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# Evaluation of the Toxic Components of Toxaphene in Lake Michigan Lake Trout

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Lake trout from Lake Michigan were analyzed for residues of the insecticide toxaphene and two of the primary toxic constituents, toxicants A and B. Using various chromatographic techniques and mass spectrometric confirmation, we have identified these toxic congeners in Lake Michigan lake trout residues. Levels of toxicants A and B have been found to be roughly 1 order of magnitude or more less than the estimated total toxaphene residue. Since the environmentally derived toxaphene is extensively altered in comparison to the technical material, measurment of toxic congeners may be a more toxicologically relevant measure of toxaphene derived residues.

## INTRODUCTION

Contaminant residues in Great Lakes fish are well documented and have been widely studied for a number of years (Schmitt et al., 1983). In the majority of cases the residues are derived from single components and represent a relatively simple task for quantitation and evaluation. Until recently, analysts have not had adequate techniques for assessment of residues derived from complex mixtures. With the development and routine usage of capillary column gas chromatography and capillary GC/mass spectrometry, a more complex variety of chemicals is being detected and analyzed at low concentrations.

One such complex mixture is the chlorinated hydrocarbon insecticide, toxaphene. Toxaphene is a chlorinated organic insecticide composed of over 177 components (Holmstead et al., 1974; Saleh, 1983) which, until recently, was the most widely used chlorinated pesticide in the United States. Recent studies of Great Lakes fish have shown high residue levels of chlorinated hydrocarbons which appear to be very similar to toxaphene (Schmitt et al., 1981, Rappe et al., 1979, Rice and Evans, 1984). Alterations in composition of the mixture prior to and during deposition as a residue creates difficulty for accurate quantitation and evaluation due to the lack of a suitable analytical standard (Musial and Uthe, 1983; Jansson et al., 1979; Wideqvist et al., 1984).

Since the changes which give rise to the residue pattern are so significant, semiquantitative residue levels of overall "toxaphene" may not accurately reflect the true toxicologic nature of the material being measured. Studies have shown that toxicity to various organisms is not distributed evenly among various fractions of technical toxaphene. Studies by Nelson and Matsumura (1975), Saleh et al. (1977), and Isensee et al. (1979) have shown that toxicity to invertebrates and fish can be attributed to several components, while mammalian toxicity is apparently more restricted. In addition, Harder et al. (1983) have shown that anaerobic sediment decomposition products are at least as toxic as technical material to two species of estuarine fish.

Because of the complexity of the technical mixture, very few of the components have been isolated and identified. Studies by Turner et al. (1977) and Saleh et al. (1977) contain excellent summaries of several chemical structures that have been isolated and their toxicities. It should be noted that only small quantities of a few of these compounds exist, or are available for routine analytical use.

In all of the studies to date, including those of Seiber et al. (1975) and Pollock and Kilgore (1980), two constituents of toxaphene have proven to be the most toxic to all organisms tested. Toxicant B (2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane, heptachlorobornane I) was originally isolated and identified by Casida et al. (1974). Toxicant A (a mixture of 2,2,5-*endo*,6-*exo*,8,9,9,10- and 2,2,5-*endo*,6-*exo*,8,8,9,10-octachlorobornanes) was isolated and identified by Turner et al. (1975) and Nelson and Matsumura (1975). These two components comprise approximately 8% of the toxaphene mixture as manufactured by the Hercules Chemical Co., Wilmington, Delaware (Casida et al., 1974; Saleh, 1983; Turner et al., 1975). This study was conducted to investigate the presence and potential use of quantitation of toxicants A and B as a more toxicologically meaningful measure of residues derived from toxaphene. Here, we report the presence and levels of these two constituents in lake trout from Lake Michigan.

## EXPERIMENTAL SECTION

**Materials.** The lake trout examined in this study were obtained as incidental catch from commercial fishing nets approximately 5 miles south of Muskegon, MI, in Lake Michigan on Aug 6, 1982. Fish (50–60 cm total length) were kept on ice approximately 10 h before being frozen in individual plastic bags.

**Methods.** For analysis, fish were partially thawed and a sample of the "belly flap" region was removed. This region is rich in adipose tissue and was chosen since it is

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expected to contain higher levels of contaminants than other portions of tissue. Fish were extracted and apparent toxaphene residues isolated essentially as described by Ribick et al. (1982), with omission of the final nitration procedure. Briefly, this method involves grinding tissue with anhydrous sodium sulfate and column extracting with methylene chloride at a flow rate of 3–5 mL/min. Lipids are removed via gel permeation chromatography on Bio-Beads (SX-3) by using 1:1 methylene chloride–cyclohexane. The residue is fractionated on florisil to remove some of the cyclodiene insecticides, followed by silica gel to remove interfering polychlorinated biphenyls (PCBs). The final extract contains toxaphene, chlordane, DDT (etc.), hexachlorocyclohexane (BHC), and several other chlorinated pesticides. Replicate samples were periodically spiked with [$^{14}$C]toxaphene in order to determine recovery. Lipid analysis was not conducted as it has been shown (Schmitt et al., 1983) that differing lipid levels do not generally explain variations in residue levels. All reagents used were pesticide residue grade. Recovery from spiked samples was generally >90%. Residue levels were not corrected for recovery.

**TLC Purification of Toxicant A.** Toxicant A is a variable mixture of two octachlorobornane components which chromatograph with identical retention times on capillary GC (Saleh and Casida, 1978; Gooch and Matsumura, unpublished data). The $\alpha$ (Nelson and Matsumura, 1975) or A-1 (Turner et al., 1975) component is 2,2,5-*endo*,6-*exo*,8,9,9,10-octachlorobornane while the $\beta$ (B-1) component is 2,2,5-*endo*,6-*exo*,8,8,9,10-octachlorobornane. Saleh (1983) contains a good description of the basic structural framework of the variably chlorinated bornane, bornene, and bornadiene components of toxaphene.

Silica gel 60 F-254 (250 $\mu$m, E. Merck) plates were heated at 130 °C prior to use. The final extracts from the procedure described above were spotted and plates developed 4 times with *n*-heptane (saturated chamber). Toxaphene chromatographs as a streak with several distinct spots under these conditions (Nelson & Matsumura, 1975). Authentic toxicant A was chromatographed under identical conditions to determine the appropriate $R_f$ region to isolate. A region corresponding to an $R_f$ of 0.30–0.43 was scraped, placed into a chromatographic tube and eluted with 25% diethyl ether in hexane. This procedure separates some of the toxaphene components from *p,p'*-DDT which migrates with an $R_f$ >0.60.

**Gas Chromatography and Quantitation of Residues.** Extracts were analyzed with a 30M × 0.25 mm i.d. DB-1 (J&W Scientific) fused silica capillary column with helium as the carrier gas (fixed pressure, 140 kPa). Samples were injected (injection temperature 200 °C) in the split mode (split ratio 3:1) with the column at 190 °C. The oven was immediately programmed to 260 °C at 2 °C min. The Sc$^3$H foil electron capture detector was operated with a nitrogen makeup gas flow of 30 mL/min at 280 °C.

Data were collected with a Spectra Physics 4270 integrator. Technical toxaphene standards, originally obtained from the Hercules Chemical Co., (Lot No. X16189-49) were used for comparison with residues. Peak (30–32) areas (after *p,p'*-DDE with the appropriate retention times, window 0.1 min) were used for quantitation of the residue. Retention times used were either absolute or relative to *p,p'*-DDE. Quantitation of "estimated total toxaphene" was done by using methods employed by several other authors, whereby peak areas are summed for selected peaks with identical retention times to those of the analytical standard. The same peaks are summed for several concentrations of a known quantity of standard for generation of a standard curve. This method assumes equal peak response factors for analogous peaks in the residue and standard, a situation which may or may not be true. For this study, 30 peaks eluting after *p,p'*-DDE were used for quantitation. Peak matching was done manually by using a light table and overlaying residues on technical standards spiked with *p,p'*-DDE. By using this method we were able to more accurately determine peak matches than by relying on consistent performance of our integrator. After viewing a large number of residue chromatograms, it is readily apparent that the residue profile, in terms of presence or absence of peaks, is very similar between fish, sights, and years (this subject will be discussed in more detail in a future report). Thus, the same peaks can be used for a large number of samples.

Quantitation of toxicants A and B was done by using peaks matched with NMR certified standards isolated in this laboratory (Nelson and Matsumura, 1975; Matsumura et al., 1974). Toxicant B was quantitated in the original extract prior to TLC purification of the residue for toxicant A. Because of the limited amount of material available (micrograms), we have used our standards for peak identification purposes only. In order to generate standard curves for toxicants A and B we have used average fractional composition estimates based on available information in the literature. Using the mass spectrometric data of Casida et al. (1974), Turner et al. (1975), Khalifa et al. (1974), Holmstead et al. (1974), and Saleh (1983), we have arrived at estimates of 4.8% for toxicant A and 3.4% for toxicant B.

Masses for the respective toxicants in the mixture can be derived by multiplying the mass of the standard times the proportional composition (0.048 or 0.34) estimate and using the corresponding mass/peak area relationship to generate a standard curve. Although this is an estimate, we feel that the proportional estimates for composition are sound and thus can be used by other investigators when no technical standard is available, as long as identification of the appropriate peak can be made.

Capillary gas chromatography/mass spectrometry was done by using a Hewlett Packard 5985A quadrupole mass spectrometer operated in the electron impact mode (70 eV). A 30 M × 0.25 mm i.d. DB-1 fused silica capillary column was used and operated essentially as described for routine ECD analysis although injections were done in the splitless mode. Approximately 700 spectra were acquired during each run (approximately 17 scans/min). Spectra of the individual peaks were analyzed manually for the appropriate characteristics.

## RESULTS AND DISCUSSION

Figure 1 demonstrates the difficulty in accurately conducting toxaphene residue analysis in Great Lakes fish. There is a dramatic difference between environmental residues and technical toxaphene, a phenomenon noted in Canadian east coast marine fish (Musial and Uthe, 1983) as well as seals from Swedish waters (Jansson et al., 1979). Zell and Ballschmiter (1980) called attention to the widespread occurrence of toxaphene (polychlorinated camphenes (PCC)) in spawn from fish from several different areas but only used four key reference peaks for quantitation, though technical toxaphene contains at least 177 different components. Part of the difficulty stems from the fact that many toxaphene components are readily degraded by microbes (Clark and Matsumura, 1979), and there are several unrelated compounds in the residue that coelute and interfere with accurate quantitation of toxaphene components. Most of the compounds found in samples after silica gel purification are related to technical

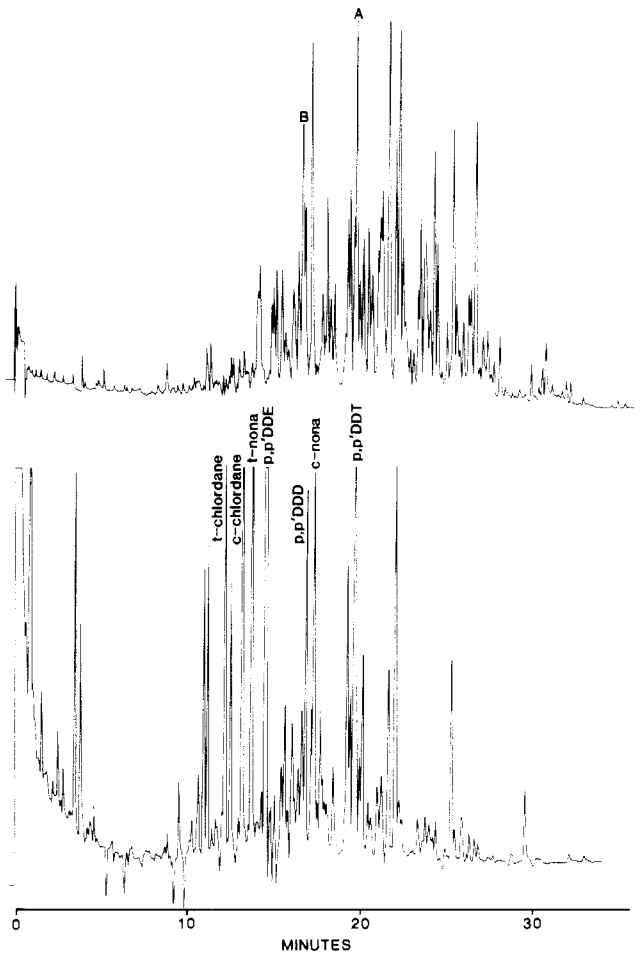


**Figure 1.** Capillary column GC-ECD traces of toxaphene (above) and the toxaphene fraction of a lake trout residue from Lake Michigan (below). Conditions are reported under the Experimental Section. Locations of toxicants A and B are indicated.

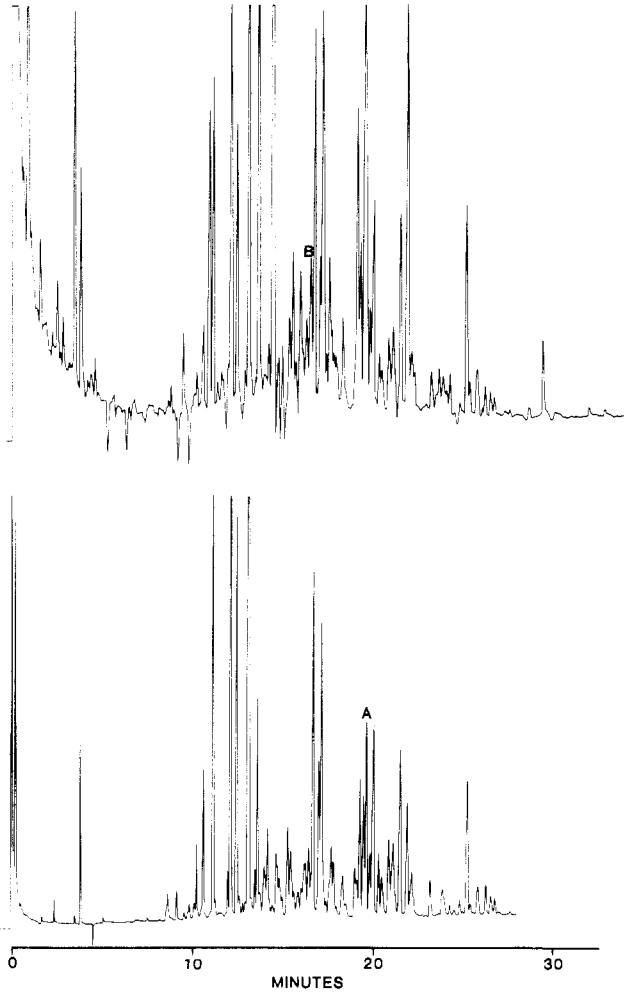


**Figure 2.** Capillary column GC-ECD traces of the toxaphene fraction of a Lake Michigan lake trout residue prior to (above) and after (below) TLC purification for toxicant A. Locations of toxicants A and B are indicated.

chlordane and the DDT complex. Nitration procedures are available for selective removal of the aromatic compounds, a procedure we feel is useful, though not absolutely necessary for reliable "overall" toxaphene analysis. However, as we will show, some type of purification procedure must be used for accurate quantitation of the toxic congeners. In our samples, the ubiquity of the DDT compounds was useful for determining relative retention times since they chromatograph in strategic regions. None of the compounds eluting prior to *p,p'*-DDE were considered for quantitation due to interferences from chlordane components. In our system this excludes very few components since the vast majority of the standard toxaphene elutes after these compounds (Figure 1). In general, we were able to match approximately 30 peaks of the residue to the toxaphene standard. Since this is only a fraction of the available peaks, other peaks must either be transformation products or compounds unrelated to toxaphene. The mass spectral data indicates that most of the peaks following *p,p'*-DDE have a polychlorinated bornane type of structure. Since accurate quantitation relies on matched peaks having the same composition and response factor, quantifying this residue can only be considered a rough estimate, a concept agreed upon by many authors (Jansson and Wideqvist, 1983; Musial and Uthe, 1983).

Since toxicants A and B exert substantial toxicity in all systems investigated (Casida et al., 1974; Nelson and Matsumura, 1975), it is important to quantify these components in relation to the overall total. This should provide a more reliable indication of the toxicologic potential of the residue. Initially, we used thin-layer chromatography of a lake trout residue and authentic toxicant A to look for this component. Figure 2 is a capillary gas chromatogram of a lake trout residue before and after being fractionated in this manner. When this procedure is carried out, a peak free of *p,p'*-DDT remains with the same $R_f$ as authentic toxicant A. It also possesses an identical retention time on capillary GLC and has the same retention characteristics on florisil and silica gel. Investigation of gas chromatograms of initial extracts for toxicant B suggested that no post silica gel purification was necessary (see Figure 1).

To confirm the presence of toxicants A and B in lake trout samples, we employed capillary GC–MS in the electron (EI) impact mode with the pesticide fraction from the silica gel column. Figure 3 (top) shows the mass spectrum of a toxaphene component coeluting with *p,p'*-DDT. The clusters at masses 352, 316, 235, and 165 are from *p,p'*-DDT with masses 352 and 316 corresponding to the parent ion ($M^+$) and a fragment from the loss of HCl, respectively. Toxicant A elutes incompletely resolved from *p,p'*-DDT in this region. For identification of toxicant A we have relied primarily on the fragment clusters at masses 375, 339, and 303. The cluster at 375 corresponds to the $[M - Cl]^+$ ion from a compound having a $C_{10}H_{10}Cl_8$ ($M_r$ 410) formula. For comparison, a mass spectrum of toxicant A as it elutes from technical toxaphene under the same GC conditions employed for the residue is shown (Figure 3, bottom). The lack of a parent ion near mass 410 is

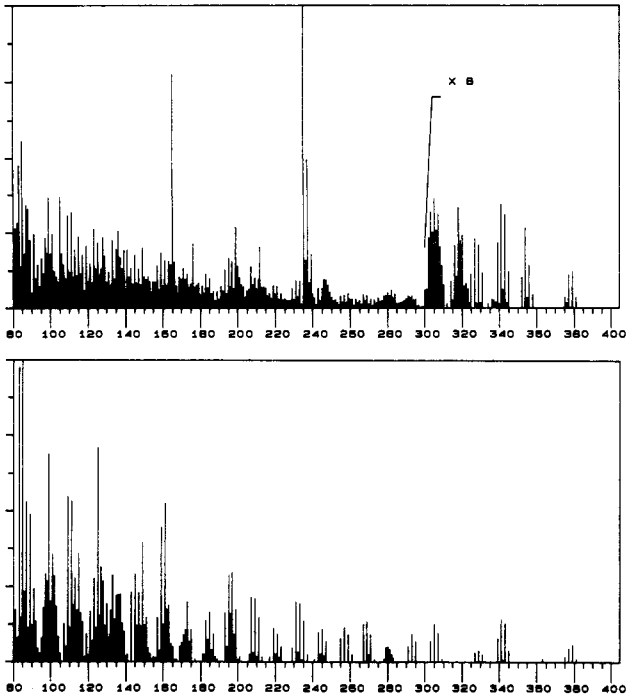

**Figure 3.** Electron impact mass spectra of toxicant A (below) and of toxicant A (above) coeluting with *p,p'*-DDT from a lake trout residue during a capillary GC–MS run. The spectrum of toxicant A (below) was acquired during a capillary GC–MS run of technical toxaphene.

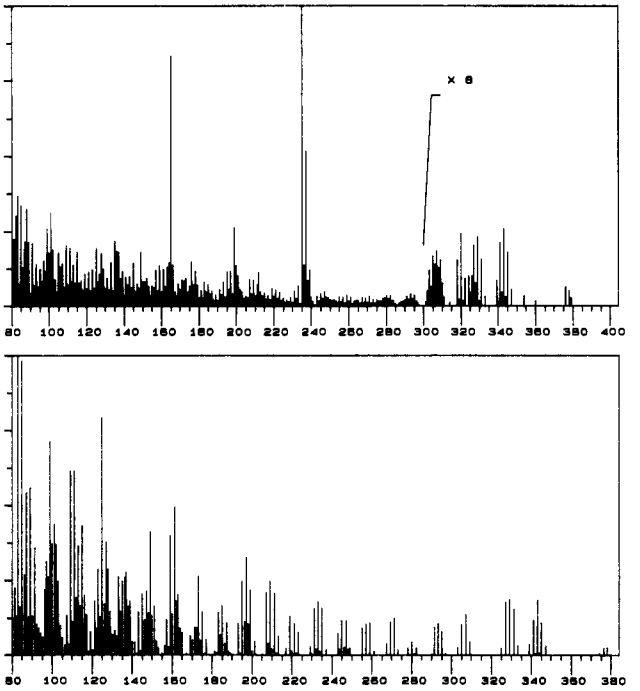

**Figure 4.** Mass spectra of toxicant B eluting from a technical standard (below) and of toxicant B coeluting with *p,p'*-DDD in a lake trout residue (above).

typical of toxaphene components (Saleh, 1983; Holmstead et al., 1974). The results supporting the identification of toxicant A are the mass spectrum indicating a bornane structure with 8 chlorines, matching $R_f$'s on thin-layer chromatography, similar retention on florisil and silica gel columns, and capillary gas chromatography with authentic toxicant A.

As stated earlier, identification of toxicant B in residues was achieved by matching retention times of an authentic standard to that of a corresponding peak in the extract

**Table I. Levels of the Two Primary Toxic Constituents and Estimated Total Toxaphene Found in Lake Trout from Lake Michigan**

| fish | estimated[a] total toxaphene | toxicant A | toxicant B |
|---|---|---|---|
| 1 | 10.7 | 0.70 (6.6)[b] | 0.21 (2.0) |
| 2 | 1.9 | 0.08 (4.4) | 0.08 (4.0) |
| 3 | 1.6 | 0.12 (7.1) | 0.06 (3.7) |
| 4 | 2.9 | 0.12 (4.2) | 0.04 (1.4) |
| X ± S.E. | 4.3 ± 3.7 | 0.26 ± 0.26 | 0.10 ± 0.07 |

[a] All residue values are in μg/g (ppm) wet weight. Estimated total toxaphene levels have been derived on the basis of equal electron capture response factors for all components (see text). [b] Numbers in parentheses indicate the percent composition of the toxic components in the estimated toxaphene total.

prior to TLC purification for toxicant A. Figure 4 shows the mass spectra acquired in this region which support our identification. Toxicant B, labeled in Figures 1 and 2, can be seen to elute just prior to *p,p'*-DDD. With the small loss in resolution we encountered in the GC–MS system and the relatively small amount of toxicant B present, the spectra acquired show a coelution with *p,p'*-DDD. The masses at 318, 235, and 165 identify *p,p'*-DDD with the mass at 318 corresponding to the parent ion ($M^+$). The lower spectrum of toxicant B as it elutes during a capillary GC–MS run shows a weak $M^+$ ion at mass 376 ($C_{10}H_{11}Cl_7$) and complex characteristic fragments at masses 339, 325, and 303. Identical fragments can be seen in the peak that coelutes with *p,p'*-DDD. If it is indeed true that these residues are derived from the use of toxaphene, we would expect both of these compounds to be present.

Table I shows the results of the analysis of four Lake Michigan lake trout for polychlorinated bornanes (estimated total) and more specifically for the toxic congeners. The level of toxicant A is more than an order of magnitude less than the total residue while toxicant B is 2–3 times less than toxicant A. Since these are two of the most universally toxic congeners, and we have no other good reference point, this residue level may be more toxicologically relevant. We are currently conducting more detailed studies to further explore this suggestion.

Estimates of the amount of toxicant A and toxicant B in technical toxaphene varies from 1.5 to 8.4% for toxicant A and from 2.5 to 4.1% for toxicant B. On average, toxicant A is approximately 4.8% and toxicant B is approximately 3.4% (Casida et al., 1974; Turner et al., 1975; Saleh, 1983; Khalifa et al., 1974; Holmstead et al., 1974) of the total mixture. When these estimates and the mean values from Table I are used, it appears that the environmental residues of toxaphene have nearly the same relative amounts of the toxic components as the technical mixture. This is somewhat surprising, and perhaps coincidental, since the estimates for the total apparent toxaphene is subject to such large assumptions during quantitation. Indeed, since toxaphene must be extensively altered by degradative processes (Musial and Uthe, 1983; Jansson et al., 1979; Zell and Ballschmiter, 1980), this similarity of ratios was unexpected. Perhaps the rate of environmental transport and metabolic degradation of toxicant A and B may be similar to the rest of toxaphene residue as a whole, an observation suggested by Clark and Matsumura (1979) who have shown that the rate of degradation of toxicant A by microorganisms and in sediment was roughly identical with the overall mixture. Experiments are currently being conducted in order to more clearly understand the nature of the processes, such as biotransformation, selective uptake, and/or depuration etc., involved in the gen-

eration of the residue pattern in lake trout.

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**Registry No.** Toxicant B, 51775-36-1; toxaphene, 8001-35-2; 2,2,5-*endo*-6-*exo*-8,9,9,10-octachlorobornane, 58002-18-9; 2,2,5-*endo*-6-*exo*-8,8,9,10-octachlorobornane, 58002-19-0.

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# Macrolide Aggregation Pheromones in *Oryzaephilus surinamensis* and *Oryzaephilus mercator* (Coleoptera: Cucujidae)

Aldona M. Pierce,* Harold D. Pierce, Jr., Allan C. Oehlschlager, and John H. Borden

*Oryzaephilus surinamensis* (L.), the sawtoothed grain beetle, and *O. mercator* (Fauvel), the merchant grain beetle, utilize male-produced, marcolide aggregation pheromones. These compounds were isolated from the Porapak Q-captured volatiles obtained from adult beetles feeding on rolled oats. The pheromones were identified as (*Z*)-3-dodecen-11-olide (I) and (*Z,Z*)-3,6-dodecadien-11-olide (II) for *O. mercator* and (*Z,Z*)-3,6-dodecadien-11-olide (II), (*Z,Z*)-3,6-dodecadienolide (III), and (*Z,Z*)-5,8-tetradecadien-13-olide (IV) for *O. surinamensis*. Structures of isolated I, II, III, and IV were confirmed by comparison with synthesized materials. Laboratory bioassays with *O. surinamensis* indicated that IV synergized the response to a mixture of II and III, while I and II were not synergistic for *O. mercator*.

## INTRODUCTION

The sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), and the merchant grain beetle, *O. mercator* (Fauvel), are stored-product pests of worldwide distribution. A recent survey of stored-product insects across the United States (Mueller, 1982) ranked *O. surinamensis* first in importance as a pest of stored products and processed food and second in importance as a problem on raw grain. Being less tolerant of low ambient temperatures (Howe, 1956), *O. mercator* has become firmly established as a household pest in North America, especially on cereal products (Loschiavo and Sabourin, 1982) and on processed food in general (Mueller, 1982). Moreover, *O. mercator* frequently has been misidentified as *O. surinamensis* due to the morphological similarity of the two species (Loschiavo and Sabourin, 1982).

Identification of pheromones and food attractants for *Oryzaephilus* species could contribute to the development

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