitoneally and with female houseflies of an insecticide-susceptible strain (SCR) treated topically (*1*), each in the presence and in the absence of the synergist piperonyl butoxide (*12*), and with goldfish exposed for 24 hours under static conditions (*3*) (Table 1). The potency of compounds resulting from introducing chlorine substituents into **1** generally decreases in the order: 9-chloro (**2**) > 8-chloro- (**3**) > no added chlorine (**1**) > 3-*exo*-chloro- or 5-*exo*-chloro- or 10-chloro (*10*). Addition of a 10-chloro substituent to the 3-*exo*-chloro or 8-chloro derivative of **1** reduces their potency. The toxicity of **1** is also decreased by reductive dechlorination at the geminal-dichloro group especially on conversion to **5** (*13*) and by dehydrochlorination, particularly at the 5,6 position on removing the chlorine from carbon-6 (*10*).

The reactions of heptachlorobornane **1** were examined in several chemical and biological systems (*13*). These systems included triphenyltin hydride in hexane in the presence of light (*6*), iron(II) hematin in both *N*-methyl-2-pyrrolidone-ace-

tic acid (*14*) and in neutral aqueous solution (*9*), rat liver microsomes under anaerobic conditions with reduced nicotinamide-adenine dinucleotide phosphate (NADPH) as the essential cofactor (*9*), bovine rumen fluid (*15*), and sewage primary effluent (*16*). Also examined was the photolysis of **1** in hexane solution with ultraviolet light (> 220 nm) [for an analogous system see (*17*)]. Surprisingly, in each of these systems heptachlorobornane **1** undergoes reductive dechlorination at the geminal-dichloro group to give hexachlorobornanes **4** and **5** (*13*). This occurs rapidly, with remarkable specificity, and often in high yields, for example, 87 percent with hematin in the nonaqueous system and 100 percent with bovine rumen fluid. These findings are similar to previous observations on reductive dechlorination of the trichloromethyl group of the insecticide DDT (*14–16, 18*). A minor product in the nonaqueous iron(II) hematin system is compound **6** from dehydrochlorination of **1** (*13*). Products **4**, **5**, and **6** were identified by proton magnetic resonance spectroscopy and mass spectrometry (*13*) and **4** and **5** by x-ray crystallography (*19*).

The observed ease of reductive dechlorination in vitro of **1** at the geminal-dichloro group by reduced microsomal cytochrome P-450 prompted studies in vivo with topically treated male and female houseflies of the SCR strain (4.5 mg/kg, applied in acetone) and orally treated male albino rats (3.1 mg/kg, administered in soybean oil) (*13*). The amounts of **4**, **5**, and **6** were determined as percentages of the applied dose in the treated animals and their excreta (*20*). Products in the flies at 24 hours after treatment were: **1**, 23 percent; **4**, 6 percent; **5**, 11 percent; and **6**, 7 percent. Those in the feces of rats at 72 hours after treatment were: **1**, 0.2 percent; **4**, 5.3 percent; **5**, 2.1 percent; and **6**, 1 percent. Each of these metabolites also appears in very low concentrations in the liver [0.8 to 10 ng per gram of wet tissue (ppb)] and fat (8 to 34 ppb) at 7 and 72 hours after treatment. At these times, little **1** is present in the liver (9 to 17 ppb) and in the fat (335 to 453 ppb) relative to the administered dose. The low overall recoveries indicate that **4**, **5**, and **6** undergo further metabolism in addition to direct excretion or that metabolic attack at the geminal-dichloro group is only one of the initial detoxication mechanisms involved.

The conversion of **1** to **4** and **5** presumably involves a radical intermediate with triphenyltin hydride and the iron(II) hematin systems (*14, 21*) (Fig. 1). A radical intermediate is probably also involved in these conversions in the biological systems examined. The mechanism of dehydrochlorination of **1** to **6** in the iron(II) hematin system and in flies and rats in vivo remains to be established.

These findings on a single toxaphene component are relevant to the more difficult problem of the metabolism of the complex mixture of components in toxaphene itself. Analyses (*13, 20*) of liver, fat, and feces of rats orally administered toxaphene show rapid loss of components **1**, **2**, and **3** and of other components with similar chromatographic properties. Only in the fat do the chlorinated hydrocarbons approximate the composition of those in toxaphene, while in the liver and feces they consist of derivatives of greatly reduced retention times on gas-liquid chromatography. Thus, almost all toxaphene components are readily metabolized. There are now eight identified polychlorobornane components of toxaphene, each with one geminal-dichloro group on the ring at carbon-2 or at carbon-3 or -5 and with none, one, or two dichloromethyl groups (*1–5, 10, 22*). It is likely that reductive dechlorination is one of the initial steps

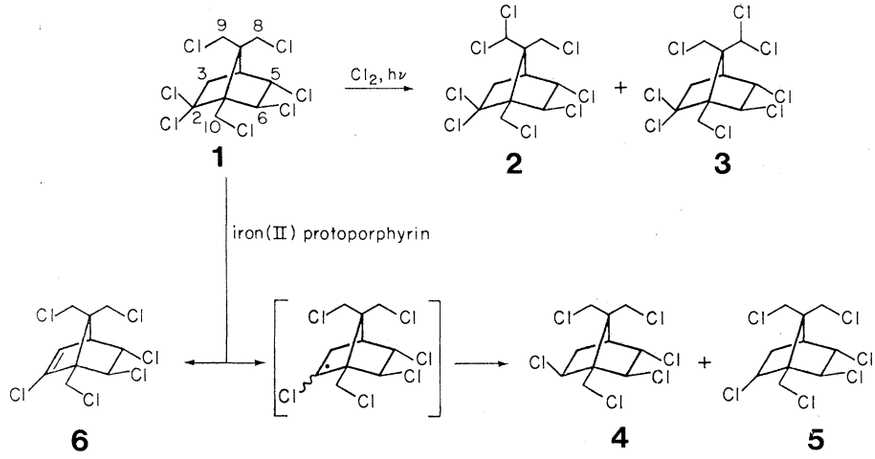

Fig. 1. Reactions of the toxaphene component 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane **1** involving photochlorination to octachlorobornanes **2** and **3**, metabolic reductive dechlorination to hexachlorobornanes **4** and **5**, and metabolic dehydrochlorination to hexachlorobornene **6**. Compound **1** is also converted to **4** and **5** on photolysis.

Table 1. Structure-toxicity relations of polychlorobornanes and polychlorobornenes (*10, 13*). The structures of the compounds are shown in Fig. 1.

Compound*	Position of chlorine addition or removal relative to **1**		Relative toxicity (**1** = 100)†		
	Added	Removed	Mice	Houseflies	Goldfish
		Heptachlorobornane			
1			100‡	100‡	100
		Octachlorobornanes			
	3-*exo*		<75	62‡	7
	5-*exo*		~312	44‡	22
3	8		2272	209‡	264
2	9		>3000§	>371§	>527§
	10		<75‡	14‡	8
		Nonachlorobornanes			
	3-*exo*,10			12	<3
	8,10			19‡	7
		Hexachlorobornanes			
4		2-*endo*		32‡	60
5		2-*exo*		5	3
		Hexachlorobornenes			
6		2		32‡	11
		6	~115	5‡	<3

*Compounds **1** to **3** and the 3-*exo*-chloro and 10-chloro derivatives of **1** are toxaphene components. Compounds **4** to **6** are metabolites of compound **1**. †The values for the lethal doses for 50 percent of the animals (LD_{50}) for **1** are 75 and 12 mg/kg for mice and houseflies, respectively, and 2.9 μg/liter for goldfish. Relative toxicity is the ratio of the LD_{50} for **1** to that of the test compound times 100. ‡These compounds are two to eight times more toxic to mice and houseflies that had been given preliminary treatments with piperonyl butoxide than to normal mice and houseflies. §Values for a mixture of **2** (43 percent) and **3** (57 percent).

in metabolism of each of these components and others with geminal-dichloro groupings, at least in the variety of systems used in examining the metabolism of toxaphene component **1**. Preliminary findings (*13*) with **3** in the iron(II) hematin system indicate that the dichloromethyl substituent is less susceptible to attack than other substituents such as the ring geminal-dichloro group.

Relatively few major polychlorobornane components of toxaphene have proper configurations for high toxicity. This structural specificity and the available knowledge concerning the potency of polychlorobornane metabolites suggest that metabolic reductive dechlorination in the geminal-dichloro substituent and dehydrochlorination are likely to detoxify many toxaphene components or initiate a series of metabolic events leading to their detoxification. The toxicity of **1** to mice and houseflies is increased by a factor of 5 to 8 by piperonyl butoxide (*10*), indicating the importance of cytochrome P-450–mediated detoxification mechanisms (*12*), either reductive (*9, 13*) or oxidative (*9*). Piperonyl butoxide also increases the toxicity of several other polychlorobornanes and polychlorobornenes (Table 1). A combination of reductive dechlorination at geminal-dichloro groups, dehydrochlorination, and oxidation of carbon substituents probably contributes to the extensive dechlorination noted for toxaphene components in rats (*8*). These findings help explain the low persistence of toxaphene as compared with that of many other chlorinated hydrocarbons in mammals (*23*). They also suggest possible pathways for environmental degradation of toxaphene residues.

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23 April 1977; revised 21 July 1977

Histone Occurrence in Chromatin from *Peridinium balticum*, a Binucleate Dinoflagellate

Abstract. Peridinium balticum *is one of two dinoflagellates known to have dissimilar nuclei together in the same cell. One nucleus (dinokaryotic) has permanently condensed chromosomes, while the other (eukaryotic) does not have morphologically distinct chromosomes. Acid extracts of chromatin prepared from a mixture of dinokaryotic and eukaryotic nuclei and purified eukaryotic nuclei give four bands that co-migrate with four of the five histones from calf thymus when analyzed in urea-containing polyacrylamide gels.*

Although it has been shown that the chromosomes of dinoflagellate algae do not contain histones, all of the studies on histone occurrence in these organisms were done on uninucleate dinoflagellates. We now report on the occurrence of histones in a binucleate dinoflagellate and provide evidence that one of the two dissimilar nuclei present in *Peridinium balticum* contains four acid-soluble proteins which comigrate with histones H1, H2a, H2b, and H4 from calf thymus in urea-containing polyacrylamide gels. Dinoflagellates are eukaryotic algae that have several bacterial traits with respect to the organization of their nuclei and chromosomes. The occurrence of prokaryotic traits in a eukaryotic nucleus has led many investigators to suggest that the nuclear organization of dinoflagellates is intermediate between that of prokaryotes and eukaryotes (*1–5*). Some of the unusual features of dinoflagellate nuclear organization are the absence of a centromere and conventional mitotic spindle (*6*), and the presence of extremely high amounts of DNA (*3, 7, 8*). The DNA is organized into numerous, identical appearing chromosomes (*4, 9*) that are attached to the nuclear envelope (*2, 6*). The nuclear envelope does not disintegrate during division (*6*), and the chromosomes remain condensed throughout the entire division cycle (*2, 6, 9*). Although little is known about the genetics of dinoflagellates, recent work suggests that the vegetative cells of dinoflagellates are haploid (*10*), and genetic recombination occurs in at least some species (*11*). One of the most interesting aspects of dinoflagellate chromosomes is the absence of histones. Although typical eukaryotic histones are absent, chromatin from isolated nuclei of *Crypthecodinium cohnii* (*12, 13*) and *Peridinium trochoideum* (*13*) log phase cells contains a histone-like protein that has a mobility in acid-urea polyacrylamide gels slightly lower than that of histone H4. The protein from *Crypthecodinium cohnii* has been partially characterized and does not appear to be analogous to any of the five vertebrate histones (*13*).

In addition to the peculiar features of the dinoflagellate nucleus, an even more intriguing condition exists in the binucleate dinoflagellates *Glenodinium foliaceum* (*14*) and *Peridinium balticum* (*15*). These organisms have two dissimilar nuclei within the confines of a single cell. One of these nuclei (dinokaryotic) is similar in ultrastructure to that of uninucleate dinoflagellates and contains permanently condensed chromosomes. The chromatin of the second nucleus (eukaryotic) is not organized into chromosomes at any stage in the cell cycle. It was recently suggested (*16*) that *Peridinium balticum* obtained its eukaryotic nucleus after the invasion of a chrysophyte-like endosymbiont. Al-