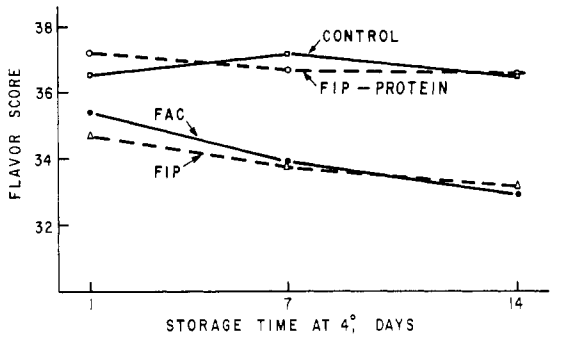

Figure 1. Effect of storage on beverage flavor quality of milks fortified with iron at the level of 10 mg/qt (FIP, ferripolyphosphate; FAC, ferric ammonium citrate).

SUMMARY

The iron from FIP–protein powders and FIP (aqueous) was found to be highly assimilable (92–100%) relative to ferrous sulfate, when fed by direct addition to animal diets. Iron from FIP, solid gel, was less well utilized (50–60%), but nonetheless can be considered a good source of biologically assimilable iron. The iron from FIP–protein remained highly assimilable in sterile concentrated whole milks despite the protocol of its addition.

No toxic or pathological effects were found in rats fed a dietary intake of 720 ppm of iron from FIP–protein for a 90-day period. Slightly increased splenic iron deposition was found when rats were fed 1000 ppm of Fe from FIP and FIP–protein for 90 days. Rats fed 10,000 ppm of Fe from FIP and $FeSO_4$ developed lesions from dietary iron overload, with $FeSO_4$ causing the more severe iron deposition.

FIP–protein, when used to fortify whole milk at 10 mg of iron/qt, maintained a beverage flavor quality comparable to the unfortified control for a period of 2 weeks.

FIP–protein and FIP solid and aqueous may serve as useful iron-enriching additives to dairy products and other commonly used foods such as flour and cereals. Processing trials in these commodities are in progress.

ACKNOWLEDGMENTS

We thank J. E. Moulton and D. Gould, veterinary pathologists, for microscopic examination of rat tissues, and Annette Kravitz and Mary Gavin for nitrogen and ash determinations.

LITERATURE CITED

Edmondson, L. F., Douglas, F. W., Avants, J. K., *J. Dairy Sci.* **54,** 1422 (1971).

Hazel, J. F., McNabb, W. H., McElroy, M. K., to Research Corp., U.S. Patent 3,403,971 (Oct 1, 1968).

Jones, S. B., Kalan, E. B., Jones, T. C., Hazel, J. F., *J. Agric. Food Chem.* **20,** 229 (1972).

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Separation and Comparative Toxicity of Toxaphene Components

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Components and fractions of toxaphene have been separated by a combination of methods including Sephadex LH-20–methanol column chromatography, thin-layer chromatography, reverse phase thin-layer chromatography, and preparative gas chromatography. Fractions separated by the above methods show varying toxicities to three aquatic organisms, a blue-green alga, brine shrimp, and mosquito larvae. Employing a combination of preparative TLC and GC methods, a toxic fraction of toxaphene 1.87, 1.75, and 1.35 times more toxic than toxaphene to mosquito larvae, brine shrimp, and algae, respectively, has been isolated. The fraction, though it behaves as a single component in various chromatographic systems, was found to consist of two components on the basis of nuclear magnetic resonance (NMR) spectroscopy. They were partially characterized by infrared and mass spectrometry as octachlorobornanes.

Toxaphene is a widely used insecticide. Two-thirds of its production is used for cotton insect control while other uses include vegetables, small grains, soybeans, and control of external insects on livestock. It has also been employed extensively in fish eradication programs (Muirhead-Thomson, 1971). Its annual production is about 50 million pounds (1971 estimate, Environmental Protection Agency, 1972) with a total usage of one billion pounds in the past 25 years. Despite this wide usage, little has been known about the chemistry, toxicity, metabolism, or environmental fate of its components. Only recently a major effort toward answering these questions has been made by Casida et al. (1974), who were successful in isolating and identifying a toxic component of toxaphene, 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane. Also isolated was a $C_{10}H_{10}Cl_8$ component which was more toxic to mice and houseflies than the above component; however, no structure was proposed for the latter component. According to them, at least 175 polychlorinated 10-carbon compounds were recognized by their methods (Holmstead et al., 1974). The components were described as polychlorobornanes, polychlorobornenes, and polychlorotricyclenes with 6 to 10 chlorine atoms per component.

Isolation and identification of all the components of toxaphene would be a monumental task. Therefore, at this stage of our understanding of toxaphene, it is necessary to limit study to the major toxic components. The definition of toxicity, however, is not so simple. The first problem is to define the target species. Casida et al. (1974), for instance, used mice and houseflies as test organisms to bioassay fraction and component toxicity.

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From an environmental standpoint, however, it is also important to assess the hazards in terms of its toxicity to nontarget organisms, particularly aquatic species. This is particularly important since results from several studies on the toxicity of toxaphene to nontarget organisms (Pimmentel, 1971) indicate toxaphene to be quite toxic to fish, many aquatic invertebrates, and most insects.

MATERIALS AND METHODS

Standard toxaphene (X-16189-49) was a gift from Hercules Incorporated, Wilmington, Del.

Gas chromatographic (GC) analyses and separations were performed on a Varian Model 1848 gas chromatograph equipped with a flame ionization detector (FI) and a ^3H electron capture detector (EC). Columns used in this work include: (1) SE-30, (2) QF-1, and (3) OV-1, each at 3% on Chromosorb G, 80–100 mesh, and packed in 1.6 m × 3 mm i.d. stainless steel columns. Preparative GC columns were 10% QF-1 and 10% SE-30 on Gas-Chrom P 60–80 and packed in 2 m × 6 mm columns. Also a 5% DC11, 3.3 m × 9 mm, was employed for some preparative GC separations. Fractions were collected in 4 mm × 15 cm glass tubes.

Column operating conditions for the gas chromatograph were: injector temperature, 215–225°; column temperature, 180 or 190°; detector temperature, 200–210°; N_2 carrier gas flow rate of 30–40 ml/min.

Thin-layer chromatography (TLC) plates were made with Brinkman-Desaga TLC equipment. Layers 0.25 mm thick were used for most separations except for preparative TLC, when plates with silica gel G of 0.5 mm thickness were used. After air drying, all plates were activated for 2 hr at 125°, and then stored in a dessicator. Four solvent developments with *n*-heptane to a distance of 15 cm above the origin were used to resolve toxaphene fractions. Plates were air dried between developments.

Reverse phase TLC (RPTLC) plates were prepared from activated plates by carefully immersing the silica gel G plates in a 5% (v/v) solution of paraffin oil (Fisher Scientific) in petroleum ether for 2 min. After this treatment the plates were kept overnight before use. Three solvent developments with methanol–water (9:1) (saturated with paraffin oil) were used to resolve toxaphene fractions. Chlorinated compounds were visualized by silver nitrate reagent spray followed by exposure to ultraviolet light. For preparative TLC, plates were spotted with a Brinkman TLC streaker. Bands were detected after solvent development by spraying only the edges of plates while the center portion was covered with aluminum foil. Bands of silica gel corresponding to spots on the edges of plates were scraped and the separated fractions were extracted with diethyl ether.

Gel permeation chromatography (GPC) of toxaphene on Sephadex LH-20 employed anhydrous methanol as the eluting solvent. The gel was swollen overnight in the solvent and slurry packed in a 1 × 110 cm glass column. Fractions were collected by a Gilson fraction collector. Preliminary experiments showed that no toxaphene eluted in the first 80 to 90 ml of solvent, and therefore a measured amount of 72–76 ml was run through the column before the start of collection of 2.0-ml fractions. Altogether 40 fractions were usually collected. The flow rate was 0.25 ml/min.

Mass spectra were taken on a Finnigan 1015 quadropole mass spectrometer (E.I., 70 eV). Both direct inlet and GC inlet systems were employed. Infrared (ir) spectra were obtained on KBr pellets of purified components using a Beckman IR33 spectrophotometer. Fourier transform NMR spectra were obtained on a 90-MHz Bruker FTNMR spectrometer.

Three test organisms were used for bioassay of toxaphene fraction toxicity. They were: (1) mosquito larvae, *Aedes aegypti;* (2) fresh-water blue-green algae, *Anacystis nidulans* (TX20); and (3) brine shrimp, *Artemia salina.*

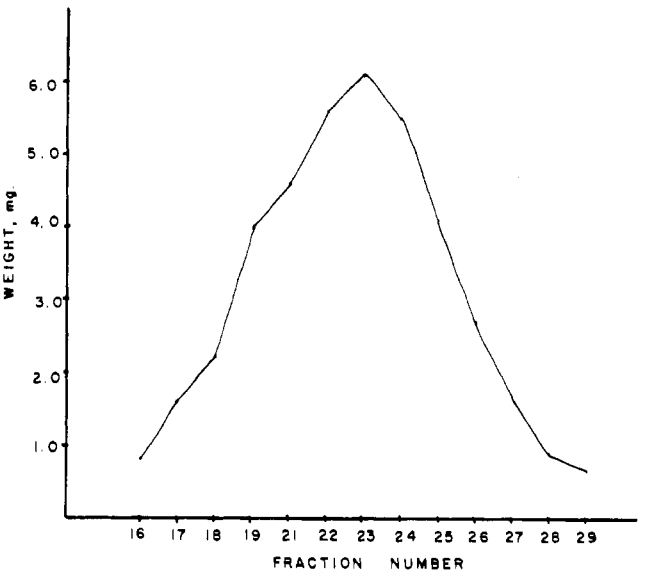

Figure 1. Elution of toxaphene from a 1.0 × 110 cm Sephadex LH-20 column with methanol. Chromatographic conditions: 40 mg of toxaphene dissolved in methanol was washed onto the column; then 74 ml of solvent was run through the column before 37 2-ml fractions were collected. Toxaphene components eluted into tubes 16 (104 ml) through 28 (130 ml). The flow rate was 15 ml/hr.

Mosquito larvae were reared in plastic containers 35 cm × 45 cm and fed a finely pulverized mixture of Purina lab chow and wheat germ (4:1). Ten CSMA strain mosquito larvae in the late third or early fourth instar were used per bioassay in 15 ml of water in a 20-ml vial. Insecticide solutions in acetone were added with a microsyringe. Volumes added ranged from 1 to 15 μl, with control insects receiving a volume of acetone equal to the largest treatment volume. Dead and moribund larvae were counted after 24 hr (24°). LC_{50} values were determined by probit analysis.

Brine shrimp were reared in artificial sea water (Instant Ocean, Aquarium Systems, Inc.), and fed a suspension of Baker's yeast. Brine shrimp (10 per vial, 6 to 7 days old) were transferred to 2-dram vials containing 7.5 ml of artificial sea water. Test solutions in acetone were added with a microsyringe as in the mosquito larvae bioassay. The mortality of brine shrimp was recorded after 24 hr (24°). LC_{50} values were determined by probit analysis.

The assay for inhibition of algal growth has been described by Batterton et al. (1971) and sensitivity to insecticidal compounds is reflected in the depression of the growth rate constant k. The only modifications were the incubation temperature which was 26 ± 1° and the solvent for adding test solutions which was acetone (10, 5, or 1 μl) in this case. Acetone-treated cultures gave k values similar to the control cultures without acetone.

RESULTS

Evaluation of Separation Methods. The isolation of toxic components of toxaphene is one of the primary goals of this work. Due to the complexity of the toxaphene mixture, it was concluded that a combination of separation methods would be necessary to isolate any one component. This would include a preliminary separation step or steps followed by preparative GC. The large number of components made it important to pursue only the most toxic components. Toxicity was monitored with three bioassay organisms, as described under Materials and Methods.

The efficiencies of preliminary separation methods were assessed by the GC patterns and toxicities of the separated fractions. Gas chromatograms of fractions were examined for the number of peaks and the relative amounts of each

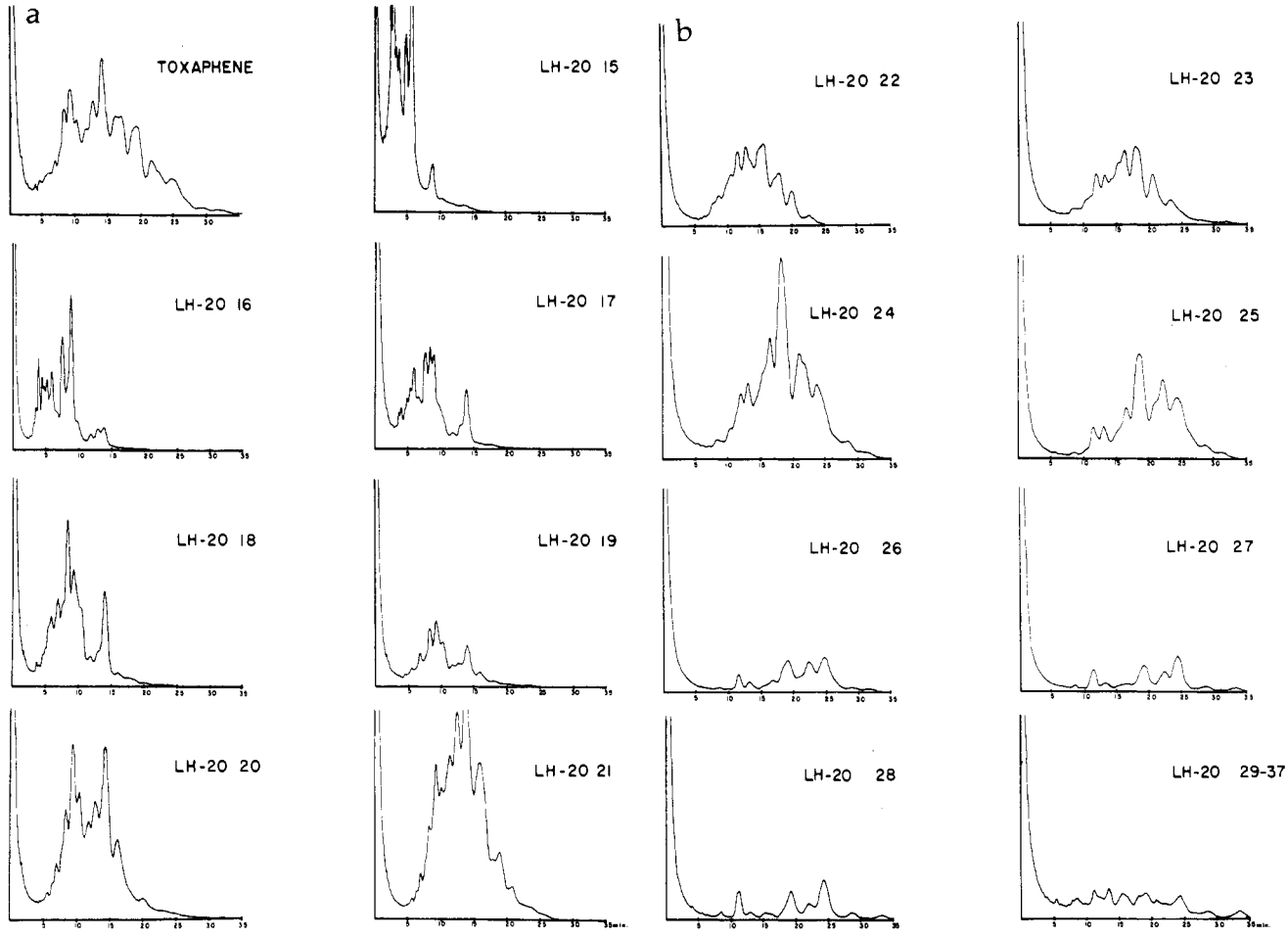

Figure 2. Gas chromatograms of toxaphene fractions collected from Sephadex LH-20–methanol column. GC conditions: 3% QF-1 on Chromosorb G, 80–100 mesh packed in a 3 mm × 1.6 m stainless steel column. Injector, column, and FI detector temperatures were 225, 190, and 200°, respectively. The N_2 carrier gas flow rate was 25 ml/min.

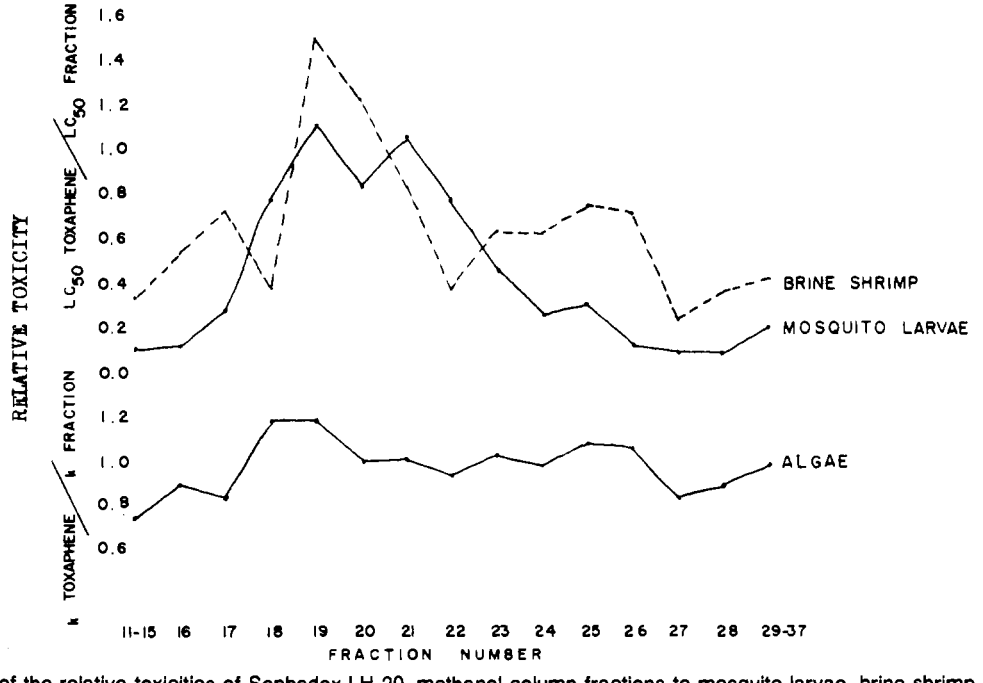

Figure 3. Graph of the relative toxicities of Sephadex LH-20–methanol column fractions to mosquito larvae, brine shrimp, and algae as compared with toxaphene (as 1). Values higher than 1 indicate toxicities greater than toxaphene. For column chromatographic conditions, see Figure 1. For the GC pattern of each fraction, see Figure 2. The *k* value for algae is a growth rate expression (the higher the *k* value the less inhibition) and the *k* value ratios were determined at an insecticide concentration of 1 ppm.

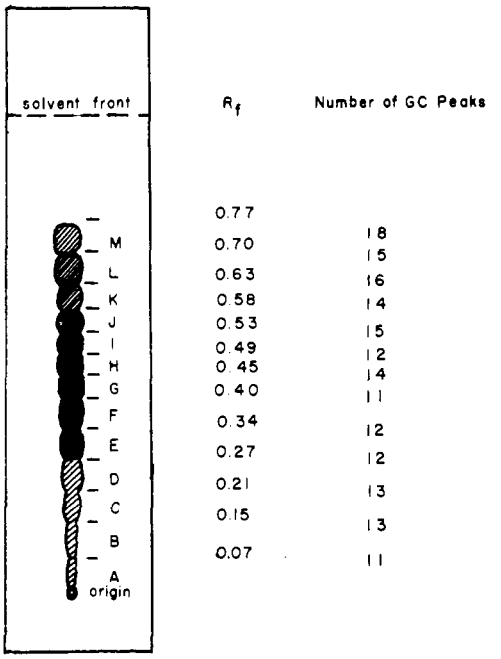

Figure 4. Representation of the separation of toxaphene on thin-layer chromatography; silica gel G plates, 0.25 mm thick, developed four times with *n*-heptane to 15 cm above the origin. R_f values refer to upper margins of spots. GC peaks were determined on 3% QF-1 column.

peak, the resolution of GC peaks, and the amount of GC peak overlap from one fraction to the next. Another consideration was the amount of material that could be handled by the method. With these criteria in mind, the following separation methods were examined: Sephadex LH-20–methanol column chromatography, preparative TLC on silica gel G developed four times with *n*-heptane, reverse phase TLC developed with methanol–water (9:1), and preparative gas chromatography on Dow 11 and QF-1 columns.

Sephadex LH-20–Methanol Column Chromatography. Toxaphene elutes from a Sephadex LH-20–methanol column in a roughly symmetrical peak (Figure 1). There is, however, a separation of components as judged by the changing GC patterns of consecutive fractions (Figure 2). The apparent complexity of the fractions follows the elution curve, i.e., early and late fractions are relatively simple while the middle fractions are more complex. This system apparently separates toxaphene on the same basis as the GC column (QF-1) as evidenced by short retention times of GC peaks in the early fractions with increasing GC peak retention times in successive fractions. There is definitely overlapping of GC peaks from one fraction to the next, but this is expected since the collection scheme of fractions was arbitrary. The elution of fractions was quite reproducible if careful attention was paid to elution volumes, as attested to by the consistency of GC patterns for corresponding fractions from several separation runs.

Fractions from a single LH-20–methanol separation of 40 mg of toxaphene were used for bioassay of toxicity to algae, mosquito larvae, and brine shrimp. The bioassay results are presented in Figure 3.

Toxaphene at 1 ppm inhibits the growth of algae, *Anacystis nidulans* (TX-20). This is reflected in an average k value of 0.327 ±0.009 (n = 10) for toxaphene vs. an average k value of 0.960 ± 0.072 (n = 33) for the acetone control. From Figure 3 it can be seen that no one fraction is clearly responsible for toxaphene's toxicity against any of the test organisms. Fractions 18 and 19 are the most toxic to algae followed by 25 and 26. In general, components of toxa-

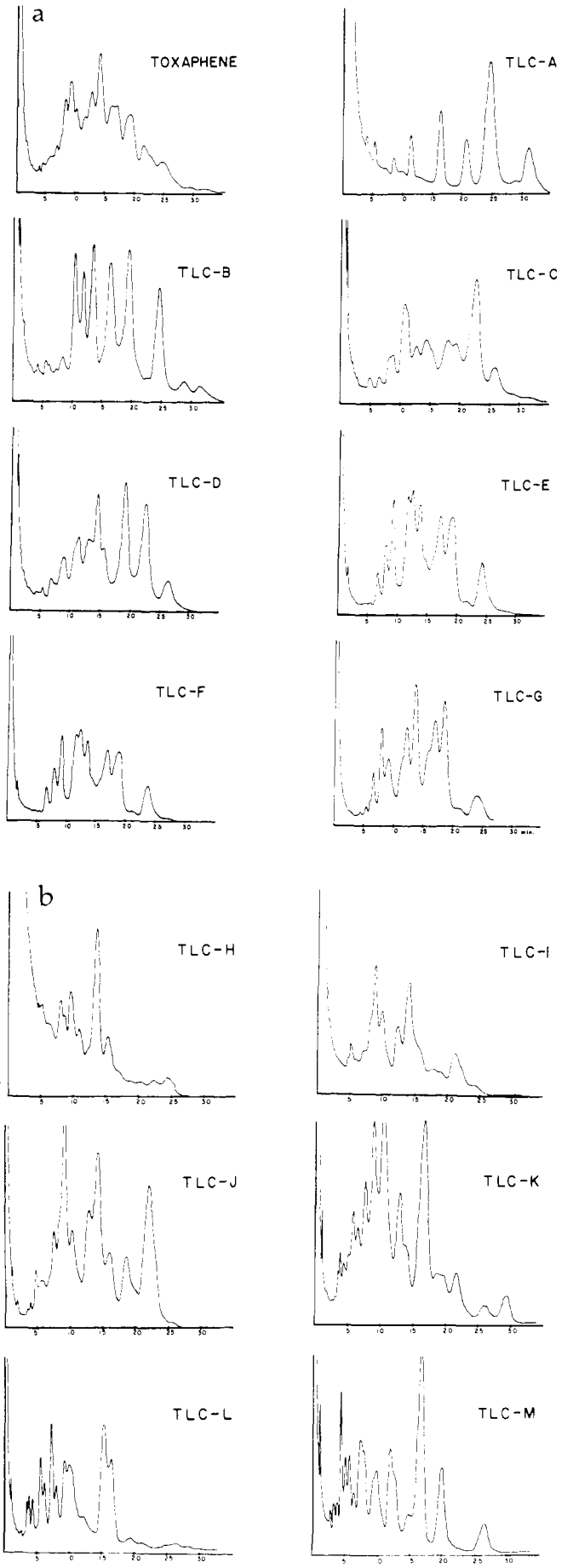

Figure 5. Gas chromatograms of toxaphene fractions separated by thin-layer chromatography. GC conditions are identical with those described in Figure 2.

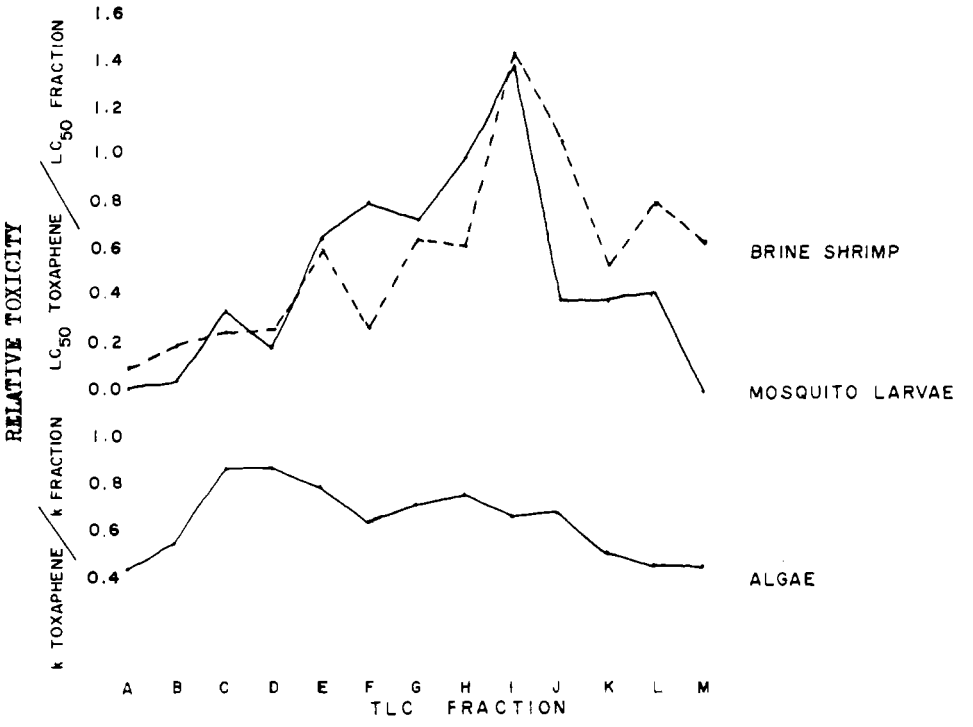

Figure 6. Graph of the relative toxicities of TLC separated fractions to mosquito larvae, brine shrimp, and algae as compared with toxaphene. For TLC conditions, see Figure 4. For GC patterns of each fraction, see Figure 5.

phene toxic to algae are spread among most of the LH-20 fractions.

Two fractions, 19 and 20, were more toxic than toxaphene to brine shrimp. All other fractions were less toxic than toxaphene. There was almost a sixfold difference in toxicity to brine shrimp between fractions 19 and 27.

Only two fractions were more toxic to mosquito larvae than toxaphene—again fraction 19 and also 21 (Figure 3). The major toxic fractions were between fractions 18 and 22. There was almost a tenfold difference in toxicity to mosquito larvae between fractions (19 vs. 27 and 28).

Only fraction 19 was consistently more toxic than toxaphene to all the bioassay organisms. The GC pattern of this fraction (Figure 2), although simpler than toxaphene, is still complex with five major peaks, several smaller peaks, and peak shoulders.

Preparative Thin-Layer Chromatography. Thin-layer chromatography of toxaphene on silica gel G with *n*-heptane gives a streak of unresolved components (Kovacs, 1963). However, if the plate is developed a second time with the same solvent, the resolution improves. If four solvent developments are used, a much better separation such as that represented in Figure 4 can be obtained. While this is still a streak in that no spot is completely resolved from the next, the spots can be distinguished as bulgings of the streak and by differences in intensities of the chromogenic reaction between spots. Gas chromatograms of the 13 spots thus separated are shown in Figure 5. The separation by this method is on a different basis than the Sephadex LH-20–methanol column since these TLC fractions give GC peaks with widely varying retention times in almost every fraction. This TLC method gives an added advantage in that the resulting GC peaks are better resolved within the TLC fractions. By this method, a total of 176 peaks are counted in 13 fractions. This figure could be misleading, since there was no good way of accounting for peak overlapping between TLC fractions, or components with identical retention times occurring in the same fraction.

Toxicity data for the TLC fractions are presented in Figure 6. Looking first at algae, again there are no outstanding toxic fractions. Fractions C and D are the most effective inhibitors of algal growth. Since the components toxic to algae are apparently quite evenly distributed among several fractions in two basically different separation systems, one must conclude that there are several components of toxaphene which are toxic to algae. If such is the case, the toxic action of toxaphene to algae is relatively nonspecific.

Fractions H, I, and J (Figure 6) were found to be relatively toxic to mosquito larvae and brine shrimp. Looking again at the GC patterns for these fractions (Figure 5), one sees that all these fractions have a major peak in common (retention time, 14 min) which later turned out to be the most toxic fraction. Among them fraction H showed the simplest GC pattern. For this reason, fraction H was used for purification of a toxic component as described later.

Reverse Phase Thin-Layer Chromatography. Reverse phase thin-layer chromatography of toxaphene on paraffin impregnated TLC plates developed three times with methanol–water (9:1) separates the components of toxaphene into 10 spots as represented in Figure 7. This system resolves toxaphene spots better than the regular TLC system described above. Despite the drawback that elimination of paraffin from toxaphene components adds another separation step, it may prove to be quite useful for checking the purity of fractions and components because of its high resolving capacity. These results suggest that new high-pressure liquid chromatographic methods using bonded phase columns in a reverse phase mode could be very useful in separation of toxaphene components.

Preparative Gas Chromatography. Two preparative GC columns (QF-1 and Dow 11) were used to separate toxaphene without preliminary separation steps. The peaks were collected as marked (Figures 8 and 9) and the fractions collected were used for mosquito larvae bioassay. Fractions 2, 4, and 5 were the most toxic fractions. This method has a high resolving power but a relatively low capacity, and therefore preparative GC was used as the final chromatographic purification step.

Isolation of a Toxic Component of Toxaphene (Figure 10). As indicated earlier, preparative TLC fraction H

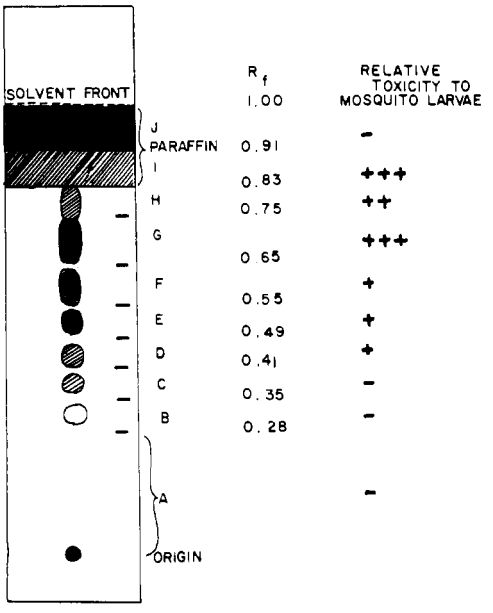

Figure 7. Representation of reverse phase thin-layer chromatographic separation of toxaphene. Paraffin-treated silica gel G plates were developed three times with a solvent mixture of methanol–water (9:1).

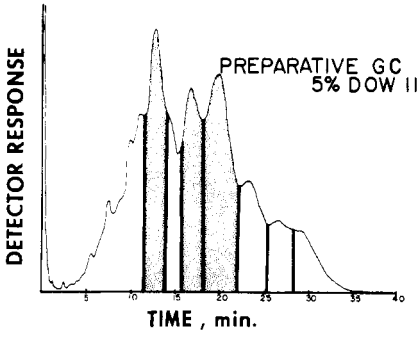

Figure 8. Preparative gas chromatogram of toxaphene on 5% Dow 11 column indicating the areas collected.

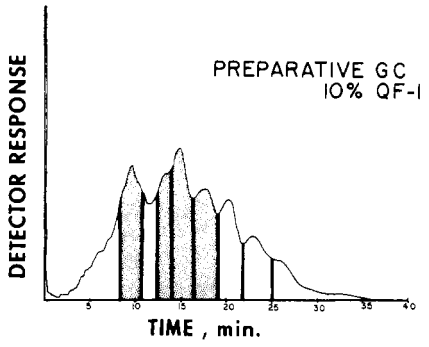

Figure 9. Preparative gas chromatogram of toxaphene on 10% QF-1 indicating the areas collected. The relative toxicities of fractions to mosquito larvae, as compared to toxaphene, are indicated by the shading.

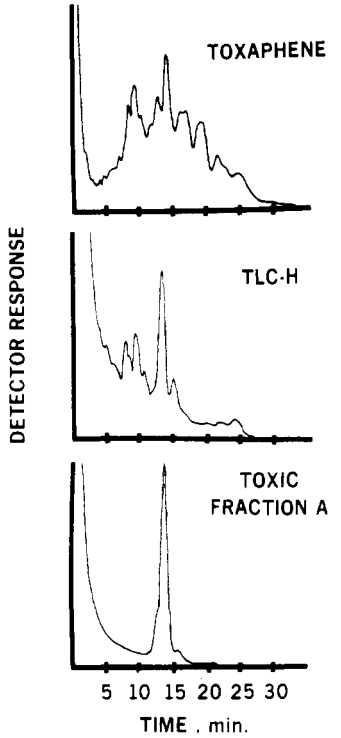

Figure 10. Chromatographic isolation of toxic fraction A. For GC conditions, see Figure 2.

was toxic to mosquito larvae and contained only one major GC peak. Twenty-four preparative TLC plates were spotted with toxaphene and developed four times with *n*-heptane. Bands of chlorinated compounds were detected by spraying the edges of plates with silver nitrate reagent followed by uv light exposure. The position of TLC band H on each plate was confirmed by scraping a small portion of silica gel from the plate adjacent to the H spot, adding solvent, and checking the GC pattern. By this way only fraction H was collected and pooled for solvent extraction, and subsequent preparative GC. The major peak, designated toxic fraction A, was purified by two preparative GC runs (QF-1). Toxic fraction A was checked on three different GC columns, 3% SE-30, 3% QF-1, and 3% OV-1, and was a single major peak in each system. There are two minor contaminants in toxic fraction A which could not be separated by the QF-1 column (Figure 10). Since the two contaminant peaks are not decreased relative to toxic fraction A by careful preparative GC, they could possibly be GC decomposition products of the main peak. On the other hand, the material in toxic fraction A formed a single spot on the reverse phase TLC.

Examination of the infrared spectrum of this fraction reveals the following absorptions: CH_2, 2920 and 2850 cm^{-1}; CH_2, 1460 cm^{-1}; CCH_3, 1450 cm^{-1}; CHCl, 1430 cm^{-1}; CH_2Cl, 1305 cm^{-1}; bicyclic five-membered ring strain, 880, 850, 840, and 805 cm^{-1}; and C–Cl stretching, 770 cm^{-1}. *gem*-Methyl absorptions at 1380 and 1365 cm^{-1} are reduced as compared to the standard reference compound, *exo*-2,10-dichlorobornane.

The mass spectrum (Figure 11) for this isolated toxic fraction is quite complex. The parent ion has a mass of 410 (Cl = 35), but the signals for this eight-chlorine cluster were very weak in comparison to other fragments. Prominent fragments are: 375 (7 Cl); 361 (7 Cl); 339 (6 Cl); a mixed cluster at 325 (6 Cl) and 327 (6 Cl); 303 (5 Cl); 291 (5 Cl); 267 (4 Cl); 243 (3 Cl); 231 (3 Cl); 193 (3 Cl); and 195 (3 Cl), another mixed cluster; 159 (2 Cl); and 83 (2 Cl). The major routes of fragmentation appear to be removal of chlorines, elimination of hydrogen chloride, and cleavage of chloromethyl and dichloromethyl moieties.

The spectral information for this fraction indicates a bicyclic ring structure with chloromethylenes, chloromethyl, and dichloromethyl groups. This fraction was more toxic to

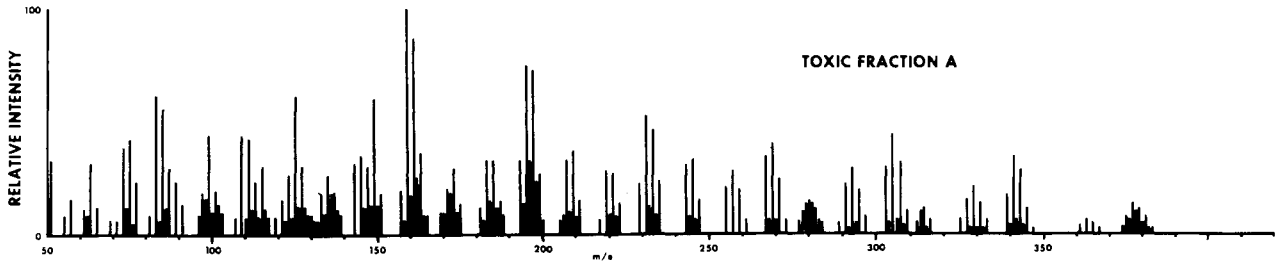

Figure 11. The mass spectrum of the toxic fraction A.

all three bioassay organisms with LC_{50} values of 0.056 ppm for mosquito larvae and 0.32 ppm for brine shrimp and a k value of 0.243 for algae growth inhibition which was, respectively, 1.87, 1.75, and 1.35 times more toxic than the toxaphene mixture.

DISCUSSION

Basic to most toxicological studies is a knowledge of the chemical structure of the compound studied. To date, only one toxic component of the complex mixture of polychloroterpenes known as toxaphene has been identified (Casida et al., 1974). The identification of individual toxaphene components hinges on one's ability to separate components from this complex mixture. To this end, this study has evaluated a variety of separation methods including Sephadex LH-20–methanol column chromatography, preparative thin-layer chromatography, reverse phase thin-layer chromatography, and preparative gas chromatography.

Separation of toxaphene by Sephadex LH-20–methanol column chromatography must be primarily by adsorption. The general pattern of toxaphene component elution from this system, lesser chlorinated compounds early and higher chlorinated later, suggests that there is a minimum of filtration occurring. This separation method, though it was not fully utilized in this study, may be developed into a useful technique to supplement preparative GLC which tends to give the problem of column bleed.

Both the TLC and RPTLC systems used in this work apparently separate toxaphene components on a polarity basis. The TLC system, silica gel G developed four times with n-heptane, has a larger sample capacity but lower resolving power than the RPTLC system, paraffin-treated silica gel G plates developed three times with methanol–water (9:1). Though the latter system was not utilized as a purification step in this attempt because of the presence of paraffin which made further purification schemes difficult, it appears to be a potentially useful system.

The TLC system in conjunction with preparative gas chromatography was used to isolate a toxaphene component which was more toxic to three bioassay organisms than the parent mixture. This toxic fraction was characterized spectroscopically as octachlorobornane.

Recently, Khalifa et al. (1974) published their method of isolation of toxaphene components. The major difference between their method and ours is that we adopt a multiple solvent development system TLC with separation based upon visual recognition of individual bands as they appear in the TLC plate while they use a liquid–liquid partition column chromatography with an arbitrary fractionation criterion. Otherwise, both methods follow a similar sequence of isolation procedures by heavily depending upon GC purification.

As judged by their chromatographic description, and by the mass spectrum (Holmstead et al., 1974), it appears that their toxicant A corresponds to the toxic fraction A isolated here. The NMR spectrum of the fraction in deuteriobenzene is complex, but there are a few isolated peaks which facilitate its interpretation. A doublet proton with a narrow splitting (2 Hz) at 6.8 ppm probably represents a $CHCl_2$ group at either C-8 or C-9 (all carbon positions represent those on the bornane skeleton), since an equivalent proton at C-10 should be a singlet. There is a proton cluster between 2.9 and 3.19 which is interpreted to represent a proton at C-4. The overall structure of the toxic component of this fraction is likely very similar to the toxicant B of Casida et al. (1974) except that it contains an extra chlorine at either C-8 or C-9. Several attempts to crystallize the material for an X-ray diffraction study were unsuccessful, nor was it possible to further purify the toxic component from this fraction.

The results of the toxicity studies show that at least several toxaphene components are responsible for the toxicity of toxaphene to three aquatic organisms. The toxic fraction A isolated here represents one of the most toxic major fractions, but there are several other toxic fractions particularly for the brine shrimp and the algae. This is in contrast to the finding of Khalifa et al. (1974) that two toxaphene components account for most of its toxicity to mice. Therefore, from an environmental standpoint, the toxicities, chromatographic character, and chemical structures of several toxaphene components need to be determined. The separation methods described in this work provide a means to that end. With such information, one can assess the fate of individual components and also the environmental implication of the use of toxaphene.

LITERATURE CITED

Anagnastopoulos, M. L., Parlar, H., Korte, F., *Chemosphere* **2,** 65 (1974).
Batterton, J. C., Boush, G. M., Matsumura, F., *Bull. Environ. Contam. Toxicol.* **6,** 589 (1971).
Buntin, G. A., U.S. Patent No. 2,565,471 (1951).
Casida, J. E., Holmstead, R. L., Khalifa, S., Knox, J. R., Ohsawa, T., Palmer, K. J., Wong, R. Y., *Science* **138,** 520 (1974).
DeVos, R. H., Peet, E. W., *Bull. Environ. Contam. Toxicol.* **6,** 164 (1971).
Environmental Protection Agency, Pesticide Study Series 5, "The Pollution Potential in Pesticide Manufacturing", 1972, p 44.
Holmstead, R. L., Khalifa, S., Casida, J. E., *J. Agric. Food Chem.* **22,** 939 (1974).
Khalifa, S., Mon, T. R., Engel, J. L., Casida, J. E., *J. Agric. Food Chem.* **22,** 653 (1974).
Kovacs, M. F., Jr., *J. Assoc. Off. Anal. Chem.* **46,** 884 (1963).
Muirhead-Thomson, R. C., "Pesticides and Freshwater Fauna", Academic Press, New York, N. Y., 1971, p 72
Pimmentel, D., "Ecological Effects of Pesticides on Non-Target Species", Executive Office of the President, Office of Science and Technology, 1971, p 70.
Stalling, D. L., Huckins, J. N., *J. Assoc. Off. Anal. Chem.* **56,** 367 (1973).

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