

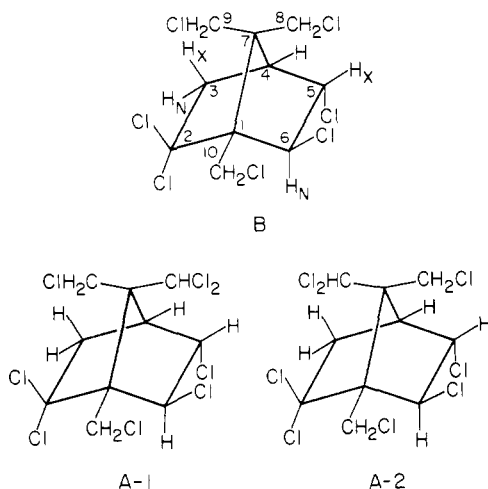
# COMMUNICATIONS

## Toxaphene Toxicant A. Mixture of 2,2,5-*endo*,6-*exo*,8,8,9,10-Octachlorobornane and 2,2,5-*endo*,6-*exo*,8,9,9,10-Octachlorobornane

Two materials, toxicants A and B, account for a significant portion of the toxicity of technical toxaphene to mice, goldfish, and houseflies. The more toxic one, toxicant A, is identified by  $^1\text{H}$  NMR studies as a mixture of 2,2,5-*endo*,6-*exo*,8,8,9,10-

octachlorobornane and 2,2,5-*endo*,6-*exo*,8,9,9,10-octachlorobornane. These components have many structural features in common with the previously identified toxicant B, 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane.

Toxaphene is a complex mixture of closely related compounds which vary over a broad range in their intraperitoneal (ip) toxicity to mice and topical toxicity to houseflies (Casida et al., 1974, 1975; Holmstead et al., 1974). Studies on the analysis, metabolism, and environmental degradation of toxaphene would be facilitated by identification of the most toxic components of the technical mixture. Accordingly, two toxic materials, designated toxicants A and B, were isolated using mouse ip toxicity as the monitoring criterion (Khalifa et al., 1974); these isolations were made from toxaphene which had been recrystallized from methanol rather than from technical toxaphene as erroneously reported. Toxicant B has been previously identified by X-ray crystallography and by  $^1\text{H}$  NMR as 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane (Palmer et al., 1975). The present report identifies the more toxic material, toxicant A, by  $^1\text{H}$  NMR spectroscopy.



Toxicant A, a crystalline material with the composition  $\text{C}_{10}\text{H}_{10}\text{Cl}_8$ , gives a single peak on capillary GLC (Khalifa et al., 1974) but can be seen by its 100-MHz  $^1\text{H}$  NMR spectrum to consist of two major components. Thus, while this 100-MHz spectrum is largely unresolved and too complex to be analyzed, there are certain well-resolved downfield resonances which appear in different ratios (50:50 to 60:40) in separate samples of toxicant A. These variations in the ratio of the two components indicate that a minor degree of separation occurs during some stage of isolation. The 300-MHz spectrum of toxicant A with the major chlorinated hydrocarbon components in the ratio 60:40 almost completely resolves the various resonances into essentially first-order patterns (Figure 1). Assignment of the resonances to component A-1 (60%) or A-2 (40%) follows from their integrals. The major components composed about 80% of the mixture except for impurities with resonances at  $\delta < 2$  introduced during the isolation.

In analyzing the  $^1\text{H}$  NMR spectrum, the assumption was made that both components of toxicant A are octachlorobornanes. This is likely to be the case since in the chlorination of camphene to produce toxaphene the early chlorination steps result in 2-*exo*,10-dichlorobornane (Jennings and Herschbach, 1965; Richey et al., 1965) and extensive chlorination yields polychlorobornanes (Casida et al., 1975; Holmstead et al., 1974). The bornane skeleton of A-1 and A-2 is further supported by the observation that the complete set of proton-proton coupling patterns of the five ring protons of the heptachlorobornane, toxicant B (Table I), is almost exactly duplicated in each component of toxicant A. There is little likelihood of so close a correspondence in all five coupling constants in structures without the bornane skeleton. Accordingly, these resonances of components A-1 and A-2 are assigned to bornane ring protons as shown in Table I. The remaining resonances for each component of toxicant A are assigned to two chloromethyl groups on the basis of their coupling constants and chemical shifts and on analogy with toxicant B (Palmer et al., 1975) and to one dichloromethyl group on the basis of its chemical shift [addition of 2.53 ppm (Silverstein et al., 1974) to the average chemical shifts of the chloromethyl protons of toxicant B gives a predicted value of  $\delta$  6.3 for the dichloromethyl protons]. In each component of toxicant A, the coupling of the dichloromethyl group proton to one proton of a chloromethyl group reveals that these groups are attached to C-7. This four-bond coupling is probably due to steric hindrance holding only one proton of the chloromethyl group in a planar W-configuration with the dichloromethyl group proton. In toxicant B the protons on C-8 are coupled to different protons on C-9. In both toxicant A and toxicant B the protons of C-10 show only geminal coupling.

The data considered above strongly indicate that the components of toxicant A differ from toxicant B only by the addition of one chlorine at C-8 or C-9. Molecular models reveal that such structures have extreme steric hindrance, which raises the question whether the components of toxicant A are positional or conformational isomers. Although the addition of the chlorine atom to groups attached to C-7 has no effect on the coupling among the ring protons, it does alter some of their chemical shifts, presumably by the magnetic anisotropy of the C-Cl bond. A comparison of the chemical shifts of these protons in toxicants A-1, A-2, and B, as in Table II, reveals that significant downfield shifts occur in H-4 of both components of A relative to H-4 of B, but that otherwise only the shifts of protons 5X in A-1 and 3X in A-2 are significantly changed, both also being shifted downfield from their positions in B. Strictly, it cannot be ruled out from these data that the differing chemical shifts of the 3X and 5X protons in A-1 and A-2 are caused by differing interactions of the isomers with the  $\text{C}_6\text{D}_6$  solvent. However, the rotations allowed by the steric crowding seem to be too small to cause such different interactions if the components were conformers, and the

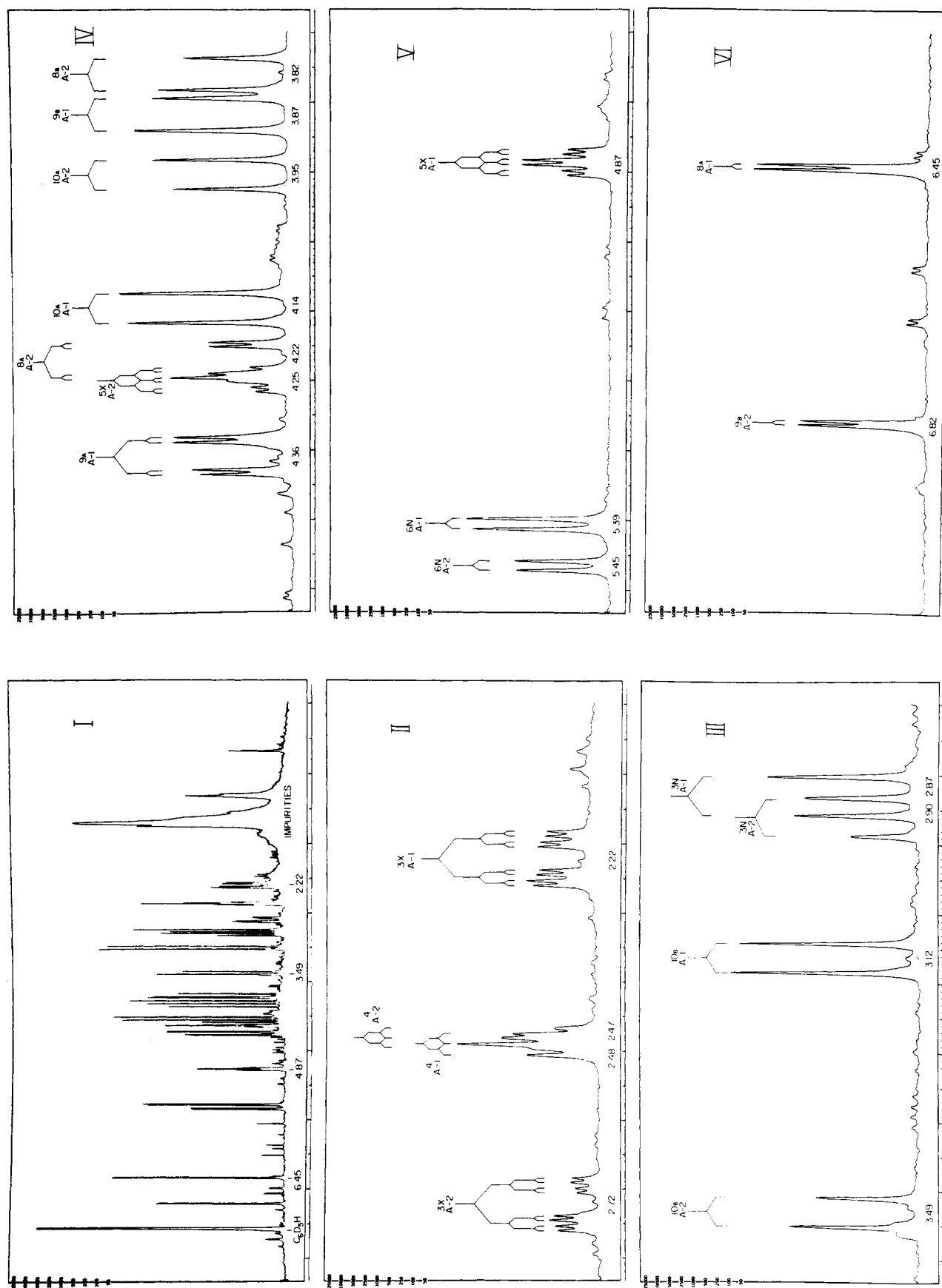


Figure 1.  $^1\text{H}$  NMR (300 MHz) spectrum of toxicant A in  $\text{C}_6\text{D}_6$ . The complete spectrum (I) is at 2500-Hz sweep width while the expansions (II-VI) are at 250-Hz sweep width. The sweep offsets for expansions II-VI are 720, 950, 1250, 1530, and 2000 Hz, respectively.

Table I.  $^1\text{H}$  NMR Spectra of Toxicants A and B<sup>a</sup>

H	Toxicant A					
	Toxicant B		A-1		A-2	
	$\delta$ ( $\text{C}_6\text{D}_6$ )	$J$ , Hz	$\delta$ ( $\text{C}_6\text{D}_6$ )	$J$ , Hz	$\delta$ ( $\text{C}_6\text{D}_6$ )	$J$ , Hz
3X	2.21	16.2, 4.5, 1.8	2.22	16.6, 4.5, 2.0	2.72	16.3, 4.2, 1.8
3N	2.87	16.2 (0.6)	2.87	16.4 <sup>b</sup>	2.90	16.3 <sup>b</sup>
4	1.75	4.5, 4.5 (0.6)	2.48	4.5, 4.5	2.47	4.5, 4.5
5X	4.22	4.6, 4.5, 1.8	4.87	4.5, 4.5, 2	4.25	4.5, 4.5, 1.8
6N	5.31	4.6	5.39	4.5	5.45	4.0
8a	3.65	12.4, 1.8	6.45	2.0	4.22	13.7, 1.8
8b	3.65	12.4, 1.8			3.82	14
9a	4.01	12.5, 1.8	4.36	14, 2.1		
9b	3.65	12.5, 1.8	3.87	14	6.82	1.8
10a	4.29	12.5	4.14	12.7	3.95	12.7
10b	3.55	12.5	3.12	12.6	3.49	12.5

<sup>a</sup> As ca. 1% solutions in  $\text{C}_6\text{D}_6$  with  $\text{C}_6\text{D}_5\text{H}$  at  $\delta$  7.17 as the reference. Assignments to a and b of protons on C-8, -9, and -10 are arbitrary. In addition, the assignments of protons to C-8 and -9 in toxicant B are arbitrary. The coupling constants are actual line separations, as if the spectra were first order. Those of toxicant B are taken from the  $\text{CCl}_4$  spectrum of Palmer et al. (1975) since the present 220-MHz spectrum was not sufficiently resolved for accurate measurements of coupling constants. <sup>b</sup> Each of these resonances is broad, suggesting small further coupling, probably to H-4 as in toxicant B. However, the protons at C-4 of the two components are so obscured by near coincidence that only the large coupling could be observed.

Table II. Differences in Chemical Shifts ( $\Delta\delta$ ,  $\text{C}_6\text{D}_6$ ) of the Ring Protons between Toxicants A-1 and B and Toxicants A-2 and B

H	(A-1) - B	(A-2) - B
3X	0.01	0.51
3N	0.00	0.03
4	0.73	0.72
5X	0.65	0.03
6N	0.08	0.14

Table III. Relative Toxicity of Chlorinated Hydrocarbon Insecticides to Goldfish

Compound	24-hr $\text{LD}_{50}$ , ppb	Toxicity rel. to tech. toxaphene
Toxaphene		
Technical	43	1
Toxicant A	1.7	25
Toxicant B	8.6	5
Endosulfan	3.9	11
Endrin	2.6	17

small effects on the remaining ring protons also argue against differing solvation. The extent to which C-Cl bond magnetic anisotropy alters chemical shifts in these molecules is evident from the diastereotopic protons of C-9 and C-10 of toxicant B, in  $\text{CCl}_4$  solution (Palmer et al., 1975) as well as in  $\text{C}_6\text{D}_6$ . It is concluded, therefore, that toxicant A consists of two positional isomers, not merely two conformers, and that in the major isomer, A-1, the dichloromethyl group is on the side of the 5X proton and in the minor isomer, A-2, it is on the side of the 3X proton.

In comparison with toxaphene itself, toxicants A and B are respectively 14- and 6-fold as toxic to mice and 4- and 2-fold as toxic to houseflies (Khalifa et al., 1974). Similar toxicity comparisons were made with goldfish (4.5–5.5 cm

length) held in groups of 4 in 4 l. of water under static conditions treated with various chlorinated hydrocarbons added in 50  $\mu\text{l}$  of ethanol. At least 3 doses were used that gave mortalities between 10 and 90%, and each series was repeated at least 3 times in determination of the log dosage-probit mortality plots for  $\text{LD}_{50}$  determinations. Table III shows the high toxicity of toxicant B and particularly A in relation to technical toxaphene and that their potency is similar to that of other chlorinated hydrocarbons known to have very high fish toxicity. When technical toxaphene is subjected to chromatography on the  $\beta$ -methoxypropionitrile-heptane column of Khalifa et al. (1974), the components most toxic to goldfish appear in exactly the same fractions and with the same peaks for potency as those most toxic to mice. Accordingly, it appears that isolation of toxaphene components based on goldfish toxicity would yield toxicants A and B as previously recovered by mouse toxicity monitoring.

Estimates of the content of toxicant A in toxaphene vary from a low of 0.3 to 0.4% based on isolation of crystalline material of the purity used here for  $^1\text{H}$  NMR studies, a procedure that involves large sacrifices in yield for the sake of product purity, up to 3 to 4% as determined by other analytical methods (Holmstead et al., 1974; Khalifa et al., 1974; Ohsawa et al., 1975). On the basis of its content in toxaphene and its biological activity, toxicant A appears to account for a major portion of the toxicity of toxaphene to both mice and goldfish.

Three toxaphene components (A-1, A-2, and B) are now known to have very similar structures. The stereochemical features they have in common appear to be important in conferring high biological activity. Further studies are necessary to separate toxicants A-1 and A-2 in order to determine if one isomer is more toxic than the other.

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## Duck Eggs as a Source of Methionine and Threonine

The amino acid composition of duck egg whites and yolks is reported, as well as distribution of protein in whites and yolks. The data indicate that duck egg whites are an excellent source of essential amino acids, especially methionine (6.3

g/16 g of nitrogen), and threonine (6.0 g/16 g of nitrogen). Cost estimations indicate that duck egg white essential amino acids are more economical than commercial sources of L-amino acids.

Essential amino acids are those amino acids that must be supplied in the diet. It is well known that fortification of many vegetable proteins with essential amino acids results in an improvement in the nutritional quality. Howe et al. (1965a,b) have shown that supplementation of major cereal and oilseed crops with only four essential amino acids (lysine, methionine, threonine, and/or tryptophan) is sufficient to raise their protein quality to a level with casein. As the human diet becomes more dependent for protein on cereals and oilseeds, supplemental sources of lysine, methionine, threonine, and tryptophan will become more important.

Amino acid supplementation of a human food is normally accomplished via the addition of free amino acids. However, supplementation with free amino acids is not without its problems, one of which is their flavor. Observations regarding taste have recently been reported and reviewed by Petritschek et al. (1972). Kies et al. (1972) have reported that methionine supplementation of oatmeal made that product unacceptable.

In addition, there are toxicity problems associated with free amino acids, as evidenced by the fact that they have been removed from the GRAS list, and with the exception of D-methionine, all D-amino acids are banned as food additives by the U.S. Food and Drug Administration (Schmidt, 1973). The same regulations also ban D-methionine in infant foods. In general, only the L-amino acids are permitted as food additives in restricted applications.

The purpose of the present paper is to provide information on the amino acid composition of duck eggs, with a view toward their use as a source of essential L-amino acids, particularly methionine and threonine.

An examination of "Amino Acid Composition of Foods" (FAO, 1970) indicated that of the 394 entries, duck egg white protein was highest in both total sulfur containing amino acids and also methionine, and fifth highest in threonine. It also ranks near the top in tryptophan, phenylalanine, and tyrosine. However, the reported analyses were based on only two samples, were for the whites only, and were from unpublished data. Therefore, it was felt that additional data were needed.

## METHODS AND MATERIALS

All duck eggs originated from Texas. Three large green-shelled (LG) eggs were obtained from a mixed flock of barnyard ducks fed milo and laying mash. Two medium white shelled (MW) eggs were obtained from the same flock. For the fresh eggs the whites were individually separated from yolks on egg separators, with clinging white scraped off yolks. Each shell was rinsed and blotted dry. Whole egg, yolk, and shell were each weighed and weight of white determined by difference.

A single egg from a wild duck (WD) was obtained, which was boiled and soaked in salt water for preservation. (This egg was about 1 month old when analysis began.) All samples were freeze-dried prior to amino acid analysis.

The amino acid analyses were performed with a Beckman Model 120 C analyzer. Cystine content was determined after oxidation to cysteic acid. Tryptophan was determined by the method of Kohler and Palter (1967). Each observation was made once for each of six eggs.

## RESULTS AND DISCUSSION

The weights of whites and yolks are reported in Table I. It may be noted that the fraction of total protein in the whites ranged from 0.43 to 0.58 for the three types of eggs, a difference which is statistically significant. This difference is also important because of the difference in amino acid content of white and yolk, to be discussed.

The amino acid contents of the whites and yolks are reported in Tables II and III. Besides the amino acids reported, an unidentified component was observed which was presumably a basic amino acid. This component is present at ca. the 1% level in the white and ca. 0.4% in the yolk (percent of protein). The unidentified peak was eluted 7 min before lysine with elution conditions of 55°, pH 5.27, 0.35 N citrate buffer. The data from the limited sample analyzed indicate that there is little difference in amino acid composition for the different kinds of eggs. The amino acid composition of the whites is in fair agreement with published data (FAO, 1970). May (1960) has shown that amino