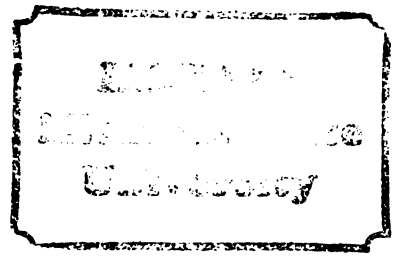

This is to certify that the

thesis entitled

AN EVALUATION OF THE CHEMODYNAMICS OF
FIREMASTER 680 IN THE AQUATIC ENVIRONMENT

presented by

Ralph Lawrence Bednarz

has been accepted towards fulfillment
of the requirements for

M.S. degree in Limnology

Frank M. D'Itri

Major professor

Date April 14, 1982

AN EVALUATION OF THE CHEMODYNAMICS OF FIREMASTER 680 IN THE AQUATIC ENVIRONMENT

by

Ralph Lawrence Bednarz

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ABSTRACT

AN EVALUATION OF THE CHEMODYNAMICS
OF FIREMASTER 680 IN THE
AQUATIC ENVIRONMENT

By

Ralph Lawrence Bednarz

The primary purpose of this investigation was to develop a base set of data to predict the fate of Firemaster 680 in the aquatic environment. This compound is an important brominated aromatic flame retardant for use in thermoplastic applications. A recent contamination incident with Firemaster 680 has been identified in the Raisin River watershed near Adrain, Michigan.

Commercial grade Firemaster 680 was identified as 1,2-bis(2,4,6-tribromophenoxy)ethane and is 99% pure. The aqueous solubility was measured as 0.04 ± 0.01 ug/L. The n-octanol/water partition coefficient was determined to be 1.38×10^7. This value was used to estimate an organic carbon normalized sediment distribution coefficient of 5.25×10^6 and a fish bioconcentration factor of 1.58×10^5. Bacteria capable of degrading Firemaster 680 were not found during acclimation, analog enrichment, or cometabolism studies.

This study revealed that the degree of adsorption to sediments and suspended matter will most likely dictate the ultimate fate of Firemaster 680 in the aquatic environment.

To Our Son, Kenton, And

Our Forthcoming Child

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INTRODUCTION

OVERVIEW

Over the past few decades the chemical industry has experienced an unparalleled expansion with the development of many new organic chemicals that have very diverse applications. Approximately four million chemicals are presently known, with about fifty thousand chemicals in everyday use, not including pesticides, pharmaceuticals, and food additives (Maugh, 1978). Under the U.S. Environmental Protection Agency's Toxic Substance Control Act, the initial Chemical Substances Inventory and Cummulative Supplement lists a total of 55,103 chemicals which are manufactured, imported, or processed for commercial purposes in the United States (U.S. EPA, 1980a). This tremendous increase in chemical development and production has resulted in a substantial burden for the environment. Iliff (1972) has suggested that, worldwide, up to 40 billion pounds of manufactured organic chemicals enter the environment annually.

The introduction of synthetic organic chemicals to the environment during the last thirty years has already had a significant impact on the biosphere. A number of catastrophes have resulted from environmental contamination by such chemical agents as mercury, cadmium, polychlorinated biphenyls (PCB), vinyl chloride, chlorinated dioxins, and numerous chlorinated pesticides (e.g., DDT, Kepone, Mirex). A more recent chemical contamination incident was the polybrominated biphenyl (PBB) tragedy which occurred in Michigan (Kay, 1977). This disaster was one of the most extreme

cases of chemical contamination of domestic animals and the human population ever recorded.

Polybrominated biphenyls are members of a diverse class of brominated aromatic flame retardants which are currently produced in large quantities (Anonymous, 1977; Anonymous, 1980; Sanders, 1978). Fire safety legislation during the last decade has greatly stimulated the synthesis, production, and application of fire retardant chemicals (Anderson, 1976). The market for brominated aromatic flame retardants in the United States rose from 20 million pounds in 1971 to 45 million pounds in 1973 (Anonymous, 1974). The amounts used in plastics alone in 1976 and 1977 were 22 and 18 million pounds, respectively (Anonymous, 1977). In 1980 the annual consumption of brominated aromatic flame retardants was 32 million pounds, and by 1985 it is expected to reach 55 million pounds (Anonymous, 1980). The potential for environmental contamination has increased along with this rapid increase in production and application.

The PBB incident in Michigan is only one of many documented instances of environmental contamination resulting from brominated aromatic flame retardants. The first reported case of environmental pollution from these compounds was in 1975 when residues of pentabromotoluene were found in sewage sludge from a wastewater treatment plant in Sweden (Mattsson et al., 1975). The source was a factory which used this chemical for flame retardant applications. Since this discovery, several reports have been published on environmental and human contamination resulting from the production and use of brominated aromatic flame retardants (Andersson and Blomkvist, 1981; DeCarlo, 1979; DiCarlo et al., 1978; Forba, 1980; Forba et al., 1980; Hesse and Powers, 1978; Jamieson, 1977; Pellizzari et al., 1978; Powers, 1976).

Although brominated aromatic flame retardant production has increased tremendously over the past ten years and numerous incidents of environmental contamination from these compounds have been identified, very little is known about their toxicity and environmental chemistry. Research so far has focused on PBBs. Recent work (Falk et al., 1980) has revealed that these chemicals may be more toxic than their chemical relatives, PCBs. Also, limited studies on the environmental transport and transformation characteristics of PBBs indicate that they may be as persistent as PCBs (Filonow et al., 1976; Jacobs et al., 1976; Jacobs et al., 1978; Matsuo, 1980; Ruzo and Zabik, 1975; Ruzo et al., 1976; Sugiura et al., 1978; Zitko, 1977; Zitko and Hutzinger, 1976). Only a few studies have been published on the toxicity and environmental chemistry of other brominated aromatic flame retardants (Hutzinger et al., 1976; Kociba et al., 1975; Liepins and Pearce, 1976; Norris et al., 1973a; Norris et al., 1973b; Norris et al., 1975; Orlando and Thomas, 1975; Zitko and Carson, 1977). These studies indicate a general lack of information on the persistence and fate of brominated aromatic flame retardants in the environment, especially aquatic ecosystems.

Our ability to respond objectively to such incidents is limited by the lack of information on this diverse group of chemical compounds. For this research project environmental fate data were developed on Firemaster 680. This compound was selected for the study because it has been designated to replace Firemaster BP-6 (PBB). Therefore, production volumes and applications are projected to be large. Additionally, a contamination incident involving Firemaster 680 has been identified in a segment of the Raisin River watershed in Michigan, thus necessitating an evaluation of the chemical dynamics of this compound in the aquatic environment.

FIREMASTER 680

Firemaster 680 is a non-reactive brominated aromatic flame retardant which is manufactured and marketed by the Velsicol Chemical Corporation, Chicago, Illinois. This flame retardant additive was designed for high performance thermoplastic resins such as acrylonitrile-butadiene-styrene terpolymer (ABS), high impact polyester and other polymer systems common in electrical and high temperature applications (Barth, 1979; Nametz and Moore, 1978; Horn, 1978). Firemaster 680 is also widely used in the manufacture of television cabinets and other electrical equipment because it retards flamability without impairing moldability, impact strength, and resistance to ultraviolet light degradation (Velsicol Chemical Company, 1977). This chemical also can be formulated into adhesives, coatings, lattices, and specialty composites (Leitheiser et al., 1978; Smith and Shukla, 1978).

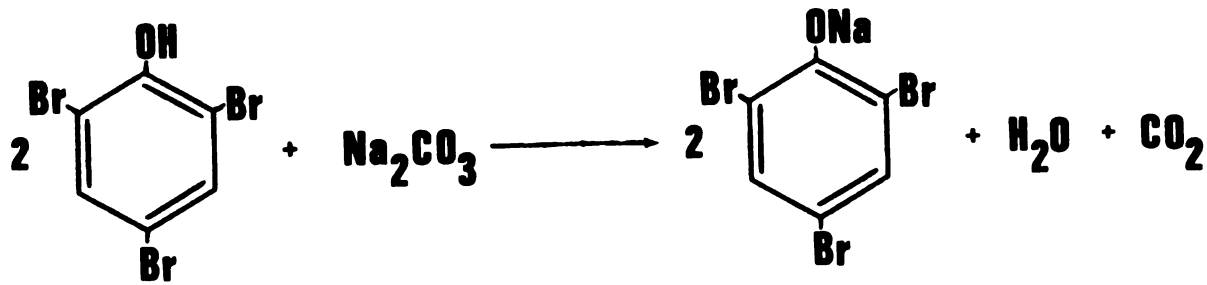
Chemically, Firemaster 680 is identified either as 1,2-bis(2,4,6-tribromophenoxy)ethane or by the Chemical Abstract Service (CAS) name of 1,1'-[1,2-ethanediylbis(oxy)]bis[2,4,6-tribromobenzene]. The CAS number is 37853-59-1 or 59764-36-2. Structurally, the compound consists of two symetrically brominated phenyl rings attached through a glycol group. The six bromine atoms give this compound a bromine content of 70% by weight and a relatively high molecular weight of 687.67 gm/mole. The known physical data include a melting point range between 223-225°C, a decomposition temperature of approximately 325°C, and a density of 2.58 gm/ml. The compound has moderate solubility in p-xylene, perchloroethylene, and boiling dichlorobenzene; a vapor pressure of 0.0019 mm Hg at 170°C; excellent thermal stability; and resistance to ultraviolet light degradation (Michigan Chemical Corporation, 1976).

The synthesis of 1,2-bis(2,4,6-tribromophenoxy)ethane is a two step process (Figure 1). In reaction I the sodium salt of 2,4,6-tribromophenol is formed from 2,4,6-tribromophenol and sodium bicarbonate in a propylene glycol mother liquor which contains 0.5% phenol. The final product is then made (reaction II) by reacting the sodium salt of 2,4,6-tribromophenol with 1,2-dibromoethane in a propylene glycol mother liquor (Heisted, 1977).

It is then washed with methanol and water to remove residual sodium bromide and tribromophenol based impurities. The impurities normally associated with this product are 2,4,6-tribromophenol and 1-(2,4,6-tribromophenoxy)-2-bromoethane. Additionally, 1-(2,4,6-tribromophenoxy)-ethanol, 1-(2,4,6-tribromophenoxy)ethylene and numerous 2,4,6-tribromophenoxypropylene glycol derivatives may be found as trace impurities. The final product specifications call for a minimum bromine content of 68.5% which corresponds to a product purity of 98.2% (Heisted, 1977).

Velsicol Chemical Corporation has conducted toxicity studies on Firemaster 680, and the results have been submitted to the U.S. Environmental Protection Agency as a substantial risk notice in compliance with Section 8(e) of the Toxic Substances Control Act (U.S. EPA, 1980b). These studies are summarized in Appendix A. The results generally indicate that Firemaster 680 is relatively non-toxic to mammals during acute, subacute, and chronic exposures. Some accumulation of Firemaster 680 in fat and other tissues of laboratory test animals were noted, but no significant changes were noted in hematological, biochemical, and urinalysis parameters. The results of an Ames mutagenicity bioassay were negative.

The bioconcentration potential of Firemaster 680 in carp (Cyprinus carpio) was also reported (U.S. EPA, 1980b). The actual test conditions of this study were not specified, but the carp were exposed to nominal



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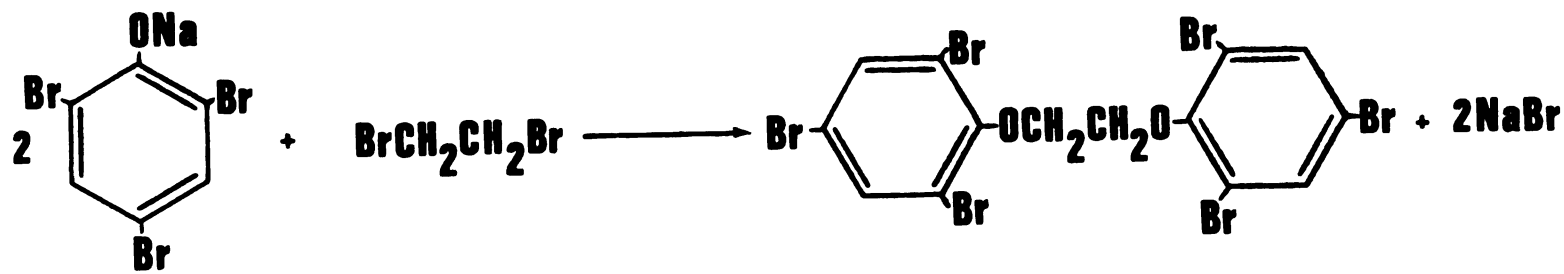


[Reaction II]

Figure 1. Chemical Synthesis of 1,2-Bis(2,4,6-tribromophenoxy)ethane

concentrations at 0.3 mg/L or 0.03 mg/L Firemaster 680 for up to 8 weeks. The highest concentration factors were 56.6 for the high exposure group and 43.6 for the low exposure group. These values indicate that Firemaster 680 has little tendency to bioconcentrate.

A biodegradation study with ^{14}C-labeled Firemaster 680 was also submitted to the Environmental Protection Agency (U.S. EPA, 1980b). Tests of aerobic biodegradability were conducted by exposing ^{14}C-labeled Firemaster 680 to acclimated sewage and garden soil microorganisms in a shake flask system. Microbial utilization of Firemaster 680 was followed by measuring respired $^{14}CO_2$. During an 18 day acclimation period, microorganisms derived from sewage and garden soil were exposed to Firemaster 680. The acclimated bacteria were then used as seed organisms and added to microbial media which contained 0.1, 1.0, or 10.0 mg/L of ^{14}C-labeled Firemaster 680. The results appeared to indicate that $^{14}CO_2$ was liberated from universally labeled Firemaster 680 in all test samples throughout the study period. However, degradation was slow and the tests were terminated after 30 weeks. Following an initially accelerated $^{14}CO_2$ liberation, the $^{14}CO_2$ absorption decreased to about 0.001 to 0.008% of the total ^{14}C-activity in the 1.0 and 10.0 mg/L Firemaster 680 test flasks. These results indicate that Firemaster 680 is relatively nonbiodegradable in the presence of sewage and garden soil microorganisms under the conditions of these tests.

The toxicity and environmental fate data on Firemaster 680 reported by Velsicol Chemical Corporation appear to indicate that this product is rather non-toxic and environmentally stable. However, only selective studies were reported and most of these data were generated in one laboratory, that of Velsicol Chemical Corporation, the manufacturer of

Firemaster 680. Before the environmental hazard potential of Firemaster 680 can be ascertained, these data must be verified, and additional toxicity and environmental fate studies will have to be conducted. Transport and transformation studies are necessary to evaluate the environmental fate characteristics and exposure potential of Firemaster 680.

ENVIRONMENTAL FATE ASSESSMENT

The fate of a brominated aromatic flame retardant such as Firemaster 680 in the environment is controlled by the transport and transformation processes acting on it. In the aquatic environment, the major transport processes include sorption, volatilization, bio-uptake, and physical transport in the water phase. Transformation processes include hydrolysis, oxidation, photolysis, reduction, and biodegradation. While the ultimate persistence of a chemical is controlled by the transformation processes, its residence time and actual exposure concentration in a segment of the aquatic environment are controlled by both transport and transformation processes.

Environmental fate and exposure assessment are extremely complex and require precise information in all of the categories identified above. At this time, the state-of-the-art has not progressed to the point where the environmental concentrations of a chemical can be predicted with a high level of accuracy by using only laboratory derived data. The actual environmental concentrations can only be ascertained by extensive monitoring studies. However, environmental fate assessment methods have advanced to the extent that they can be used to predict the interactions of a chemical contaminant with the aquatic environment and to begin to estimate, in a qualitative sense, the concentration of the chemical in specific compartments of the aquatic environment.

A variety of methods have been proposed to evaluate the fate of chemicals in the environment (Kimerle et al., 1978; Stern and Walker, 1978). These range from predictions based on chemical and physical property relationships to laboratory studies which focus on one or more of the fate processes, microcosm studies which attempt to simulate the natural environment, and field studies to validate the predictions. Recently, the Environmental Protection Agency has proposed standard protocols for environmental fate testing which are mandated under Sections 4 and 5 of the Toxic Substances Control Act (U.S. EPA, 1980c; U.S. EPA, 1981) and Section 3 of the Federal Insecticide, Fungicide, and Rodenticide Act (U.S. EPA, 1980d). Additionally, the Organization for Economic Cooperation and Development (OECD) has published a complete set of environmental fate test guidelines for use by its member countries (OECD, 1981). Chemical environmental fate assessments usually follow a step-sequence (tier) testing scheme. The lowest level generally contains a base set of tests which provide the minimum data for estimating the environmental fate of a chemical while the highest level consists of actual field studies.

The minimum data base studies have received much attention over the last few years because they require relatively short periods of time and the least capital expenditure. The base set of chemical fate tests are primarily for the purpose of providing data to determine the dominant transport and transformation processes that the chemical may undergo in natural environments. Results from these studies are used to make decisions on whether to move up to the next level of the testing hierarchy. The U.S. Environmental Protection Agency (1981) proposed that the base set of data for chemical environmental fate assessment include water solubility, vapor pressure, n-octanol/water partition coefficient,

boiling and melting points, density, dissociation constant, particle size distribution, UV and visible spectra, sediment and soil adsorption/desorption, hydrolysis, biodegradation, and bioaccumulation.

Thus, an essential first step in a chemical environmental fate analysis is to develop an understanding of the chemical, physical, and toxicological properties of the compound being studied. For common, high volume chemicals, this information is usually well known and readily available. For specialty chemicals, such as brominated aromatic flame retardants, basic physical and chemical data may be difficult to obtain and toxicological information might be non-existent. Some physical and chemical information is usually available from the manufacturer through product bulletins and advertisements appearing in trade journals. In most cases, however, the investigator has to develop much of the information in the laboratory.

STATEMENT OF OBJECTIVES

This project was undertaken to assess the impact of Firemaster 680 contamination on the south branch of the Raisin River in Michigan. The overall objective of this investigation was to obtain information to predict the fate of Firemaster 680 in the aquatic environment. The specific objectives were:

1. To characterize Firemaster 680 chemically and to determine the chemical purity of the technical grade product.
2. To develop a method for the analysis of Firemaster 680 in water.
3. To determine the water solubility of Firemaster 680.
4. To determine the n-octanol/water partition coefficient of Firemaster 680 and to use this parameter to estimate its

potential for sediment partitioning and biological up-take by aquatic organisms.

5. To determine microbial degradation of Firemaster 680.

MATERIALS AND METHODS

FIREMASTER 680 CHEMICAL STRUCTURE AND PURITY

Commercial grade Firemaster 680 (Lot No. 61114-F) obtained from Velsicol Chemical Corporation (Chicago, Illinois) was characterized for chemical structure and purity. Elemental analyses with a Perkin-Elmer Model 240 Elemental Analyzer detected 24.35% carbon, 1.15% hydrogen, and 0% nitrogen. With a total bromide determination method (Liggett, 1954; Michigan Chemical Company, 1975), the bromine content was determined to be 68.72%. These data indicate a product purity of approximately 99%. The melting point was sharp, within 0.5°C of 225°C, which also indicates a relatively pure product. Gas chromatographic analyses for organic impurities revealed less than 0.1% of 2,4,6-tribromophenol and 1-(2,4,6-tribromophenoxy)-2-bromoethane. The ultraviolet, infrared, nuclear magnetic resonance and mass spectra clearly identified this product as 1,2-bis(2,4,6-tribromophenoxy)ethane.

For a more complete discussion of these methods and results, see Appendix B.

ANALYTICAL METHOD

An XAD resin analytical scheme was devised to extract low levels of Firemaster 680 and potential metabolities from aqueous media. This method is a modification of a procedure developed by Junk et al. (1974). The extraction efficiencies for Firemaster 680 and 2,4,6-tribromophenol were

determined with XAD-2 and XAD-4 resins separately and in a mixture of XAD-2/XAD-4. Standard solutions of Firemaster 680 and 2,4,6-tribromophenol were prepared in diethyl ether and spiked water samples were prepared in a concentration range of 0.1 to 2.0 ug/L with deionized water. The spiked water samples were extracted through the XAD resin columns. The sorbed fraction of Firemaster 680 and 2,4,6-tribromophenol was eluted with diethyl ether, and the eluate was dried, concentrated and analyzed by gas chromatography. The extraction efficiencies for each resin with Firemaster 680 and 2,4,6-tribromophenol were determined by comparing the gas chromatogram peaks of the XAD resin extract for each spiked water sample with the gas chromatogram peaks for the theoretically equivalent amounts of the standard ether solutions of Firemaster 680 and 2,4,6-tribromophenol. The XAD-2 resin system was the most efficient with recoveries of approximately 50% for Firemaster 680 and 100% for 2,4,6-tribromophenol. A standard calibration curve was developed with the spiked water samples for the concentration range of 0.1 to 2.0 ug/L in order to correct for the lower extraction efficiency for Firemaster 680.

See Appendix C for a complete description of these methods and results.

WATER SOLUBILITY DETERMINATION

The time-dependent equilibrium procedure described by Haque and Schmedding (1975) was used to determine the aqueous solubility of Firemaster 680. Approximately 0.5 mg Firemaster 680 were dissolved in 25 ml of diethyl ether and swirled onto the wall of a 20 liter glass carboy. The residual ether was evaporated by using a nitrogen stream, resulting in a thin film of Firemaster 680. Nineteen liters of deionized, carbon and membrane filtered water were carefully added to the carboy so as not

Firemaster 680 film on its side. A teflon coated magnetic stir bar was added and a magnetic stirrer was used to stir the contents of the carboy. The solution was isolated from the magnetic stirrer by a one inch sheet of styrofoam to prevent temperature gradients caused by heat from the stirrer motor. Finally, the carboy was fitted with a glass siphon tube which had a fine frit that extended below the surface of the liquid. This was used to collect samples for analysis.

The solution was sampled through the glass frit-siphon tube at weekly intervals for the first month, monthly for the next two months, and once every three months for a complete year. The solution was stirred at approximately 250 rpm for the first month, and then the stirring was stopped. The carboy was held at room temperature (22 $\pm$ 2°C) for the entire study. Triplicate 500 ml samples were collected at each sampling time and then were concentrated according to the XAD-2 analytical scheme previously described. The concentrates were analyzed by gas chromatography.

A Beckman, model GC-65, gas chromatograph equipped with a non-radioactive electron-capture detector and a Beckman 10-inch linear recorder (Beckman Instruments, Inc., Fullerton, California) were used to obtain the gas chromatography data from which the aqueous solubility was calculated. The test samples and standards were chromatographed on a 0.2 (i.d.) X 183 cm glass column packed with 60/80 mesh Gas Chrom Q coated with 3% silicon OV-1 liquid phase (Applied Science Laboratories, Inc., State College, Pennsylvania) with a helium carrier gas flow rate of 55 ml/min. The gas chromatograph conditions were as follows: inlet temperature, 290°C; column temperature, 280°C; detector line temperature, 310°C; detector temperature, 360°C. The polarizing voltage, carbon

dioxide flow, and bias voltage were set for optimum detector response. With this column and appropriate operating conditions, the retention time for Firemaster 680 was 3.9 minutes, and the minimum detectable quantity was 1.0 pg at 2.5 times the background noise level.

N-OCTANOL/WATER PARTITION COEFFICIENT DETERMINATON

The n-octanol/water partition coefficient for Firemaster 680 was estimated by using a reverse-phase HPLC method similar to the procedure described by McDuffie (1981). Six chemical standards for which K_{ow} have been reported were used to calibrate the elution time in units of K_{ow}. The calibration mixture included benzene (Burdick and Jackson Laboratories, Inc., Muskegon, Michigan), 1,4-dichlorobenzene (Aldrich Chemical Company, Milwaukee, Wisconsin), biphenyl (J. T. Baker Chemical Company, Phillipsburg, New Jersey), phenanthrene (J. T. Chemical Company, Phillipsburg, New Jersey), p,p'-DDE (Aldrich Chemical Company, Milwaukee, Wisconsin), and 2,4,6,2',4',6'-hexachlorobiphenyl (Analabs, Inc., North Haven, Connecticut). Methanol solutions containing 100 mg/L of each standard, except 2,4,6,2',4',6'-hexachlorobiphenyl, were prepared with chromatography grade methanol. A 75:25 (V/V) methanol-hexane mixture was used to prepare solutions of 2,4,6,2',4',6'-hexachlorobiphenyl and Firemaster 680 at 100 mg/L concentration. The eluting solvent was 75:25 (V/V) methanol-water which was prepared from chromatography grade methanol and distilled-carbon filtered water, and it was degassed by sonication before use.

The liquid chromatograph consisted of an Altex Model 110 solvent metering pump (Altex, Inc., Berkeley, California) and a Hitachi Model 100-40 variable wavelength UV detector (Hitachi, Ltd., Tokyo, Japan) fitted with an Altex Model 155-00 spectrophotometer flow cell module. The flow cell module had a 1.0 cm path length and a 20 ul cell volume. A

0.46 (i.d.) X 25 cm Zorbax-ODS C_{18}-microparticulate reverse-phase column (DuPont Company, Wilmington, Deleware) was used. The column was held at a constant operating temperature in a DuPont Model 860 column compartment (DuPont Company, Wilmington, Deleware). Retention times were recorded on a Linear Model 255 recorder (Linear Instruments Corp., Irvine, California).

Twenty ul of each standard solution was injected onto the column which was held at a constant operating temperature of 35°C. The solutes were eluted isocratically with the methanol-water solvent mixture which was pumped through the column at 1.0 ml/min at a pressure of approximately 2000 psi. The detector was set at a wavelength of 254 nm for the calibration standards and 290 nm for Firemaster 680. Absorbance peaks were recorded at a chart speed of 30 cm/hr. The retention times of the calibration standards and Firemaster 680 were determined directly from the recorder chart.

MICROBIAL DEGRADATION OF FIREMASTER 680

A modified version of a technique for isolating PCB degrading bacteria was employed to isolate natural populations of microorganisms which can utilize Firemaster 680 as a carbon and energy source for growth (Kaiser and Wong, 1974). The bacterial cultures were then used in a cometabolism study to check for primary degradation of Firemaster 680 and to identify the degradation products.

Enrichment and Isolation

Microorganisms were obtained from sediment and water samples which were collected in the vicinity of Adrian, Michigan. These locations were previously exposed to Firemaster 680 and other chemicals in process wastes

Table 1. Environmental Samples for Bacteria Isolation

Sample Code	Sample Type	Location of Collection	Firesmater 680 Content[a]
W2	Water	WWTP[b]-influent	ND[c]
W3	Water	WWTP-return sludge	ND
W4	Water	WWTP-effluent	ND
S2	Sludge	WWTP-digested sludge	0.11 mg/kg
W5	Water	WWTP-sludge disposal bed	ND
S5	Sludge	WWTP-sludge disposal bed	1.2 mg/kg
W6	Water	WWTP-lagoon	NA[d]
S6	Sludge	WWTP-lagoon	5.9 mg/kg
S7	Sludge	WWTP-sludge disposal bed	5.3 mg/kg
S7B	Sludge	WWTP-sludge disposal bed	NA
S8	Sediment	East Side Drain-Anderson Development Company	55.0 mg/kg
S9	Sediment	East Side Drain-end of storm sewer	1.3 mg/kg
S10	Sediment	East Side Drain-Academy Road	6.3 mg/kg
S11	Sediment	East Side Drain-Oakwood Street	1.1 mg/kg
S12	Sediment	South Branch Raisin River-East Side Drain	1.3 mg/kg
S13	Sediment	South Branch Raisin River-Howell Highway	0.49 mg/kg

[a]Data obtained from Michigan Department of Natural Resources surveys (Boerson, 1979a; Boerson, 1979b; Jackson, 1979)

[b]Adrian, Michigan Wastewater Treatment Plant

[c]None Detected

[d]Not Analyzed

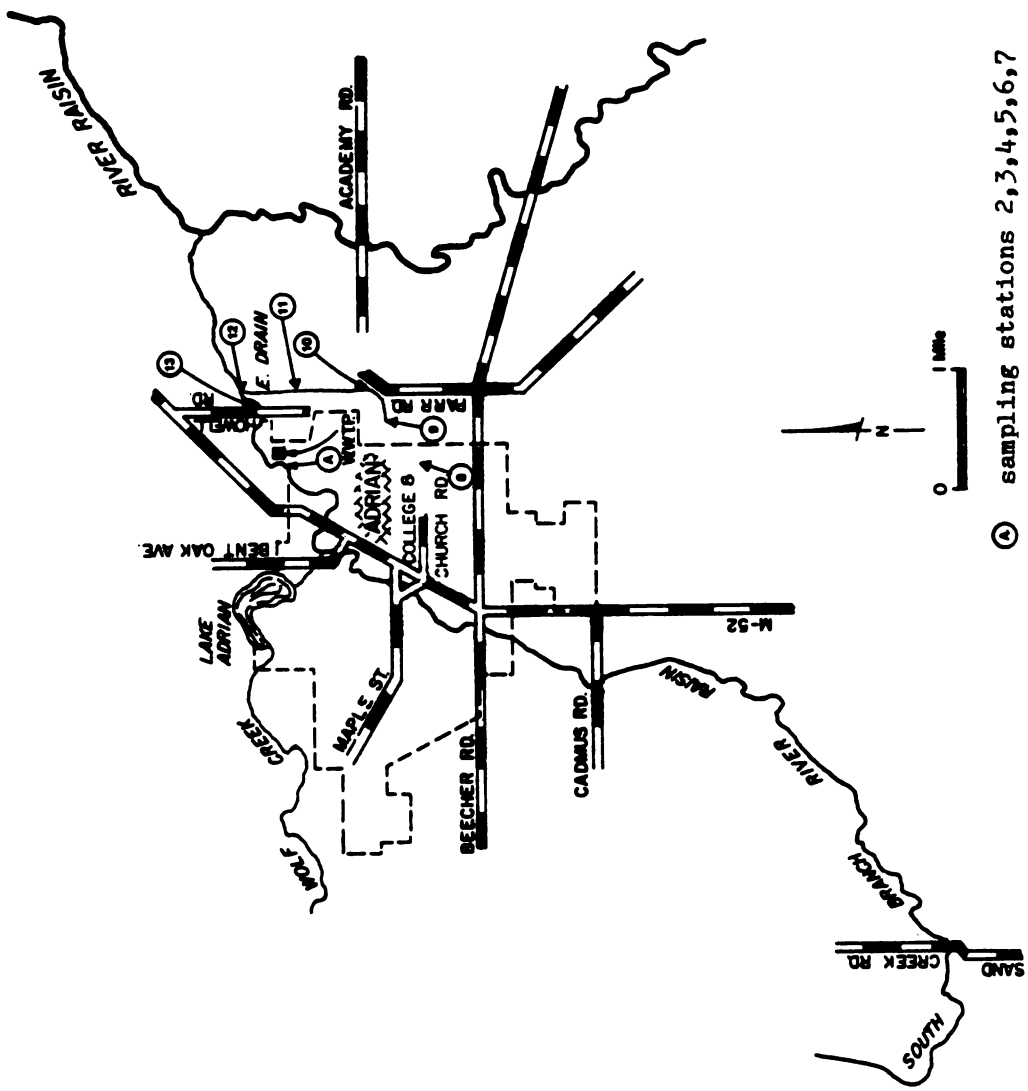


Figure 2. Sampling Stations for Firemaster 680 Degrading Bacteria

from the Anderson Development Company. A description of the location and types of samples and their content of Firemaster 680 is included in Table 1 and Figure 2.

Subsamples consisting of approximately 2 g of the sediment or sludge or 100 ml of water, were placed in 300 ml Erlenmeyer flasks and fortified with 200 mg Firemaster 680 and 100 ml of mineral salt solution. The mineral salt solution was prepared according to Smith et al. (1977) and it contained: K_2HPO_4, 1.4 g; KH_2PO_4, 0.6 g; $(NH_4)_2SO_4$, 0.5 g; NaCl, 0.1 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; $CaCl_2 \cdot 2H_2O$, 0.02 g; $FeSO_4$, 0.005 g; in 1 liter H_2O and 1 ml of trace elements solutions. The trace elements solution contained 0.1 g H_3BO_3 and 0.05 g each of $CuSO_4 \cdot 5H_2O$, $MnSO_4 \cdot H_2O$, $ZnSO_4 \cdot 7H_2O$, Na_2MoO_4, and $CoCl_2 \cdot 6H_2O$ per liter of water. The samples were incubated for one week at room temperature on a rotary shaker. This procedure presumably selected for those bacteria that could use Firemaster 680 as the sole carbon and energy source for growth.

After incubation, 0.1 ml aliquots of the enriched samples were plated onto sterile growth medium agar plates with a standard spread plate technique (Rodina, 1972). The growth medium agar consisted of the mineral salt solution, agar, and a carbon source. Three carbon sources were used separately or in combination to prepare four growth medium agar substrates with carbon source contents of 0.1%. These included: Firemaster 680; Firemaster 680 and plate count agar which contained glucose, tryptophane, and yeast extract (Difco Laboratories, Detroit, Michigan); diphenoxyethane (Aldrich Chemical Company, Milwaukee, Wisconsin); and a 1:1 mixture of Firemaster 680 and diphenoxyethane. Diphenoxyethane is the nonbrominated analog of Firemaster 680. A growth medium agar without any carbon source was also prepared.

The inoculated growth medium agar plates were incubated at room temperature for one week and were observed for bacterial growth. On plates showing good growth, colonies of bacteria were removed and purified by repeated streaking onto sterile growth medium agar plates. This was an attempt to isolate specific bacteria capable of utilizing the individual carbon source. The inoculated plates were incubated at room temperature for 16 days. Four observations for bacterial growth were made during this period.

The capability of the purified cultures to use Firemaster 680 directly or indirectly as a cometabolite for growth was further tested by growing them in a mineral salt solution that was fortified with the respective carbon source. Colonies of bacteria were taken from the growth medium agar plates and placed in 300 ml Erlenmeyer flasks which contained 200 ml of the mineral salt solution with 0.01% Firemaster 680, diphenoxyethane, Firemaster 680/diphenoxyethane, or Firemaster 680/glucose. The samples were incubated for thirty days at room temperature on a rotary shaker and each flask was observed daily for bacterial growth.

Cometabolism Study

A specially designed chamber was used for this study. This system consisted of a 9 liter glass carboy to which was fitted a glass frit and a condenser. The glass frit supplied a constant air flow and facilitated sampling the growth medium in the carboy. The condenser was added to eliminate evaporation of the sample.

The growth medium was prepared by adding Firemaster 680 to the basal salts medium to obtain a final concentration of 5 ug/L. The basal salts medium consisted of a 1:1 dilution of the mineral salts solution with deionized water. Five liters of Firemaster 680 growth medium were added

to each of the biodegradation chambers. Besides the Firemaster 680, two of the growth chambers received an additional carbon source. Diphenoxyethane was added to one growth chamber to obtain a final concentration of 5 ug/L. The third growth chamber received glucose at 50 mg/L.

Each growth chamber was inoculated with the S9a strain of bacteria which had been obtained in the isolation and enrichment phase. This culture was selected because it appeared to grow better than any other that was isolated on FM680+ agar. The bacteria, which were growing on Firemaster 680 plate count agar slants, were suspended in 25 ml of the basal salts medium and then were added to each biodegradation chamber.

Samples consisting of 500 ml of the growth medium were collected from each growth chamber at the time of inoculation (t_0) and at 1 (t_1), 3 (t_2), 6 (t_3), 12 (t_4), 90 (t_5), and 153 (t_6) days after inoculation. A 0.1 ml aliquot was taken from each one at the time of sampling and transferred to growth medium agar plates which contained the same carbon source as the sample. The inoculated plates were incubated at room temperature for two weeks and observed periodically for bacterial growth. Each set of samples was analyzed for Firemaster 680 and 2,4,6-tribromophenol according to the XAD-2 analytical scheme described in the analytical methods section.

RESULTS

WATER SOLUBILITY DETERMINATION

The results obtained in this study are tabulated in Table 2 and presented graphically in Figure 3. During the first month, the concentration of Firemaster 680 increased, probably approaching an equilibrium value. However, after the stirring was stopped, the concentration of Firemaster 680 decreased sharply over the next two months, perhaps because aggregates of Firemaster 680 may have been formed during the stirring that gave higher solubility values. This possibility is strengthened by the fact that the error associated with the analyses during the first five sampling periods was much greater than during the last five sampling periods. This large variance could be due to the random capture of microcrystals or aggregates of Firemaster 680 during sampling as discussed by May et al. (1978). The equilibrium solubility value of 0.04 ug/L was reached at 24 weeks and was consistent through the end of the 60 week study period. These data show that the equilibrium solubility, including 95% confidence limits, for Firemaster 680 in deionized, carbon and membrane-filtered water was 0.04 ± 0.01 ug/L.

N-OCTANOL/WATER PARTITION COEFFICIENT DETERMINATION

The results obtained in this experiment are tabulated in Table 3. The corrected solute retention times (t_C) were determined according to the equation:

Table 2. Firemaster 680 (FM680) Water Solubility Determination

Sample	Time (Weeks)	FM680 (ug/L)
t_0	0	1.31 (.21)[a]
t_1	1	1.37 (.24)
t_2	2	1.41 (.36)
t_3	3	1.50 (.28)
t_4	4	1.56 (.59)
t_5	8	0.98 (.22)
t_6	12	0.13 (.02)
t_7	24	0.04 (.02)
t_8	36	0.04 (.01)
t_9	48	0.04 (.01)
t_{10}	60	0.04 (.03)

[a]( ) = standard deviation, N=3

$$t_C = t_R - t_0 \qquad \text{(Equation 1)}$$

where t_R is the solute retention time after injection and t_0 is the solvent retention time. The logarithm, base 10, of t_C for each standard was calculated and correlated with the reported log K_{ow} values for these chemicals. The log t_C values were used instead of log t_R to obtain an improved linear correlation as discussed by McDuffie (1981).

Figure 4 graphically illustrates this correlation which is best described by the equation:

$$\log K_{ow} = 3.53(\log t_C) + 2.52 \qquad \text{(Equation 2)}$$

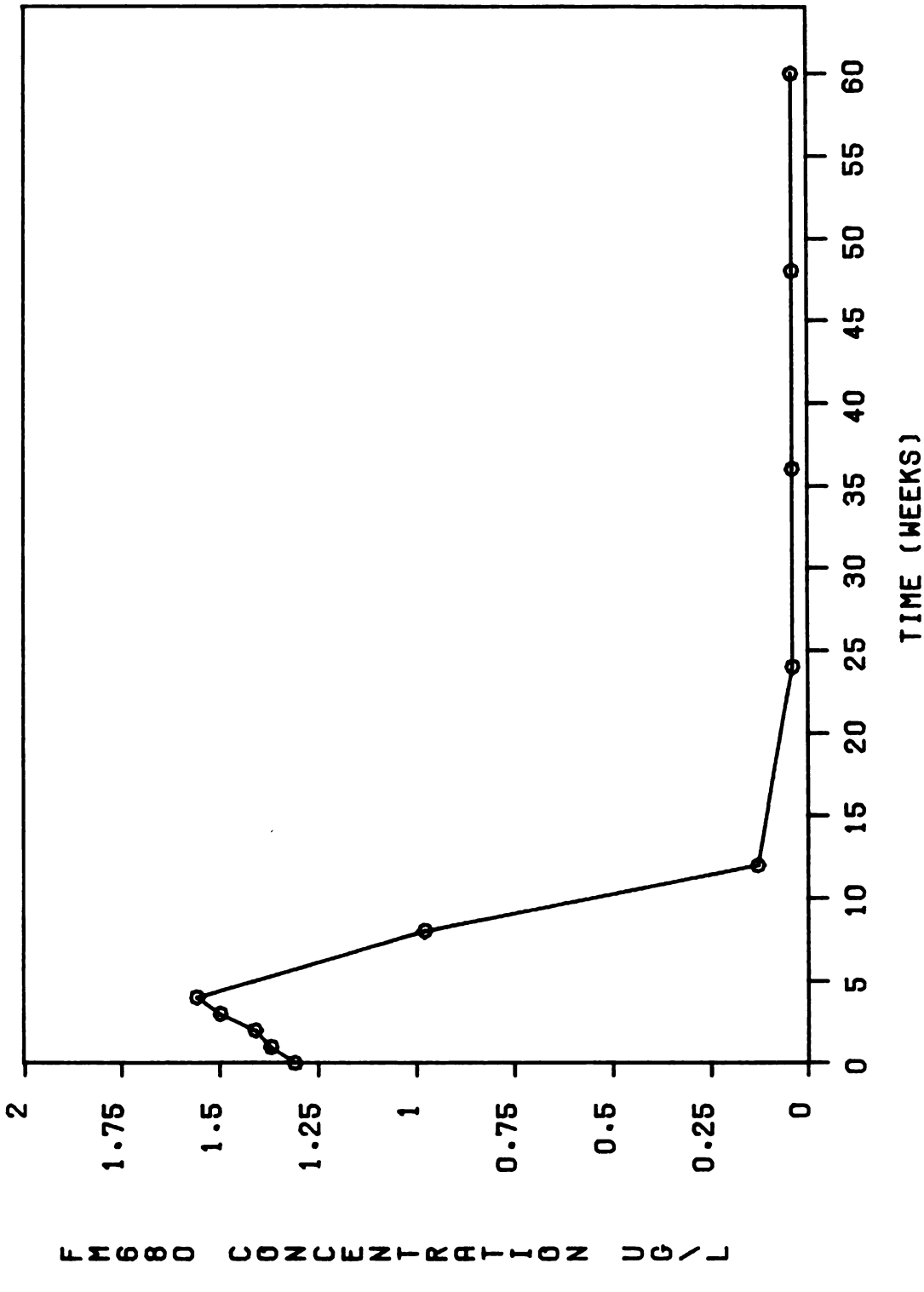


Figure 3. Firemaster 680 (FM680) Water Solubility Determination

Table 3. HPLC Retention Times and Partition Coefficients for Calibration Standards and Firemaster 680

Compound	t_O(min)	t_R(min)	t_C(min)	log t_C	log K_{OW} (reference)
Benzene	3.2	4.2	1.0	0.00	2.11 (Karickhoff *et al.*, 1979)
1,4-Dichlorobenzene	3.2	5.0	1.8	0.26	3.39 (Leo *et al.*, 1971)
Biphenyl	3.2	5.4	2.2	0.34	4.09 (Leo *et al.*, 1971)
Phenanthrene	3.2	6.2	3.0	0.48	4.57 (Karickhoff *et al.*, 1979)
p,p'-DDE	3.2	11.4	8.2	0.91	5.69 (Leo *et al.*, 1971)
2,4,6,2',4',6'-PCB	3.2	17.0	13.8	1.14	6.34 (Karickhoff *et al.*, 1979)
Firemaster 680	3.2	23.5	20.3	1.31	7.14[a]

[a]Calculated from Equation 2

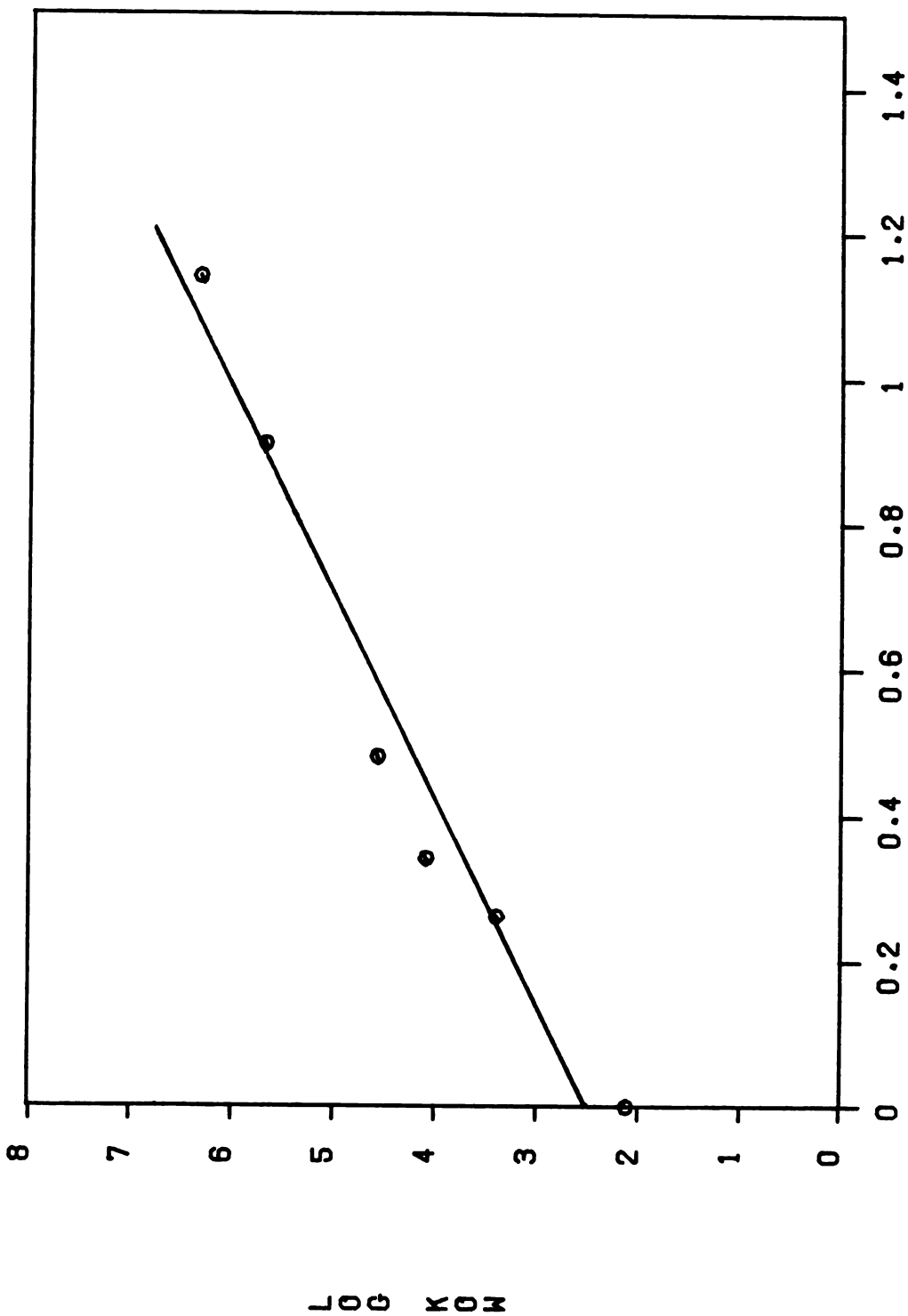


Figure 4. Correlation Plot for Log K_{OW} and Log t_C of the Test Standards

It has a correlation coefficient (r) equal to 0.980. With this equation, the estimated log K_{ow} for Firemaster 680 was 7.14.

To determine the accuracy of this method of estimating K_{ow}, a comparison was made between the literature log K_{ow} values reported for the calibration standards and their estimated log K_{ow} values by using equation 2 (Table 4). The mean absolute error was 0.24 with a standard deviation of 0.17. The error associated with the estimated log K_{ow} for Firemaster 680 could be larger because it was necessary to extend the linear regression line past the log K_{ow} range of the calibration standards. However, if the regression line is linear above this range, then the log K_{ow} for Firemaster 680 is 7.14 $\pm$ 0.42 with 95% confidence.

It is interesting to compare the log K_{ow} value for Firemaster 680 determined by using the HPLC procedure with the calculated log K_{ow} values

Table 4. Comparison of Estimated Log K_{ow} Values Using Equation 2 and Reported Log K_{ow} Values

Compound	Estimated log K_{ow}	Literature log K_{ow}	Absolute Deviation
Benzene	2.52	2.11	0.41
1,4-Dichlorobenzene	3.44	3.39	0.05
Biphenyl	3.72	4.09	0.37
Phenanthrene	4.21	4.57	0.36
p,p'-DDE	5.73	5.69	0.04
2,4,6,2',4',6'-PCB	6.54	6.34	0.20
Mean Absolute Deviation = 0.24			

derived from equations which incorporate other physical properties. Chiou et al. (1977) correlated experimental values of the aqueous solubility and n-octanol/water partition coefficient for 34 chemicals. Their regression equation showed:

$$\log K_{ow} = 5.00 - 0.67(\log S) \qquad \text{(Equation 3)}$$

where S = aqueous solubility, in umole/L. When the aqueous solubulity of Firemaster 680, 5.82×10^{-5} umole/L, determined in the previous experiment, was substituted into equation 3, the log K_{ow} for Firemaster 680 was calculated to be 7.84.

Kenaga and Goring (1980) extended this correlation to include 90 chemicals and developed the regression equation:

$$\log K_{ow} = 4.158 - 0.800(\log S) \qquad \text{(Equation 4)}$$

where S = aqueous solubility, in umole/L. Using this equation and the experimentally determined water solubility, 5.82×10^{-5} umole/L, for Firemaster 680, the log K_{ow} was calculated as 7.55.

Banerjee et al. (1980) reported that the correlation between water solubility and K_{ow} developed by Chiou et al. (1977) could be invalid for high-melting solids due to the crystal structure of these chemicals. To correct for this error, these authors developed a relationship between the aqueous solubility and n-octanol/water partition coefficient by using data on 27 chemicals which included a melting point correction term. Their regression equation showed:

$$\log K_{ow} = 6.5 - 0.89(\log S) - 0.015(mp) \qquad \text{(Equation 5)}$$

where S = aqueous solubility, in umole/L, and mp = melting point, in ^{o}C. By using this equation and the experimentally determined water solubility, 5.82×10^{-5} umole/L, and melting point 225^{o}C for Firemaster 680, the log K_{ow} was calculated to be 6.89.

Mackay and co-workers (1980a) extended the melting point correction approach by including a correction factor based on the fugacity concept. These authors used water solubility values and n-octanol/water partition coefficients for 45 organic chemicals and developed the regression equation:

$$\ln K_{ow} = 6.79(1 - T_M/T) - \ln C^s - \ln(a_o v_o) \quad \text{(Equation 6)}$$

where C^s = aqueous solubility, in mole/m^3; T_M = triple point; T = system temperature, in ^oK; v_o = molar volume for octanol which is approximately 115 x 10^{-6} m^3/mole; and a_o = octanol phase activity coefficient which is estimated as 64.20 for chemicals with molecular weights greater than 300. With this equation and specific parameters for Firemaster 680, C^s = 5.82 x 10^{-8} mole/m^3, T_m = 498^{o}K, T = 295^{o}K and a_o = 64.20, the log K_{ow} was calculated as 7.34.

In summary, the HPLC derived log K_{ow} value for Firemaster 680 agreed well with the log K_{ow} values calculated by using equation 5 or 6 and appeared to be within experimental error of the true log K_{ow} value.

MICROBIAL DEGRADATION OF FIREMASTER 680

Enrichment and Isolation

The results obtained from the initial spread-plate inoculation are summarized in Table 5. The growth medium containing Firemaster 680 and plate count agar (FM680+) supported abundant growth for all of the environmental samples that were tested. These results were anticipated because the FM680+ medium contained glucose as one of the carbon sources. Such abundant growth was not found on the agar plates which contained Firemaster 680 (FM680), diphenoxyethane (DPE) or Firemaster 680 and diphenoxyethane (FM680/DPE) as the carbon source. These plates appeared

Table 5. Enrichment and Isolation - Spread-Plate Step

	Carbon Source[a]				
Sample	FM680	FM680+	DPE	FM680/DPE	Control
W2	+/-	+++	+/-	+	+/-
W3	+	+++	+	+	+
W4	+/-	+++	+	-	+/-
S2	-	+++	+/-	-	-
W5	-	+++	-	-	-
S5	-	+++	-	-	-
W6	-	+++	-	-	-
S6	+/-	+++	+/-	+/-	+/-
S7	+/-	+++	+/-	+/-	+/-
S7B	-	+++	-	+/-	-
S8	-	+++	+	-	-
S9	+	+++	+	+/-	+/-
S10	+/-	+++	+	+/-	+/-
S11	-	+++	+	-	-
S12	+/-	0	+	+/-	+/-
S13	+	+++	+	+/-	+/-

[a]Data Key
- = No Growth
+ = Slight Growth
+++ = Abundant Growth
+/- = Possible Growth
0 = No Plate

to support some growth, but the colonies were very small and scarce. The growth medium agar without an added carbon source (Control) also supported marginal growth of bacteria from samples W2, W3, W4, S6, S7, S9, S10, S12, and S13. This low level of bacterial growth could have been sustained by organic substrates which were transfered from the enriched samples when the agar plates were inoculated or they could have resulted from impurities in the agar. With these results, no conclusions could be drawn as to whether or not bacteria capable of utilizing Firemaster 680 and/or diphenoxyethane for growth had been isolated. These results did reveal, however, that Firemaster 680 is not toxic to the bacteria growing on the FM680+ agar plates.

The results of the streak-plate isolation step are summarized in Table 6. Basically, this rather complex set of data revealed essentially the same bacterial growth pattern that was found during the spread-plate isolation step. The FM680+ agar supported abundant growth while the FM680, DPE, and FM680/DPE agar supported only a few very slow growing colonies. The W4a, W4e, and S9a strains initially appeared to be growing on Firemaster 680, but the small colonies which occurred after two days of incubation never proliferated during the course of the study. This marginal growth pattern was also observed for strains W2a, W2b, W2c, W2f, W4b, W4g, S2d, S7a, S7d, S9b, S10d, S11c, S13a, and S13b. While the results are ambiguous, it can be reasonably concluded that these cultures are not capable of utilizing Firemaster 680 or diphenoxyethane to support active population growth. The spurious results suggest that these bacteria were using the agar or impurities therein as sources of carbon. This phenomenon has been previously descried by Marshall *et al.* (1960), who found a substantial number of soil microorganisms that were capable

Table 6. Enrichment and Isolation - Streak-Plate Step

Sample (Strain)	Spread-Plate Initial Agar Medium	Streak-Plate Final Agar Medium	Results[a] (Date) 6/4/79	6/5/79	6/10/70	6/18/79
W2a	FM680	FM680	+/-	+/-	+	+
W2b	DPE	DPE	+/-	+/-	+/-	+
W2c	FM680/DPE	FM680/DPE	+/-	+/-	+	+
W2d	FM680	FM680+	+++	++++	+++++	+++++
W2e	FM680+	FM680	-	+/-	+/-	-
W2f	FM680+	FM680/DPE	+/-	+/-	+	+
W2g	FM680+	DPE	-	+/-	-	-
W3a	FM680	FM680	+	+/-	+/-	+/-
W3b	FM680/DPE	FM680/DPE	+/-	+/-	+/-	+/-
W3c	FM680	FM680+	+++	++++	+++++	+++++
W3d	FM680+	FM680	+/-	+/-	+/-	-
W3e	FM680+	FM680/DPE	-	-	-	-
W4a	FM680	FM680	+	+	+	+
W4b	FM680/DPE	FM680/DPE	+/-	+/-	+	+
W4c	FM680	FM680	-	-	-	-
W4d	FM680	FM680/DPE	-	-	-	+/-
W4e	FM680+	FM680/DPE	+	+	+	+
W4f	FM680+	DPE	+/-	+/-	+/-	+/-
W4g	FM680+	FM680	-	-	+/-	+
W4h	FM680	FM680+	+++	++++	+++++	+++++
S2a	FM680	FM680+	+++	++++	+++++	+++++
S2b	FM680+	FM680	-	-	-	+/-
S2c	FM680+	FM680/DPE	-	-	-	-
S2d	FM680+	DPE	-	+/-	+/-	+
S5a	FM680	FM680+	+++	++++	+++++	+++++
S5b	FM680+	FM680	+/-	+/-	+	+/-

Table 6 (cont'd.)

Sample (Strain)	Spread-Plate Initial Agar Medium	Streak-Plate Final Agar Medium	Results[a] (Date) 6/4/79	6/5/79	6/10/70	6/18/79
S5c	FM680+	FM680/DPE	-	-	+/-	+/-
S5d	FM680+	DPE	+/-	+/-	+/-	+/-
S6a	FM680	FM680+	+++	++++	+++++	+++++
S6b	FM680+	FM680	f	f	f	f
S6c	FM680+	FM680/DPE	+/-	+/-	+/-	+/-
S6d	FM680+	DPE	+/-	+/-	+/-	+/-
S7a	FM680+	FM680	+/-	+/-	+/-	+
S7b	FM680+	FM680/DPE	+/-	+/-	+/-	+/-
S7c	FM680+	DPE	+/-	+/-	+/-	+/-
S7d	DPE	DPE	+/-	+/-	+	+
S9a	FM680	FM680	+/-	+	+	+
S9b	DPE	DPE	+/-	+/-	+	+
S9c	DPE	FM680+	+++	++++	+++++	+++++
S9d	FM680+	FM680/DPE	+/-	+/-	+/-	+/-
S9e	FM680+	DPE	+/-	+/-	+/-	+/-
S10a	FM680	FM680+	+++	++++	+++++	+++++
S10b	DPE	DPE	+/-	+/-	+/-	-
S10c	FM680+	FM680	-	-	-	-
S10d	FM680/DPE	FM680/DPE	-	-	+/-	+
S10e	FM680+	FM680/DPE	-	+/-	+/-	+/-
S10f	FM680+	DPE	-	-	+/-	+/-
S11a	FM680	FM680+	+++	++++	+++++	+++++
S11b	FM680+	FM680	+/-	+/-	-	-
S11c	FM680+	FM680/DPE	+/-	+/-	+	+
S11d	FM680+	DPE	-	-	-	-

Table 6 (cont'd).

Sample (Strain)	Spread-Plate Initial Agar Medium	Streak-Plate Final Agar Medium	Results[a] (Date)			
			6/4/79	6/5/79	6/10/70	6/18/79
S13a	FM680	FM680	-	+/-	+	+
S13b	FM680	FM680/DPE	+/-	+/-	+	+
S13c	FM680	DPE	-	-	-	-

[a]Data Key
- = No Growth
+ = Slight Growth
+++++ = Very Abundant Growth
+/- = Possible Growth
f = Fungal Growth

of growth in a medium with no source of carbon other than agar. Alexander (1977) referred to these microorganisms as oligocarbophiles.

In order to distinguish between oligocarbophiles and bacteria capable of growing on Firemaster 680 or diphenoxyethane, a final enrichment step was conducted with a liquid medium. Twenty flasks were prepared and inoculated as described in Table 7. Growth was only observed in the flask containing the FM680/glucose supplemented mineral salts solution. The FM680, DPE, or FM680/DPE growth medium did not support observable growth during the 30 day study. These results substantiate the former conclusions. The microorganisms that were isolated were probably oligocarbophiles rather than bacteria capable of growing on Firemaster 680 or diphenoxyethane. Thus, it appears that Firemaster 680 is not readily degradable as a sole carbon source or cometabolite in these systems.

Cometabolism Study

The biodegradation chamber containing FM680/glucose fortified growth medium was the only one that supported visible growth. The bacterial population grew rapidly but died off seven days after inoculation, leaving a bacterial cell floc at the bottom of the growth chamber. No growth was observed in the FM680 or FM680/DPE biodegradation chambers during the entire study. The growth medium agar plate results were consistent with these observations. No colonies were formed on the plates which received samples from the FM680 or FM680/DPE growth chambers. However, substantial growth was observed on the plates that were inoculated with bacteria from the FM680/glucose biodegradation chamber.

Gas chromatography analyses for Firemaster 680 and 2,4,6-tribromophenol revealed a relatively rapid decrease in the concentration of Firemaster 680 and no 2,4,6-tribromophenol in each growth chamber. These

Table 7. Enrichment and Isolation - Shaker Flask Step

Sample (Strain)	Spread-Plate Initial Agar Medium	Streak-Plate Final Agar Medium	Shaker-Flask Growth Medium
1) W2b	DPE	DPE	DPE
2) W2c	FM680/DPE	FM680/DPE	FM680/DPE
3) W2f	FM680+	FM680/DPE	FM680/DPE
4) W4a	FM680	FM680	FM680
5) W4b	FM680/DPE	FM680/DPE	FM680/DPE
6) W4e	FM680+	FM680/DPE	FM680/DPE
7) W4g	FM680+	FM680	FM680
8) S2d	FM680+	DPE	DPE
9) S5b	FM680+	FM680	FM680
10) S7c	FM680+	DPE	DPE
11) S7b	DPE	DPE	DPE
12) S9a	FM680	FM680	FM680
13) S9a	FM680	FM680	FM680/glucose
14) S9b	DPE	DPE	DPE
15) S9b	FM680+	FM680/DPE	FM680/DPE
16) S10d	FM680/DPE	FM680/DPE	FM680/DPE
17) S10f	FM680+	DPE	DPE
18) S11c	FM680+	FM680/DPE	FM680/DPE
19) S13a	FM680	FM680	FM680
20) S13b	FM680	FM680/DPE	FM680/DPE

data are tabulated in Table 8 and presented graphically in Figures 5, 6, and 7. The concentration of Firemaster 680 in the FM680/glucose growth chamber decreased exponentially over time which is characteristic of a substrate being metabolized by an actively growing bacterial population. However, the results were similar for the FM680 and FM680/DPE growth chambers which did not support bacterial growth. The concentration of Firemaster 680 in the FM680 growth medium initially increased slightly, but after 3 days it also decreased exponentially with time. The concentration of Firemaster 680 in the FM680/DPE growth chamber followed a similar pattern, but its rate of decline was less than that observed in other growth chambers. The concentration of Firemaster 680 eventually reached a level which was consistent with its equilibrium solubility in deionized water.

These data suggest that a mechanism other than microbial metabolism was responsible for the disapearance of Firemaster 680 from the growth medium. Some process of physical removal, such as volatilization or adsorption, may explain these results. Most likely, Firemaster 680 adsorbed on the glass walls of the biodegradation chambers and thus was removed from solution. In addition, sorption of Firemaster 680 by bacteria could have occurred in the FM680/glucose growth chamber. This would explain the increased rate of loss of Firemaster 680 from that growth medium. No matter which mechanism is responsible for these results, it appears that Firemaster 680 was not cometabolized under the conditions of this study.

Table 8. Firemaster 680 (FM680) Cometabolism

Sample	Time (Days)	FM680 (ug/L)	TBP (ug/L)	% Initial FM680 Concentration
A. FM680/glucose Growth Medium				
t0	0	1.96	ND[a]	39.2
t1	1	1.37	ND	27.4
t2	3	0.84	ND	16.8
t3	6	0.69	ND	13.8
t4	12	0.23	ND	4.6
t5	90	0.15	ND	3.0
t6	153	0.03	ND	0.6
B. FM680 Growth Medium				
t0	0	1.45	ND	29.0
t1	1	1.88	ND	37.6
t2	3	1.96	ND	39.2
t3	6	1.57	ND	31.4
t4	12	0.18	ND	3.6
t5	90	0.22	ND	4.4
t6	153	0.05	ND	1.0
C. FM680/DPE Growth Medium				
t0	0	2.34	ND	46.8
t1	1	2.04	ND	40.8
t2	3	1.98	ND	39.6
t3	6	1.75	ND	35.0
t4	12	1.03	ND	20.6
t5	90	0.02	ND	0.4
t6	153	0.04	ND	0.8

[a]ND - Not Detected (Limit of Detection 0.01 ug/L)

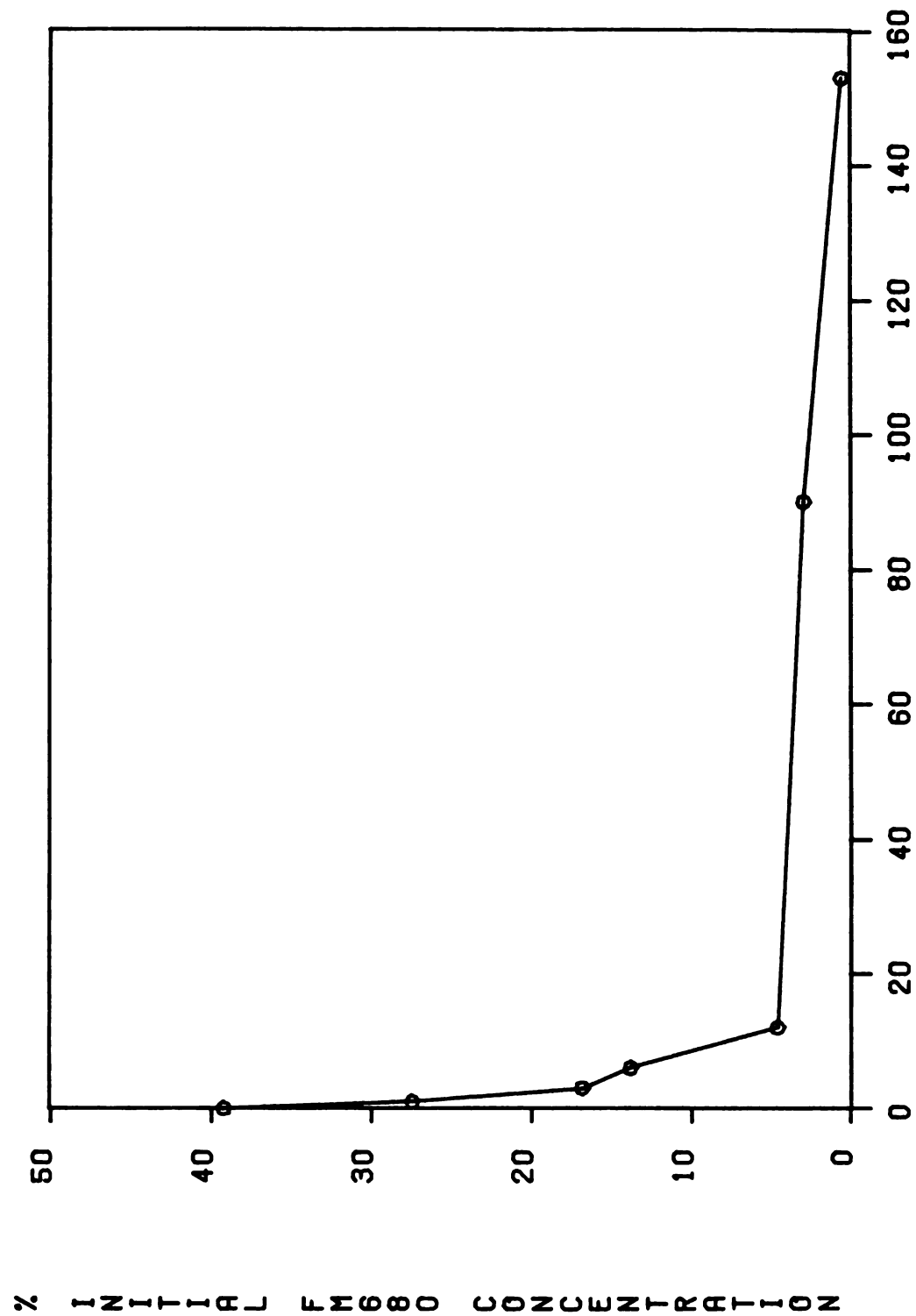


Figure 5. Progressive Loss of FM680 from FM680/glucose Growth Medium

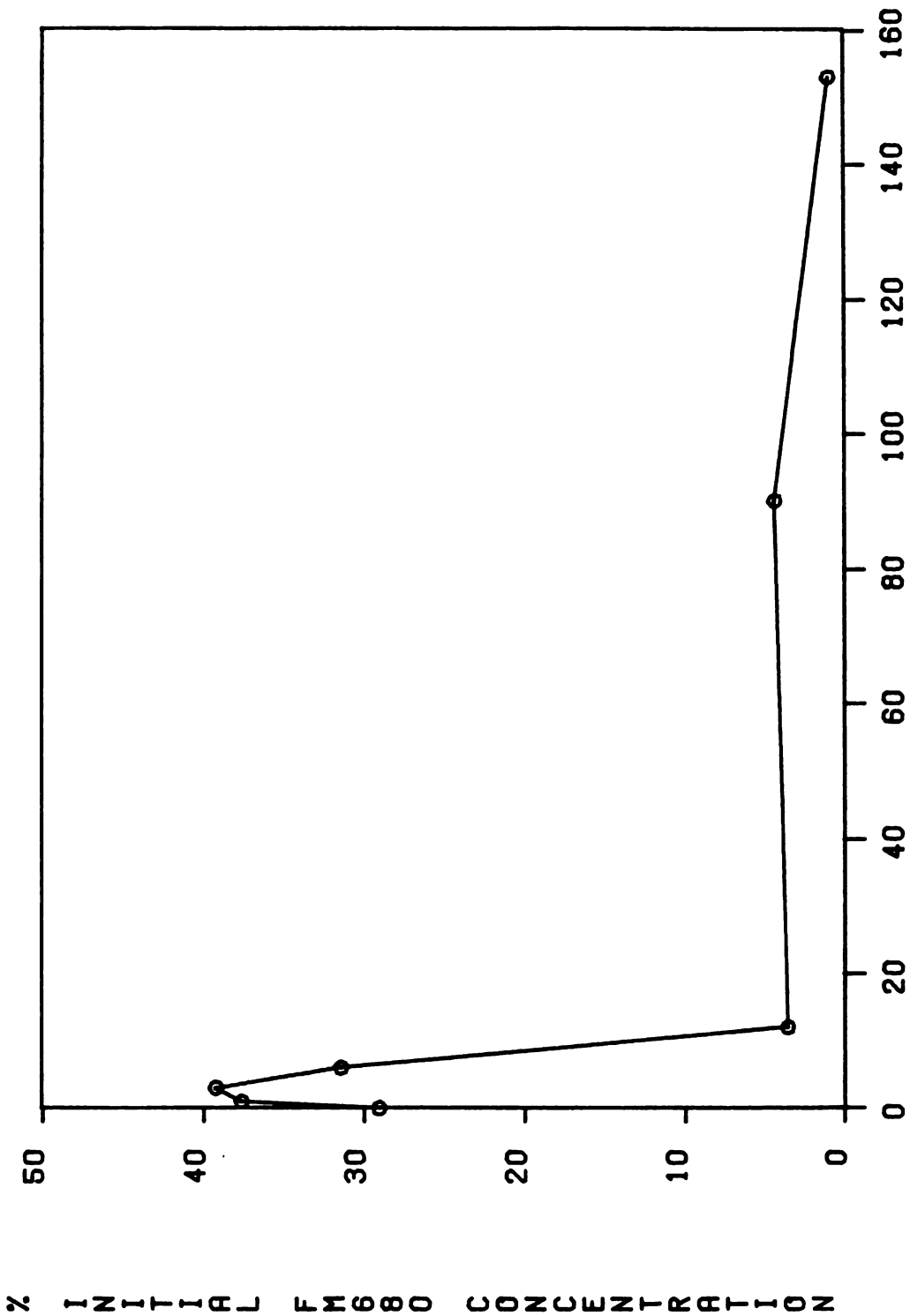


Figure 6. Progressive Loss of FM680 from FM680 Growth Medium

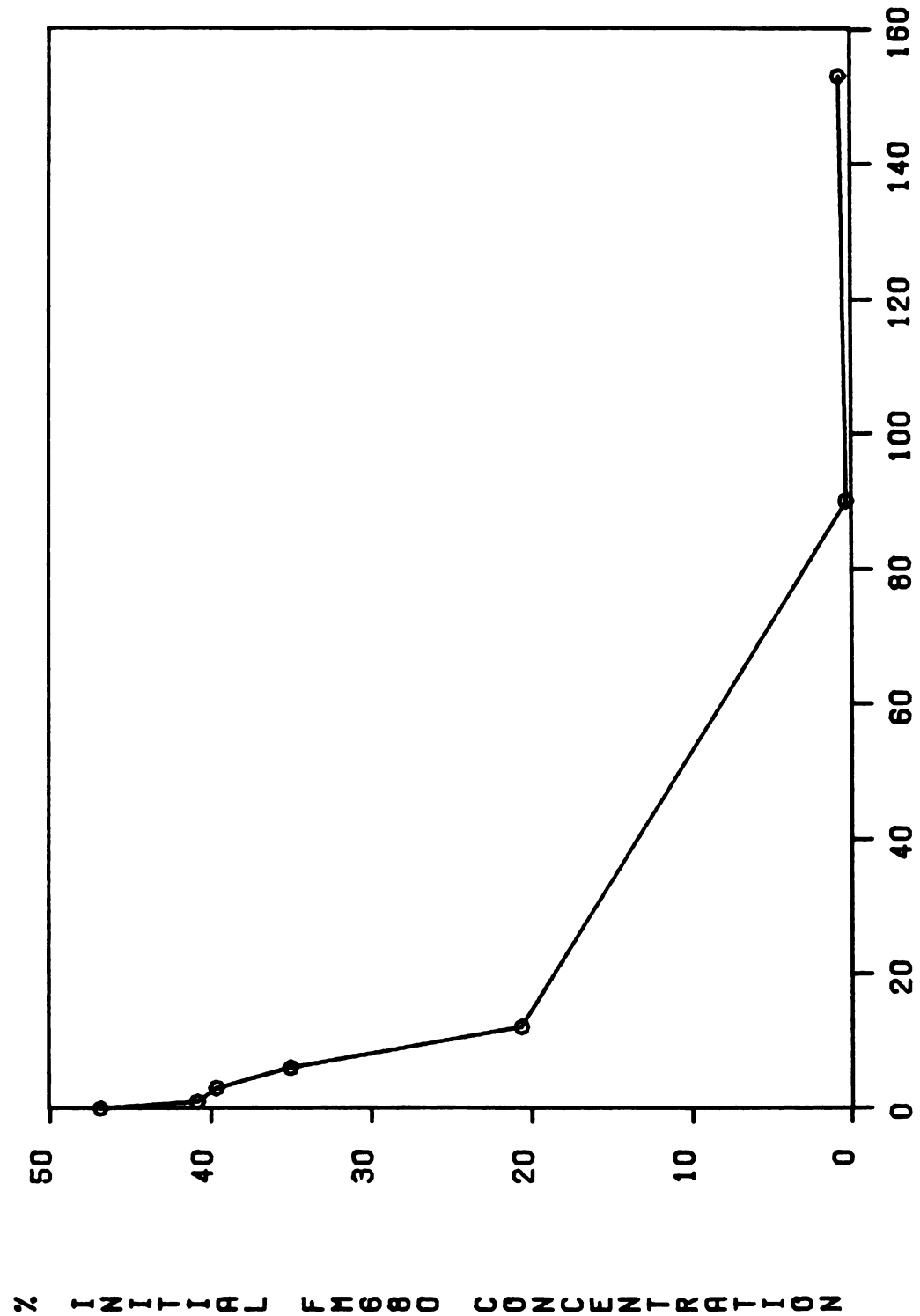


Figure 7. Progressive Loss of FM680 from FM680/DPE Growth Medium

DISCUSSION

WATER SOLUBILITY

Of the parameters that affect the fate and transport of organic chemicals in the environment, water solubility is one of the most important (Kenaga and Goring, 1980). In general, chemicals that are water soluble are likely to be more widely distributed by the hydrologic cycle than those which are relatively insoluble. The aqueous solubility of a chemical can have an effect on its adsorption and desorption on soils and sediments, on its bioconcentration potential, and on its potential for volatilizing from aqueous media (Freed et al., 1977). The more insoluble a chemical is, the more likely it is to sorb to soils and sediments and to bioconcentrate in aquatic organisms. Water solubility can also affect the possiblity of transformation via hydrolysis, photolysis, oxidation, reduction, and biodegradation in water.

In this experiment, the water solubility of Firemaster 680 was determined to be 0.04 ug/L. This value is low when compared with the aqueous solubilities of other known environmental contaminants. For example, Haque and Schmedding (1975) reported a water solubility of 10.3 ug/L for 2,2'4,5,5'-pentachlorobiphenyl and 0.95 ug/L for 2,2'4,4',5,5'-hexachlorobiphenyl. The lowest solubility value found for PCBs was 0.016 ug/L for decachlorobiphenyl (Weil et al., 1974). The highly toxic 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has a water solubility of 0.2 ug/L (Neely, 1979). For comparison with other brominated aromatic flame

retardants, technical grade octabromobiphenyl has a water solubility of 20 to 30 ug/L (Norris et al., 1974) and the solubility of Firemaster BP-6 in water is 11 ug/L (Jamieson, 1977).

In natural waters, the solubility of Firemaster 680 could be higher than reported here due to dissolved organic matter. A number of studies have shown that the presence of dissolved organic material, such as the naturally occurring humic and fulvic acids in rivers and other surface waters, leads to an increase in the solubility of many organic compounds (Hasset and Anderson, 1979; Matsuda and Schnitzer, 1971; Wershaw et al., 1969). However, this probably will not affect the solubility of Firemaster 680 by more than one order of magnitude.

Because of the low apparent aqueous solubility of Firemaster 680, the dissolved concentrations in water would be expected to be low. This indicates that Firemaster 680 tends to be associated with sediments, suspended solids, and biota in aquatic environments.

N-OCTANOL/WATER PARTITION COEFFICIENT

In recent years, the n-octanol/water partition coefficient (K_{OW}) has become a key parameter in studies of the environmental fate of organic chemicals. In particular, the n-octanol/water partition coefficient has become a critical property for predicting water solubility (Banerjee et al., 1980; Chiou et al., 1977; Kenaga and Goring, 1980; Mackay et al., 1980b; Tulp and Hutzinger, 1978), soil sorption (Briggs, 1969; Hassett et al., 1980; Karickhoff et al., 1979), bioconcentration in aquatic organisms (Chiou et al., 1977; Kenaga and Goring, 1980; Neely et al., 1974; Southworth et al., 1978; Veith et al., 1979), lipophilic storage (Davies et al., 1975), and biomagnification (Metcalf et al., 1973; Metcalf

et al., 1975; Lu et al., 1978; Lu and Metcalf, 1975; Tulp and Hutzinger, 1978).

The measured values of n-octanol/water partition coefficients for organic chemicals have been found to be as low as 10^{-3} and as high as 10^{7} (Hansch and Leo, 1979). In terms of log K_{ow}, this range is from -3 to 7. Estimated values for this parameter have been recorded as high a 15.5 (Tulp and Hutzinger, 1978). However, for most environmental contaminants that are persistent and tend to accumulate in sediments and aquatic biota, log K_{ow} values generally are in the range of 5 to 7. Some examples of these include 2,2',4,5,5'-pentachlorobiphenyl; 2,2',4,4',5,5'-hexachlorobiphenyl; p,p'-DDT; and TCDD with log K_{ow} values of 6.11, 6.72, 6.19, and 6.19, respectively (Chiou et al., 1977; Neely, 1979). Sugiura et al. (1978) have reported a log K_{ow} value of 7.41 for 3,3',5,5'-tetrabromobiphenyl. In the present study, the log K_{ow} for Firemaster 680 was estimated to be 7.14.

For neutral organic chemicals, the degree of sorption to sediments is dominated by their interaction with the organic content of the particulates. Karickhoff (1981) has shown that the organic carbon partition coefficient (K_{oc}) for natural sediments correlates well with the water solubility and n-octanol/water partition coefficients for hydrophobic pollutants. In his study, least squares fitting of the K_{ow} and K_{oc} data gave:

$$\log K_{oc} = 0.989 \log K_{ow} - 0.346 \qquad \text{(Equation 7)}$$

If the K_{oc} value for the environmental contaminant is known or estimated with equation 7, the partition coefficient for sediment sorption (K_p) can be predicted for a number of sediments where the organic content is known or designated.

A log K_{OC} value of 6.72 for Firemaster 680 was calculated by using equation 7 and the experimentally determined log K_{OW}, 7.14. For sediment with 3% organic carbon content, the corresponding K_p value would be 157,500. This indicates a strong tendency for Firemaster 680 to sorb onto suspended particulates and sediment in aquatic ecosystems.

The accumulation of organic chemicals in fish and other aquatic organisms can also be estimated from correlations between bioconcentration and n-octanol/water partition coefficients (Baughman and Paris, 1981; Geyer et al., 1981; Southworth et al., 1978; Veith et al., 1979). For fish, the following equation can be used for estimating the bioconcentration factor (BCF):

$$\log BCF = 0.76 \log K_{OW} - 0.23 \qquad \text{(Equation 8)}$$

This regression equation was derived by Veith et al. (1980) from the results of laboratory experiments by several investigators with a variety of fish species and 84 different organic chemicals. The bioconcentration factor for Firemaster 680 was calculated as 158,500 with equation 8 and 7.14 for the log K_{OW} value.

The structure-bioaccumulation relationship has one limitation which has not been thoroughly investigated (Veith, 1982). The equation implies that the bioconcentration factor will increase without bounds as the log K_{OW} increases. However, an increase in the log K_{OW} is often accompanied by an increase in molecular volume which eventually is sufficiently large to inhibit membrane permeability (Lieb and Stein, 1969).

A number of studies of the accumulation of polybrominated biphenyls by juvenile Atlantic salmon (Salmo salar) have shown that penta- and higher bromobiphenyls accumulate in the fish to a lesser extent than similar chlorobiphenyls (Zitko, 1977; Zitko, 1979; Zitko and Hutzinger,

1976). Octa- and higher brominated biphenyls were accumulated by the fish to a small extent only when administered in food. Low water solublity, possibly acting in conjunction with low membrane permeability, was considered the main factor for the lack of accumulation of the highly brominated biphenyls (Zitko, 1979). Sugiura et al. (1978) have shown that the accumulation factors for di-, tri-, and tetra-bromobiphenyls in killifish (Oryzias latipes) were proportional to n-octanol/water partition coefficients when the coefficients were below 10^6, but not when the coefficients were above 10^6.

The molecular size and steric configuration of Firemaster 680 may inhibit membrane permeability. Thus, the calculated bioconcentration factor for Firemaster 680 may significantly overestimate its bioaccumulation potential. The study reported by Velsicol Chemical Corporation on the uptake of Firemaster 680 by carp (Cyprinus carpio) revealed a maximum accumulation factor of 56.6 (U.S. EPA, 1980b). These data suggest that direct accumulation of Firemaster 680 from water by fish may not occur to a significant extent. However, further investigation of the bioconcentration and ecological magnification of Firemaster 680 in aquatic organisms is necessary before the bioaccumulation potential of this compound is fully understood.

MICROBIAL DEGRADATION

The transformation of organic compounds by living organisms is a very important factor for the evaluation of their persistence in the environment. Degradative processes caused by microorganisms are the most important degradative mechanisms for organic compounds in nature, with respect to both the mass of material transformed and the extent to which

it is degraded (Brink, 1981). Biodegradation is often a desirable mechanism for environmental transformations of organic molecules because the enzymatic degradations generally form the metabolites used for growth or potential energy storage, or simple inorganic molecules (Brink, 1981).

Considerable progress has been made in understanding how microorganisms degrade synthetic chemicals in the environment. Basically, a man-made compound will be biodegraded if it is susceptible to attack by the enzymes acquired by microbes during the course of evolution (Dagely, 1978). This depends on the ability of the microbial enzymes to accept as substrate compounds having chemical structures similar to those found in nature and the ability of these substrates to induce or derepress the synthesis of necessary enzymes (Dagely, 1978).

Numerous metabolic reactions in which microbes degraded a variety of synthetic chemicals have been identified in the laboratory (Alexander, 1981; Chapman, 1972; Chapman, 1979; Kobayshi and Rittmann, 1982). Almost all of the reactions involved in biodegradation can be classified as oxidative, reductive, hydrolytic, or conjugative. Examples of the first three kinds of reactions include beta-oxidation, hydroxylation, hydrolysis, dehalogenation, and decarboxylation. Conjugative reactions such as methylation, acetylation, and dimerization have also been observed in the presence of microorganisms. Reactions take place both in the presence and in the absence of oxygen. Some compounds, such as DDT, are transformed under both aerobic and anaerobic conditions (Meikle, 1972).

This diversity of microbial mediated reactions of chemical substances influenced many microbiologists to believe in the theory of microbial infallibility a few years ago (Alexander, 1965). They were convinced that every organic compound was able to sustain microbial growth and would

be completely degraded or mineralized. This theory has been proven incorrect because many synthetic organic molecules are mineralized very slowly or not at all. They endure for long periods of time in nature because microorganisms are not able to degrade them rapidly, if at all. Thus, they have been referred to as recalcitrant molecules (Alexander, 1981). Once released to the environment, these chemicals can be transported great distances and many of them are susceptible to biomagnification. Examples of recalcitrant chemicals include PCBs, DDT, and alkylbenzene sulfonates. A number of scientists are studying these types of chemicals and are trying to understand the basis for recalcitrance and to predict which molecules may not be subject to microbial transformation, especially mineralization (Alexander, 1981).

The results from this study indicated that Firemaster 680 was not readily degraded by microorganisms under the experimental conditions. Some loss of Firemaster 680 from the growth medium was observed in the cometabolism study, but no distinct metabolites were detected. It was assumed that the loss of the chemical was primarily the result of adsorption to the glass walls of the growth chambers and, in the case of the FM680/ glucose growth chamber, adsorption, and/or accumulation by microorganisms in the growth medium. Microorganisms that can metabolize Firemaster 680 were not found in any of the environmental samples collected for this study. These data suggest that Firemaster 680 should be classified as a recalcitrant chemical.

Resistance of an organic compound to microbial degradation may be attributable to the structure of the chemical or to one of several ecological parameters such as dissolved oxygen, oxidation-reduction potential, temperature, pH, availability of other compounds, salinity,

particulate matter, competing organisms, and concentrations of compounds and organisms (Alexander, 1965; Alexander, 1973; Alexander, 1975; Alexander, 1981). For Firemaster 680, its structure and physico-chemical characteristics are key factors influencing its resistance to biodegradation. The compound's low water solubility and large n-octanol/water partition coefficient suggests that it may not be readily available to the microorganism for biodegradation. The "bulky" chemical structure of Firemaster 680 could inhibit membrane permeability and the ability of the compound to reach the reaction site in the microbial cell. Furthermore, the extent and pattern of bromine substitution on the Firemaster 680 molecule would tend to inhibit microbial mediated dehalogenation, hyroxylation, and ring cleavage reactions for this compound.

Based on the results of this study, it appears that microbial degradation is not an important transformation process for Firemaster 680 in the aquatic environment. However, further research on the biodegradation of Firemaster 680 under anaerobic conditions is required since this chemical will most likely reside in the sediments of aquatic systems. Anaerobic degradative pathways such as reductive dehalogenation are now considered important processes in the biodegradation of certain classes of compounds (Kobayashi and Rittman, 1982).

SUMMARY AND CONCLUSIONS

Firemaster 680 has become an important brominated aromatic flame retardant for use in thermoplastic applications. Production of this compound has increased over the last five years and environmental contamination from this chemical has occurred near a number of production facilities. A contaminaton incident with Firemaster 680 has been identified in the Raisin River watershed in the vicinity of Adrian, Michigan. The impact of this pollutant on the aquatic ecosystem has not been assessed.

Very little is known about the behavior of Firemaster 680 in aquatic environments. The primary purpose of this investigation was to develop a base set of data which could be used to predict the water-related environmental fate of Firemaster 680. The aqueous solubility was measured as 0.04 ± 0.01 ug/L under controlled laboratory conditions during a 60 week experiment. The logarithm of the partition coefficient for Firemaster 680 between n-octanol and water was determined to be 7.14 ± 0.42. This value was then used to estimate an organic carbon normalized sediment distribution coefficient of 5.25×10^6 and a fish bioconcentration factor of 1.58×10^5. Bacteria capable of degrading Firemaster 680 were not found during acclimation, analog enrichment, or cometabolism studies. The UV spectrum of Firemaster 680 revealed absorption maxima at 282 and 290 nm which indicates the potential for direct photodegradation in the environment.

The melting point and density were found to be 225°C and 2.58 g/ml, respectively. The vapor pressure for Firemaster 680 at room temperature is expected to be low. These data may be used for a preliminary assessment of the chemodynamics of Firemaster 680 in the aquatic environment.

Based on the results of this study, it is apparent that physico-chemical processes will largely determine the persistence of Firemaster 680 in the environment. The degree of adsorption to sediments and other suspended matter will, to a large degree, dictate the ultimate fate of Firemaster 680 in aquatic environments. Adsorption on suspended particulates may lead to a wide translocation of this chemical from its original site of entry, and possibly to accumulation through trophic levels by the action of detritus-feeding organisms. In contrast, sedimentation of these materials may lead to burial and removal of Firemaster 680 from aquatic systems. This association with sediments may also have a negative effect on other fate processes. Compared to sorption, processes such as volatilization, photolysis, and biodegradation appear to be minor components governing the behavior of Firemaster 680 in the aquatic environment.

RECOMMENDATIONS

In view of the apparent importance of sorption of Firemaster 680 to sediments and suspended matter, further studies should be initiated to determine actual distribution coefficients for a variety of sediment types as a function of particle size distribution. The kinetics of adsorption and desorption for this chemical should be examined in the laboratory. Furthermore, studies should be undertaken to determine the ability of Firemaster 680 to undergo biomagnification through trophic levels. Finally, microbial degradation should be assessed under anaerobic conditions and the role of photodegradation in the fate of Firemaster 680 in aquatic systems should be investigated.

The results of this study suggests that an assessment of the impact of Firemaster 680 contamination on the south branch of the Raisin River should focus on sediments, especially in zones of deposition. A monitoring program should be developed for Firemaster 680 in suspended and bottom sediments and an assessment should be made on the effects of Firemaster 680 contamination on the benthic community. Additionally, an aquatic biota monitoring program should be initiated to determine possible biomagnification and the potential for wildlife and human exposure to this contaminant.

As a final note, similar studies on other brominated aromatic flame retardants should be undertaken to determine the environmental contamination potential of this diverse class of chemical substances.

APPENDICES

APPENDIX A

FIREMASTER 680 TOXICITY

The Velsicol Chemical Corporation has developed a toxicity profile on Firemaster 680 from acute, subacute, and chronic exposure studies with laboratory animals. Based on the results of these studies, the company claims that Firemaster 680 has a very low order of toxicity (Velsicol Chemical Corporation, 1977). These studies have been reported to the U.S. Environmental Protection Agency as a substantial risk notice in keeping with Section 8(e) of the Toxic Substances Control Act (U.S. EPA, 1980b). A synopsis of these data are reported below.

A number of acute toxicity studies with Firemaster 680 were reported and results from these studies are summarized in Table 9. These data appear to indiciate that this chemical is relatively non-toxic during acute exposure.

In addition to the acute studies, results from several subacute studies also were submitted (U.S. EPA, 1980b). A subacute dust inhalation toxicity study was reported with two exposure groups with 5 rats per sex in each group and one control group. The exposure group received micronized Firemaster 680 dust at concentrations of 5 or 20 mg/L for a 21-day period at 4 hr/day, 5 days/week. At the end of the 21-day period, hematological, biochemical, and urinalysis values were obtained prior to necropsy. Bromine neutron activation analysis of liver, fat, kidney,

Table 9. Acute Toxicity of Firemaster 680

Acute Study	Test Animal	Results
Oral Toxicity	Rat (male)	LD50 = 10,000 mg/kg
	Rat (female)	LD50 = 10,000 mg/kg
	Beagle Dog (male)	LD50 = 10,000 mg/kg
	Beagle Dog (female)	LD50 = 10,000 mg/kg
Dermal Toxicity	Rabbit (male)	LD50 = 12,000 mg/kg
	Rabbit (female)	LD50 = 10,000 mg/kg
Skin Irritation	Rabbit	Non-irritating
Eye Irritation	Rabbit	Non-irritating
Inhalation Toxicity	Rat (male)	LC50 = 36.48 mg/L
	Rat (female)	LC50 = 36.48 mg/L

lung, and blood samples was conducted at necropsy. There were no reported deaths in either the control or treated groups. A slight ocular porphyrin discharge was observed only in the treated groups while a clear nasal or prophyrin discharge, soft feces, and respiratory congestion was observed in animals from each group. All of the animals showed similar growth rates and the food consumption values obtained from rats in the control and treated groups were essentially similar. Results of the study indicated no significant differences between the hematologic, clinical chemistry, or urinalysis values of the control animals and the treated animals. However, very marked increases in bromine content, approximately 2,000 times, were reported in the lungs of treated rats. Increases in bromine from 2 to 3 times were also reported in liver, fat, kidney, and blood samples obtained at necropsy. No gross pathologic lesions or organ weight variations related to exposure to Firemaster 680 were reported. Compound related histopathologic lesions were limited to the lungs of

rats from both experimental groups which included small local accumulations of foamy alveolar macrophages scattered throughout the lungs.

A 28-day dermal toxicity study on albino rabbits also was reported (U.S. EPA, 1980b). In this study, the rabbits received doses of 50, 500, and 5,000 mg/kg of Firemaster 680 applied to the shaven intact or abraded skin for 5 days a week for 4 weeks. Rabbits in the control group and in each of the treatment groups exhibited very slight to slight erythema during the study period. One rabbit dosed at the 5,000 mg/kg/day level was reported to exhibit very slight to moderate erythema. The observed erythema was attributed to the normal saline carrier fluid and not the applications of Firemaster 680. No changes related to the compound were reported in hematological, biochemical, or urinalysis analyses which were obtained at day 14 and 28 of the study period. Nor were any compound related gross pathogenic lesions or variations in organ weight found in any rabbits from the experimental groups.

In addition to the inhalation and dermal subacute studies, a 28-day rat feeding study was reported in which male rats were exposed to 100 or 1,000 mg/kg Firemaster 680 (U.S. EPA, 1980b). At the end of the 28-day test period, rats in the test group showed less body weight gain and slightly poorer feeding efficiency when compared with the controls. Organ weight data were interpreted to indicate a definite difference between test and control animals with regard to the size of the organs. In general, all organ weights were decreased in the dosed animals when compared with the controls. Also, Firemaster 680 appeared to accumulate in the fat and remained there for some time.

A 90-day chronic oral toxicity study was reported in which albino rats were fed dietary concentrations of 0, 1, 10, or 100 mg/kg of Firemaster

680 (U.S. EPA, 1980b). The results of this study indicate that the average body weight gains and average food consumption among test animals were similar to those of the control group. However, the animals receiving the highest dose showed histological liver changes which were reflected by increased alkaline phosphatase in the blood. In addition, the kidney weights of the female rats on the 100 mg/kg diet were significantly lower than those of the control group. Also, the ratio of kidney weight to body weight was significantly greater in male rats on the 10 mg/kg Firemaster 680 diet. No data were presented to show the extent of bromine retention in tissues.

A mutagenicity evaluation of Firemaster 680 was also conducted (U.S. EPA, 1980b). This study was performed by using the Ames test on tester strains of Salmonella typhimurium and Saccharomyces cerevisiae. Tests were conducted both in the absence and presence of the rat liver activation system and the dose range was from 0.25 to 50 ug Firemaster 680 per plate. All results were reported to be negative.

In addition to mammalian toxicity studies, results from aquatic toxicity studies also were submitted (U.S EPA, 1980b). The 96-hour TL50's of Firemaster 680 reported for rainbow trout (Salmo gairdneri) and bluegill sunfish (Lepomis macrochirus) were 1410 mg/L and 1531 mg/L, respectively. In these studies, the test material was suspended in the water by sonication. Thus, the fish were exposed to particles of Firemaster 680 along with that dissolved in solution.

A Japanese testing company determined the 48-hour TL50 for Firemaster 680 for orange-red killifish (Oryzias latipes) to be 230 mg/L (U.S. EPA, 1980b). This value is significantly lower than the TL50's reported for bluegills and rainbow trout, although the test compound is still only

moderately toxic. The difference in the TL50's might be due to a different method of dissolving Firemaster 680. In this study, dimethyl sulfoxide and castor oil carriers, plus sonication were used to drive Firemaster 680 into solution.

In a recent study, the porphyrinogenic potential of some recently marketed fire retardants was determined using a primary tissue culture of chick embryo cells assay (Koster et al., 1980). The chemicals that were tested included decabromobiphenyl, decabromobiphenyl oxide, octabromobiphenyl oxide, N,N'-ethylenebistetrabromophthalimide, Firemaster 680, and Firemaster BP-6. Decabromobiphenyl and decabromobiphenyl oxide did not cause porphyria in the chick embryo liver cell culture. Firemaster 680 and N,N'-ethylenebistetrabromophthalimide were found to be slightly porphyrinogenic only after pretreatment of cultures with B-naphthoflavone to induce drug enzymes. Octabromobiphenyl oxide and Firemaster BP-6 were found to be strongly porphyrinogenic, even without pretreatment with B-naphthoflavone. The authors suggest that the nonporphyrinogenic effect of decabromobiphenyl and decabromobiphenyl oxide may be explained by their less planar molecular structure and their resistance to metabolic degradation.

These studies suggest that Firemaster 680 is relatively non-toxic to mammals and fish when tested under controlled laboratory conditions. However, the initial signs of Firemaster 680 toxicity are very similar to those found for PBBs. For example, several acute and chronic studies have shown that the initial indicators for PBB toxicity in mammals are weight loss or reduced weight gain (Falk et al., 1980). Additionally, increase in liver size is indicative of this toxicant. One study revealed that a dose as low as 50 mg/kg Firemaster BP-6 in the diet of male rats

for ten weeks produced an enlarged liver (Harris et al., 1978a). Another study showed decreased weight gain and an increase or decrease in the activity of some kidney enzymes in female rats when they were fed 100 mg/kg Firemaster BP-6 for 90 days (McCormack et al., 1978). When Firemaster FF-1 (Firemaster BP-6 with 2% calcium trisilicate) was fed to rats at doses of either 30, 100, 300, or 1,000 mg/kg of body weight per day, all of the animals exposed to doses greater than 30 mg/kg died within 73 days with the exception of 62% of the males at the 100 mg/kg dose (Gupta and Moore, 1979). All of these rats demonstrated depressed body weights, anemia, and enlarged livers.

Further evaluation of the carcinogenic and teratogenic potential of Firemaster 680 should be made before it is designated as non-toxic. Although the Ames test with Firemaster 680 produced negative results, this compound may still possess genotoxic properties. An Ames study with PBB was also negative (Rall et al., 1980), but several long-term animal studies have revealed carcinogenic activity (Gupta et al., 1981; Kimbrough et al., 1978; Kimbrough et al., 1981). Also, the reproductive and the teratogenic effects of PBB are well known (Allen, 1978; Aulrich and Ringer, 1979; Beaudoin, 1977; Corbett et al., 1975; Durst et al., 1978; Ficsor and Wertz, 1976; Harris et al., 1978b; Jackson and Halbert, 1974; Preache et al., 1976; Mercer et al., 1976; Moorehead et al., 1977; Wastell et al., 1978; Wertz and Ficsor, 1978).

APPENDIX B

CHEMICAL CHARACTERIZATION OF FIREMASTER 680

The reported chemical structure of Firemaster 680 was confirmed by using various analytical instrumental techniques which included gas chromatography, ultraviolet spectrometry, infrared spectrometry, nuclear magnetic resonance spectrometry, mass spectrometry, and elemental analysis. The purity of Firemaster 680 was determined from the elemental analysis, melting point, and trace organic impurity content.

EXPERIMENTAL

Materials

Firemaster 680 was obtained from Velsicol Chemical Corporation (Chicago, Illinois) as part of Lot No. 61114-F. The 2,4,6-tribromophenol standard was purchased from Aldrich Chemical Company (Milwaukee, Wisconsin) and it was 99+% pure. The 1-(2,4,6-tribromophenoxy)-2-bromoethane standard and sodium biphenyl reagent were obtained from the former Story Chemical Company (Muskegon, Michigan). All other reagents were of analytical or spectral grade.

Analytical Methods and Instruments

Elemental Analysis. The Firemaster 680 was subjected to elemental analyses to determine the carbon, hydrogen, and bromine content. The carbon and hydrogen content were determined with a Perkin-Elmer model 240 Elemental Analyzer (Perkin-Elmer, Norwalk, Connecticut).

The bromine content was measured by using a total bromide determination method (Liggett, 1954; Michigan Chemical Company, 1975). The sample was decomposed with sodium biphenyl reagent which converted the organic bromine into the inorganic form. Following decomposition, the bromide content was determined by potentiometric titration with a silver nitrate solution and calculated as bromine.

Melting Point. The melting point of Firemaster 680 was determined with a Fisher Melting Point Apparatus (Fisher Scientific Company, Pittsburgh, Pennsylvania). A small amount of sample was placed between two glass cover slips on the hot plate and heated with a temperature rate of increase of 10 ^{o}C/min.

Impurity Determination. The organic chemical impurities in the Firemaster 680 were determined by electron-capture gas-liquid chromatography. Ether solutions of 2,4,6-tribromophenol and 1-(2,4,6-tribromophenoxy)-2-bromoethane standards at concentrations of 10.0 and 1.0 ug/L and Firemaster 680 at 10.0 mg/L were prepared. These samples were subjected to gas chromatography analyses with a Beckman model GC-65 gas chromatograph equipped with a non-radioactive electron-capture detector and a Beckman 10-inch linear recorder (Beckman Instruments, Inc., Fullerton, California). The polarizing voltage, carbon dioxide flow, and bias voltage were set for optimum detector response. A 0.2 (i.d.) x 120 cm glass column packed with 1% SP-1240DA on 100/120 Supelcoport (Supelco, Inc., Bellefonte, Pennsylvania) was used with a helium carrier gas flow rate of 40 ml/min. The inlet, column, detector line, and detector temperatures were 290, 170, 310, and 360 ^{o}C, respectively. Using the above column and operating conditions, the retention times for 1-(2,4,6-tribromophenoxy)-2-bromoethane and 2,4,6-tribromophenol were 2.8 and 6.6 min.,

respectively; and the minimum detectable quantity was 1.0 pg for each standard at 2.5 times the noise level.

Ultraviolet Spectrometry. A Gilford model 2600 microprocessor-controlled ultraviolet-visible spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) equipped with a Hewlett Packard model HP7225A graphics plotter (Hewlett Packard, San Diego, California) was used to obtain UV absorption spectra of Firemaster 680. A 100 mg/L solution of Firemaster 680 in spectrophotometric grade cyclohexane (Aldrich Chemical Company, Milwaukee, Wisconsin) was placed in a 1.0 cm quartz cuvette and scanned through the near UV region between wavelengths of 220 to 340 nm.

Infrared Spectrometry. Infrared spectra of Firemaster 680 were obtained with a Perkin-Elmer model 337 Spectrophotometer (Perkin-Elmer, Norwalk, Connecticut). A KBr macropellet containing 1.0% Firemaster 680 was prepared and scanned from 2.5 to 25 microns.

Nuclear Magnetic Resonance Spectrometry. The nuclear magnetic resonance characteristics of Firemaster 680 were studied by using a Varian model EM-360 NMR Spectrometer System (Varian Instruments, Palo Alto, California). The NMR spectra were obtained for Firemaster 680 dissolved in carbon tetrachloride with tetramethylsilane (TMS) as an internal standard. The spectra were obtained at an applied field frequency of 60×10^6 cps.

Mass Spectrometry. A DuPont model 321 Mass Spectrometer (DuPont Company, Wilmington, Deleware) was used with a direct probe inlet system to obtain mass spectra data. Operating conditions for the mass spectrometer were as follows: source temperature, 250°C; ionizing potential, 70 eV; total scan time, 5.1 seconds (m/e 45 to 700); internal mass marker, perfluoro-tributylamine (PFA).

RESULTS

Elemental Analyses. Analyses of Firemaster 680, Lot. No. 61114-F, for elemental carbon, hydrogen, and nitrogen revealed that this sample contained 24.35% carbon, 1.15% hydrogen, and 0.00% nitrogen. Experimental data and calculations for these values can be found in Figures 8 and 9.

The total bromine analysis showed that Firemaster 680, Lot No. 61114-F, contained 68.72% bromine. This value was calculated with the equation:

$$\% \text{ Bromine} = \frac{(\text{ml } AgNO_3) \times (\text{N } AgNO_3) \times 0.079909 \times 100}{\text{sample weight (g)}}$$

(Equation 9)

Experimental data for this calculation included: volume $AgNO_3$ = 2.15 ml; normality $AgNO_3$ = 0.1002 N; sample weight of Firemaster 680 = 0.02505 g. Only one total bromine determination was made because of the limited amount of sodium biphenyl reagent, thus no statistical significance could be given to this value. However, it has been reported that organic bromine can be determined within 0.5% of the true value by this method (Ligget, 1954).

A comparison of the theoretical and experimentally determined elemental content of this sample of Firemaster 680 is presented in Table 10. The experimentally determined values are in close agreement (approximately 99%) with the theoretical values.

Melting Point. The melting point of Firemaster 680 was determined to be 225°C. The crystals began to melt at 225°C and were completely melted at 226°C.

Impurity Determination. Gas chromatography analyses of Firemaster 680 for organic impurities showed less than 0.1% of 2,4,6-tribromophenol and 1-(2,4,6-tribromophenoxy)-2-bromoethane. A comparsion of the gas chromatograms obtained from this experiment is presented in Figure 10.

CALIBRATION DATA SHEET

A. Standard Name: A_2 B. Date: 12-6-77

C. Standard Weight: 2393 ug

D. Theoretical Percentages: 10.36% N; 71.09 % C; 6.71 % H

E. Theoretical Weights: 247.91 ug N; 1701.18 ug C; 160.57 ug H

F. Total Signals:

	N Signal	C Signal	H Signal
Reads (uV)	537 x 4 = 2148	876 x 10 = 8760	653 x 16 = 10445
+C Suppression (uV)	***	Setting 3 = 30000	***
- Blank Values (uV)	= 210	= 190	= 450
- Zero (uV)	= 42	= 1330	= 192
Total Signals (uV)	= 1896	= 37240	= 9806

G. Sensitivities:

$$K = \frac{\text{Total signal (uV)}}{\text{Theoretical weight (ug)}}$$

$$K_N \frac{7.65}{(7.6)} \frac{uv}{ug}; \quad K_C \frac{21.81}{(21.8)} \frac{uv}{ug}; \quad K_H \frac{61.07}{(61.1)} \frac{uv}{ug}$$

Figure 8. CHN Analyzer Calibration Data Sheet

A. Sample No.: FM680 B. Sample Name: Firemaster 680

C. Date: 12-6-77 D. Sample Weight: 2033 ug

E. Theoretical Percentages: -- % N; 24.45 % C; 1.17 % H

F. Theretical Weights: -- ug N; 497.1 g C; 23.8 ug H

G. Sensitivities: K_N = 7.6 $\frac{uv}{ug}$; K_C = 21.8 $\frac{uv}{ug}$; K_H = 61.1 $\frac{uv}{g}$

	N Signal	C Signal	H Signal
Reads (uV)	210 x 1 = 210	234 x 10 = 2340	518 x 4 = 2072
+C Supression (uV)	***	Setting 1 = 10000	***
- Blank Values (uV)	= 210	= 200	= 450
- Zeros (uV)	= 50	= 1350	= 188
Total Signals (vV)	= 0	= 10790	= 1434

I. Calculated Weights and Percentages:

Weight (ug) of N, C, or H in a sample = $\frac{\text{Total Signal (uV)}}{\text{Sensitivity K } \frac{(uV)}{ug}}$

Weights 0 ug N; 494.95 ug C; 23.47 ug H

Percentage of N, C, or H in a sample = $\frac{\text{Weight (ug) x 100}}{\text{Sample Weight}}$

Percentages: 0 % N; 24.35 % C; 1.15 % H

Figure 9. CHN Analyzer Data Sheet for Firemaster 680

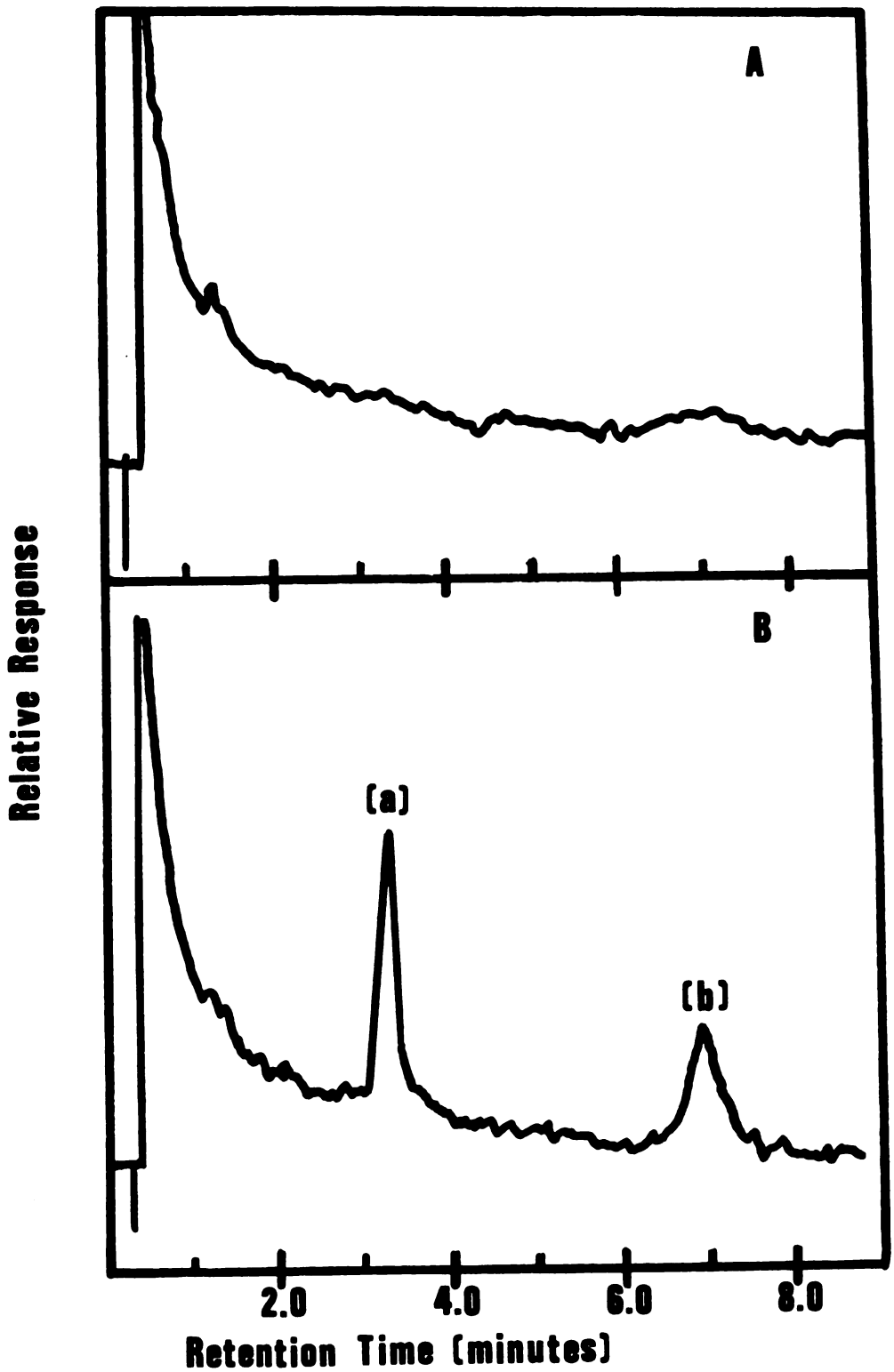


Figure 10. Gas Chromatogram - (A) Firemaster 680 (10,000 pg) (B) 1-(2,4,6-tribromophenoxy)-2-brommoethane (a) and 2,4,6-tribromophenol (b) (10 pg)

Table 10. Comparison of Theoretical and Experimentally Determined Elemental Content of Firemaster 680 (FM680)

Element	Theoretical Content	Experimental Content
	----------%	----------
Br (6)[a]	69.72	68.72
C (14)	24.46	24.35
H (8)	1.17	1.15
O (2)	4.65	ND[a]

[a]( ) = Number of atoms of each element in Firemaster 680
[b]Oxygen content was not determined.

Ultraviolet Spectrometry. The UV absorption spectrum of Firemaster 680 is presented in Figure 11. The spectrum shows B-band absorptions at max of 282 and 290 nm. This absorption pattern is characteristic of a benzene chromophore with auxochromic group substitution. A characteristic bathochromic shift (red shift) of the B-bands of the benzene chromophore is seen in the Firemaster 680 spectrum. The UV absorption spectrum of Firemaster 680 is consistent with the reported chemical structure of this product.

Infrared Spectrometry. The infrared spectrum of Firemaster 680 is shown in Figure 12. The characteristic absorption bands are as follows: (a) aromatic C-H stretch, 3060, 3030 cm^{-1}; (b) methylene C-H stretch, 2930, 2860 cm^{-1}; (c) aromatic C-H overtone band, 1715 cm^{-1}; (d) aromatic ring C-C stretch, 1550, 1520, 1430, 1430, 1410 cm^{-1}; (e) asymetric C-O-C stretch, 1252 cm^{-1}; (f) symmetric C-O-C stretch, 1002 cm^{-1}; (g) out of plane aromatic ring C-H bend, 860, 745, 737 cm^{-1}; (h) out of plane aromatic

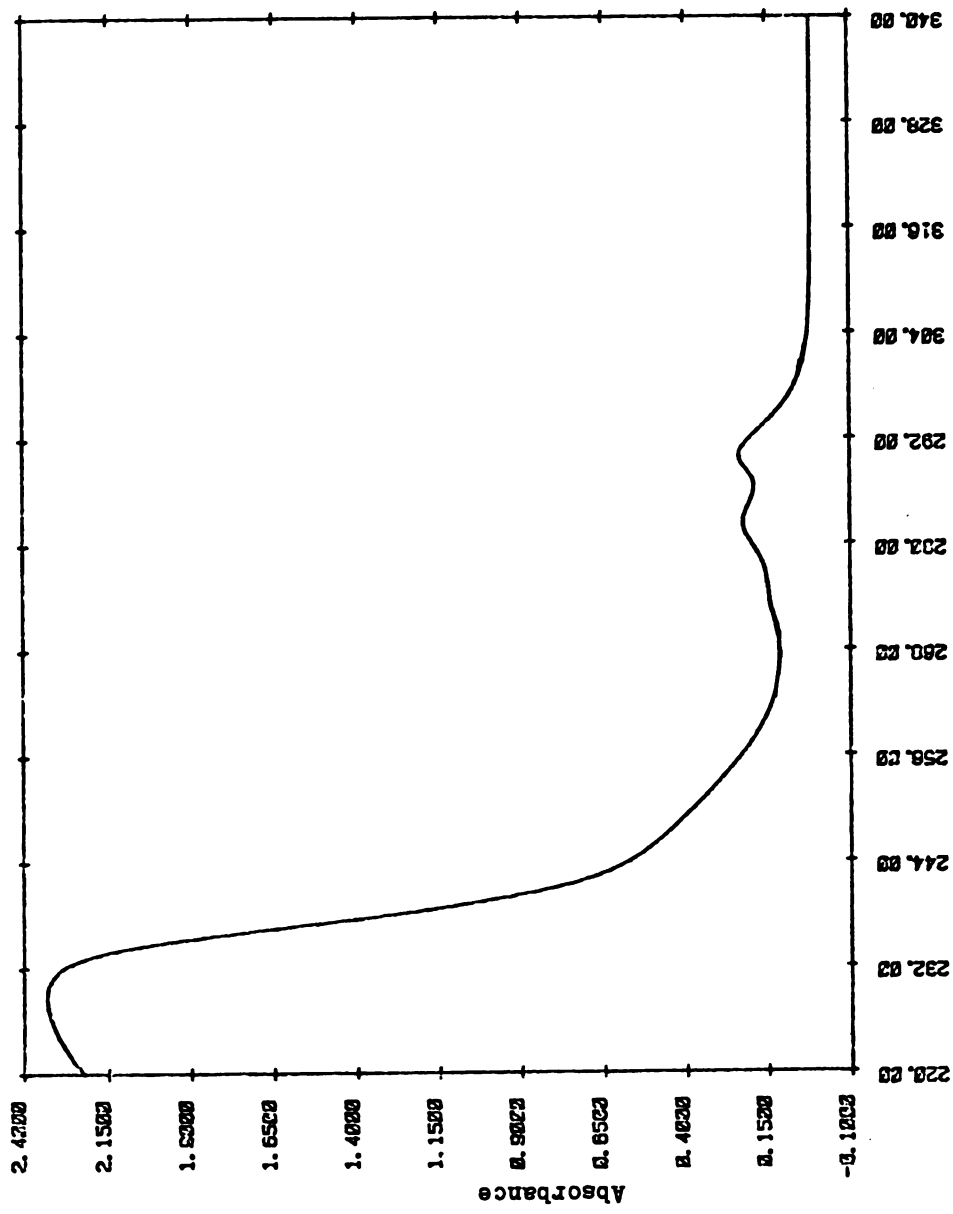


Figure 11. Ultraviolet Absorption Spectrum of Firemaster 680

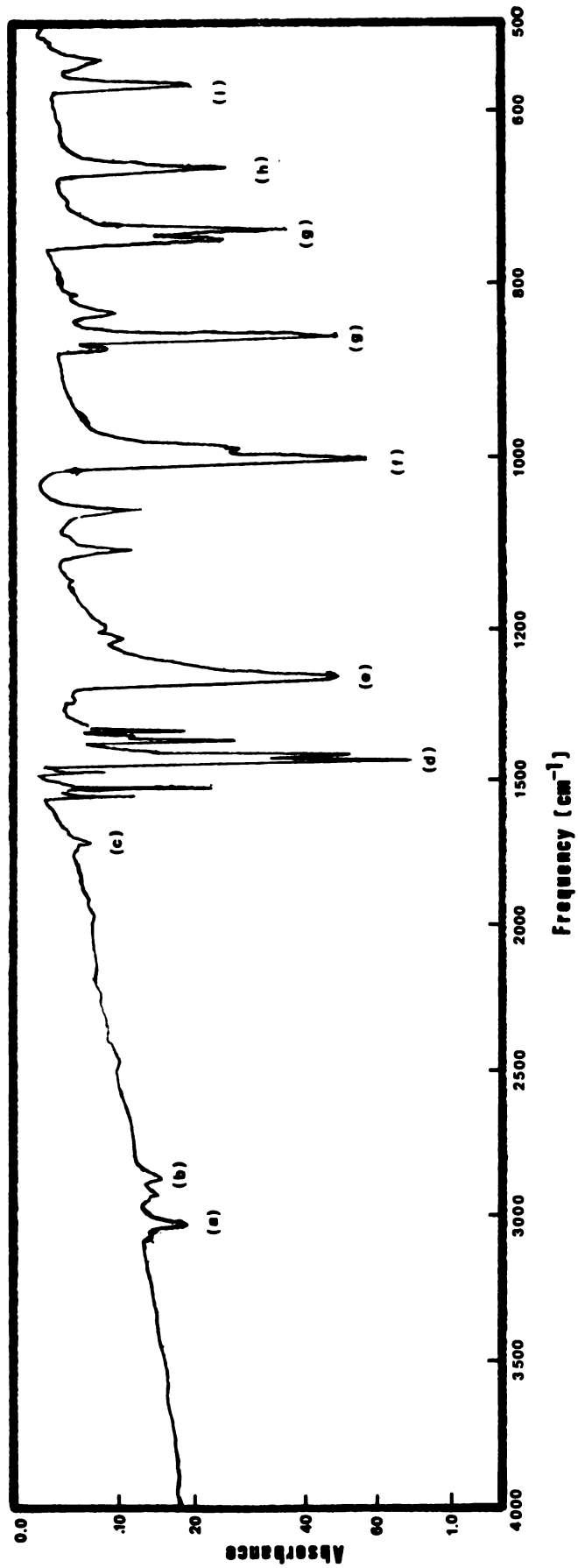


Figure 12. Infrared Spectrum of Firemaster 680

ring C-C bend, 663 cm^{-1}; (i) C-Br stretch, 569 cm^{-1}. This absorption pattern is indicative of an aryl-alkyl ether with the aryl moiety substituted with bromine. This spectrum also reveals that there were no alcohol, amine, acid, nitrile, ketone, or aldehyde functional groups in the molecule. The infrared absorption spectrum obtained for Firemaster 680 is in very good agreement with the reported structure of this product.

Nuclear Magnetic Resonance Spectroscopy (NMR). The NMR spectrum of Firemaster 680 is presented in Figure 13. The proton NMR spectrum of Firemaster 680 showed resonance signals from two different protons at 4.45 ppm and 7.70 ppm. Both signals were singlets, which indicates chemically equivalent protons contributing to each resonance signal. Furthermore, the resonance signals intigrate 1 to 1 which indicates equivalent number of protons for each signal. The singlets at 4.45 ppm is indicative of a methylene proton adjacent to an oxygen atom, specifically an alkyl-aryl ether group. The singlet at 7.70 ppm is indicative of an aromatic proton, specifically one that had been shifted paramagnetically as a result of the inductive effect of a neighboring electronegative atom such as bromine. This resonance pattern is in agreement with the reported structure of Firemaster 680, with four methylene protons of an alkyl-aryl ether group and four equivalent aromatic protons separated by electronegative bromine atoms.

Mass Spectrometry. A graphic representation of the mass spectrum of Firemaster 680 is presented in Figure 14. The molecular ion (M+) was found at m/e 682 and the base peak (B) was at m/e 354. The molecular ion peak was followed by a M+2, M+4, M+6, M+8 pattern which is characteristic of a molecule containing six bromine atoms.

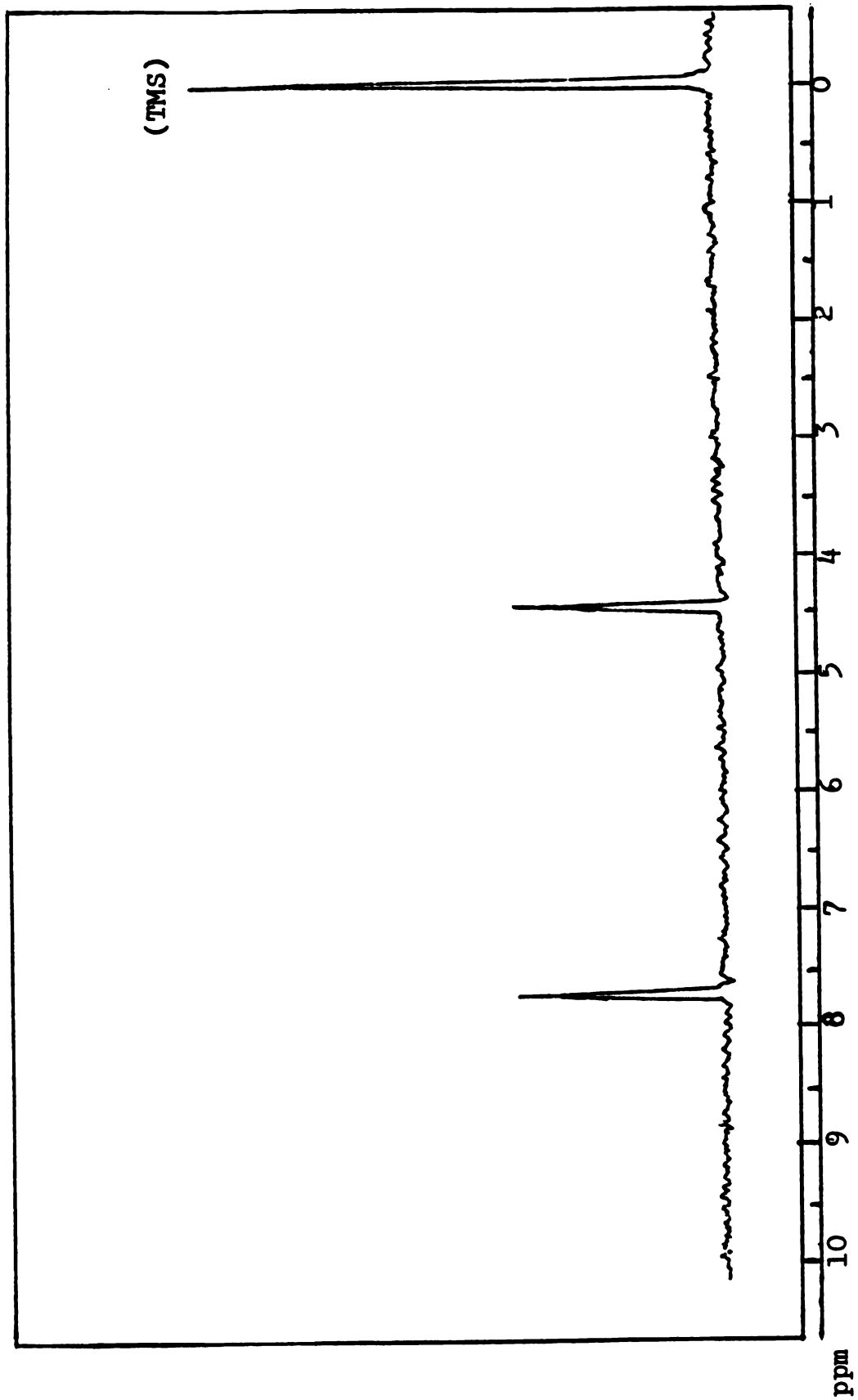


Figure 13. NMR Spectrum of Firemaster 680

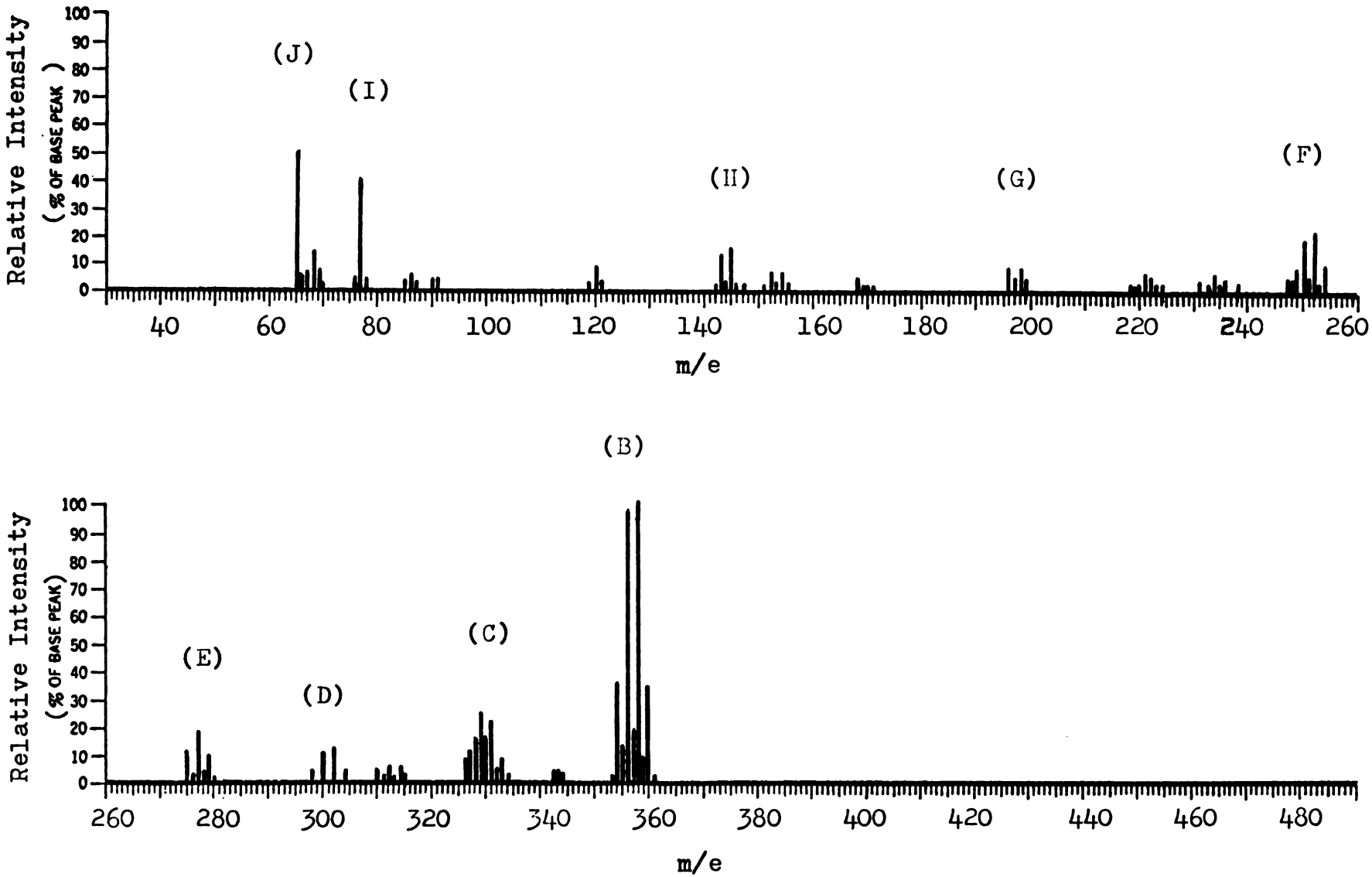


Figure 14. Graphic Representation of Mass Spectrum of Firemaster 680

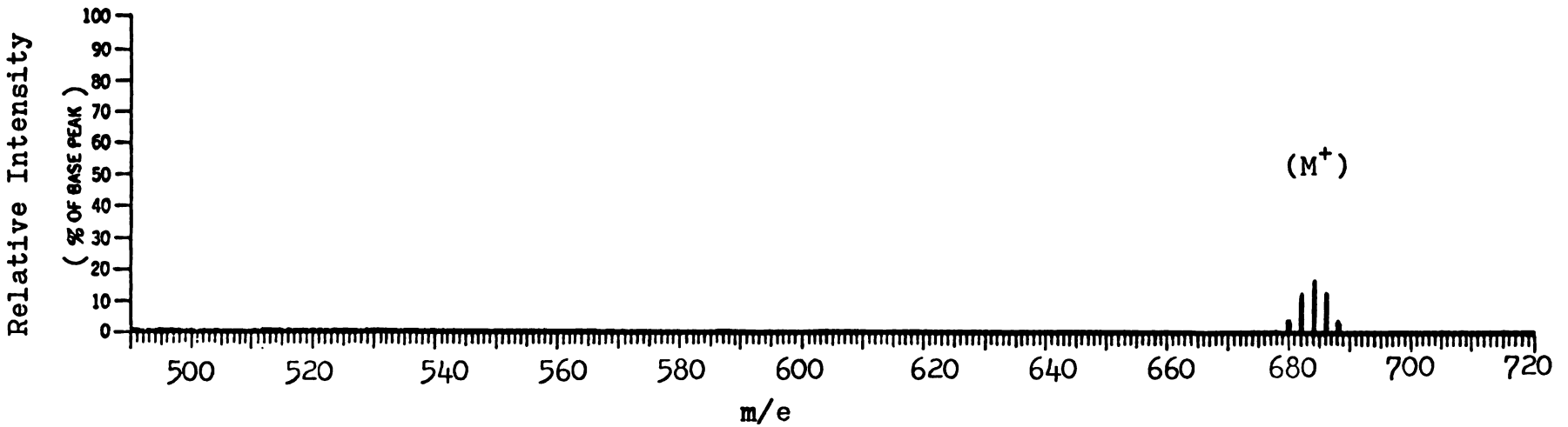
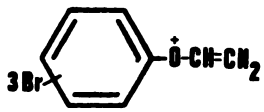


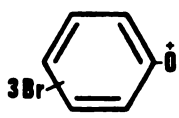
Figure 14. (cont'd.)

The base peak at m/e 354 was followed by a M+2, M+4, M+6 pattern characteristic of three bromine atoms. This fragment was most likely a tribromophenoxyethylene ion with the structure:



(B)

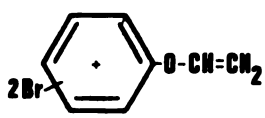
which was produced by cleavage of the parent compound through the ether bond. The other fragment resulting from this cleavage was the tribromophenol cation with the structure:



(C)

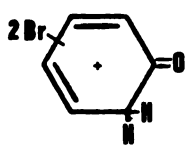
which was found at m/e 327 (C). Once again, the M+2, M+4, M+6 pattern was present which indicated three bromine atoms.

The next major peak was located at m/e 275 (E) which showed an M+2, M+4 pattern, typical of two bromine atoms. This peak was 79 mass units from the base peak (m/e 354) and resulted from cleavage of a bromine atom from the base peak ion. The resulting fragment had the structure:



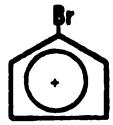
(E)

The peak at m/e 250 (F) is typical of the fragment which was produced by cleavage of the ethylene group of fragment E along with hydrogen rearrangement. The characteristic two bromine substituent M+2, M+4 pattern is also apparent here. The structure of this fragment was:



(F)

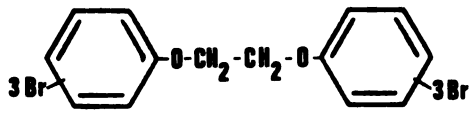
Cleavage of a Br and CO group from compound F resulted in a bromocyclopentadiene cation which was found at m/e 143 (H) and had the structure:



(H)

Additional major peaks were found at m/e 77 (I) and m/e 65 (J) which are indicative of phenyl and cyclopentadienyl cations, which are typical fragments of aromatic compounds. A number of minor peaks (i.e., D and G), which are consistent with Firemaster 680 fragments, were also found.

The mass spectrum fragmentation pattern of Firemaster 680 is in agreement with the reported chemical structure of this product. With these data alone, the chemical structure for Firemaster 680 is best described by the structure:

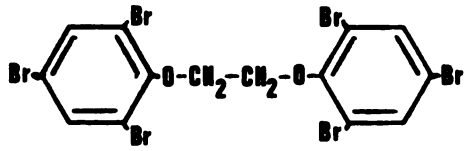


The exact location of the bromine atoms on the aromatic rings could not be determined from the mass spectral data.

DISCUSSION

The ultraviolet, infrared, nuclear magnetic resonance, and mass spectra of Firemaster 680, Lot No. 61114-F, identified this product as 1,2-bis(2,4,6-tribromophenoxy)ethane. Infrared and mass spectra data revealed that Firemaster 680 consists of a double alkyl-aryl ether connected through the ethane C-C bond with three bromine substitutes on each phenyl ring. The NMR spectrum, showing only two singlets, confirmed that the bromine atoms are placed at positions 2, 4, and 6 on each phenyl

ring. From these data, the structure of Firemaster 680 was determined to be:



This chemical is structurally symmetrical with the axis of symmetry through the C-C bond of the ethylene group. This explains the relatively simple NMR and mass spectra for Firemaster 680.

The purity of Lot No. 61114-F of Firemaster 680 appeared to be between 98 and 99%. This sample contained approximately 68.7% organically bond bromine which equates to a purity of 98.6% for Firemaster 680. There were less than 0.1% bromine containing organic impurities in the sample. The melting point was sharp, within 0.5°C, which indicates a relatively pure product. Commercial grade products are not normally as pure as Firemaster 680. However, the chemical reactions in the synthesis of this product are specific and the raw materials are relatively pure which explains the high purity of the final product.

APPENDIX C

ANALYTICAL METHOD DEVELOPMENT

An XAD macroreticular resin analytical scheme was developed for extracting Firemaster 680 and potential metabolites from aqueous media. The method described here is a modification of an analytical scheme reported by Junk et al. (1974). The extraction efficiency for Firemaster 680 and 2,4,6-tribromophenol is reported for tests with XAD-2 and XAD-4 alone, and a mixture of XAD-2/XAD-4.

EXPERIMENTAL

Materials and Apparatus

Reagents. Deionized water was freed of organic matter by passing it through a column containing activated charcoal. All of the solvents were analytical grade and were obtained from Burdick and Jackson Laboratories (Muskegon, Michigan). The concentrated HCl (approximately 37%) was obtained from Mallinckrodt (St. Louis, Missouri).

The macroreticular resins, XAD-2 and XAD-4, were obtained from Rohm and Haas (Philadelphia, Pennsylvania). The fines were removed by slurrying in methanol and then decanting. The remaining resin beads, predominantly 20-60 mesh, were purified by sequential solvent extractions with methanol, acetonitrile, and diethyl ether in a soxhlet extractor for

8 hours with each solvent. The purified resins were stored under methanol in glass-stoppered bottles to maintain their purity.

Test Solutions. Firemaster 680 and the 2,4,6-tribromophenol used to prepare standard samples and spiked water samples were obtained from Velsicol Chemical Corporation and Aldrich Chemical Company. Standard solutions containing 20, 40, 60, 80, 100, 200, 400, 600, 800, and 1000 ug/L of Firemaster 680 and 2,4,6-tribromophenol were prepared in diethyl ether. Spiked water samples were prepared to contain 0.0, 0.1, 0.3, 0.5, 0.7, 0.8, 0.9, 1.0, 1.2, 1.6, and 2.0 ug/L of Firemaster 680 and 2,4,6-tribromophenol.

Instruments. A Beckman, model GC-65, gas chromatograph equipped with a non-radioactive electron-capture detector and a Beckman 10-inch linear recorder were used to obtain the gas chromatography data. The polarizing voltage, carbon dioxide flow and bias voltage were set for optimum detector response. A 0.2 (i.d.) x 120 cm glass column packed with 1% SP-1240DA on 100/200 Supelcoport (Supelco, Inc., Bellefonte, Pennsylvania) was used with a helium carrier gas flow rate of 60 ml/min and a column temperature of 160°C to analyze the 2,4,6-tribromophenol. For Firemaster 680, a 0.2 (i.d.) x 183 cm glass column packed with 60/80 mesh Gas Chrom Q coated with 3% OV-1 liquid phase (Applied Science Laboratories, Inc., State College, Pennsylvania) was used with a helium carrier gas flow rate of 60 ml/min and a column temperature of 270°C. The inlet, detector line, and detector temperatures were 290, 310, and 360°C, respectively. With these columns and operating conditions, the retention times for Firemaster 680 and 2,4,6-tribromophenol were 4.3 and 2.7 minutes, respectively; and the least detectable quantity was 1.0 pg for each at 2.5 times the noise level.

Analytical Procedure

Column Preparation. The apparatus used to remove trace quantities of Firemaster 680 and 2,4,6-tribromophenol from water consisted of a 1.5 (i.d.) x 30 cm glass column which was fitted with a 300 ml sample reservoir and a teflon stopcock. A clean, ether-extracted silanized glass wool plug was inserted near the stopcock. The purified resin was added as a methanol slurry until a resin bed approximately 7.0 cm high was obtained (6.0 g dry resin); then a second silanized glass wool plug was inserted above the resin. The methanol was drained through the stopcock until the level reached the top of the resin bed; then the resin was washed with three 20 ml portions of deionized water. Each portion of the flow was stopped when the liquid level reached the top of the resin bed. An XAD-2, XAD-4, and 50/50 mixture of XAD-2/XAD-4 column were prepared by using this procedure.

Sample Preparation. All of the standard solutions were prepared with diethyl ether. All of the spiked water samples were prepared by injecting a calibrated volume of a standard solution of Firemaster 680 and 2,4,6-tribromophenol into a 500 ml volumetric flask containing deionized water. The spiked water samples were then acidified (pH 2.5) by adding 2.5 ml of concentrated HCl.

Column Extraction. The acidified spiked water samples were added to the glass columns containing XAD resin. The sample was allowed to pass through the XAD resin column by gravity flow at a rate of 30 to 50 ml/min. When most of the sample had passed through the column and the liquid level was at the top of the resin, the reservoir walls were carefully washed with a 20 ml portion of deionized water and drained through the column until the level reached the top of the resin bed. This wash was

repeated twice, letting the water drain completely only after the last wash.

Elution and Regeneration. The reservoir walls were washed with two 10 ml portions of diethyl ether, and each wash was allowed to drain into the XAD resin but not through the column. The column was then capped and the diethyl ether was allowed to equilibrate with the resin for 10 minutes. Then the cap was removed, the stopcock was opened, and the ether was allowed to flow through the column into a 30 ml screw-top test tube. An additional 5 ml of diethyl ether was added to the column and immediately was allowed to flow through the resin into the test tube. The last traces of diethyl ether in the column was eluted with purified air under pressure.

The XAD column was regenerated immediately after the ether was eluted. Methanol was added to the column, and the air bubbles were removed by shaking the XAD resin with the methanol. A total of 30 ml of methanol were passed through the column. The stopcock was closed when the methanol reached the top of the resin bed, and the silanized glass wool plug was inserted. An additional 15 ml of methanol was added, and the reservoir was capped with a stopper. The XAD resin column was ready for subsequent analyses without further treatment beyond wetting the resin with deionized water as outlined in the column preparation step.

Drying. Most of the residual water was removed from the diethyl ether eluate by freezing. After the water was frozen, the diethyl ether eluate was decanted through anhydrous sodium sulfate into a 20 ml graduated glass centrifuge tube. The walls of the screw-top test tube were immediately washed with 1 ml of diethyl ether and the ether was added to the eluate in the centrifuge tube.

Concentration of Eluate. In the graduated glass centrifuge tube, the eluate was concentrated by evaporating the diethyl ether with a stream of purified air. The eluate was allowed to concentrate to 1 ml, then the final volume was increased to 2 ml with additional diethyl ether.

Separation and Quantification. A 1.0 ul aliquot of each 2.0 ml concentrate was injected into the gas chromatograph with a syringe. When the gas chromatography separation of the Firemaster 680 or 2,4,6-tribromophenol in the concentrates was completed, this process was immediately repeated with the set of standard ether solutions of Firemaster 680 and 2,4,6-tribromophenol. The concentration range was similar to that expected in the 2.0 ml concentrates, assuming complete recovery of the solute from the water sample. The gas chromatographic conditions were held rigidly constant for both sample and standard during the tests. The chromatogram peaks were integrated by a computer which was interfaced with the gas chromatograph. The peak areas were used to calculate the percentage of the organic solutes that were removed.

RESULTS

The extraction efficiencies for each resin for Firemaster 680 (FM680) and 2,4,6-tribromophenol (TBP) at each concentration are summarized in Table 11. The experimental data and calculations are presented in Tables 12-17.

The extraction efficiency results show that the XAD-2 resin system was most efficient in extracting Firemaster 680 and 2,4,6-tribromophenol at all concentrations tested. The mean recovery for 2,4,6-tribromophenol was slightly greater than 100%, whereas the mean recovery for Firemaster 680 was approximately 50%. The XAD-4 system recovered 41% of Firemaster

Table 11. XAD Resin Extraction Efficiency of Firemaster 680 (FM680) and 2,4,6-tribromophenol (TBP)

FM680 Recovery (%)			Spiked Samples FM680/TBP Concentrations	TBP Recovery (%)		
XAD-2	XAD-4	XAD-2/4	(ug/L)	XAD-2	XAD-4	XAD-2/4
50.8	33.2	20.4	0.1	109.6	80.4	133.6
51.7	40.9	39.6	0.3	94.1	81.3	61.1
52.4	47.0	42.0	0.5	117.6	60.6	87.6
57.3	41.3	43.8	0.7	111.4	92.1	75.4
48.3	40.9	46.3	0.8	97.2	80.4	79.7
44.0	49.2	44.1	0.9	118.2	108.8	80.8
47.8	40.5	45.0	1.0	103.7	95.2	88.3
53.0	38.4	57.6	1.2	121.7	88.2	82.2
46.8	39.8	48.0	1.6	106.3	87.0	82.1
45.5	39.7	48.5	2.0	103.4	109.5	70.5
49.8±2.9	41.0±3.2	43.5±6.8	Mean Recovery (%)	108.3±6.5	88.4±10.3	84.1±13.7

Table 12. XAD-2 Extraction Efficiency-FM680

Spiked Water Sample FM680 Concentration (ug/L)	Theoretical Amount FM680 Injected (pg)	Spiked Water Sample GC Analysis (Relative Area)	Actual Amount FM680 Injected (pg)[a]	Recovery (%)[b]
0.0	0	ND[e]	ND	--
0.1	25	25.3(-)[d]	12.7	50.8
0.3	75	62.2(6.5)[c]	38.8	51.7
0.5	125	100.0(5.1)	65.5	52.4
0.7	175	149.4(3.3)	100.3	57.3
0.8	200	144.0(12.4)	96.5	48.3
0.9	225	147.3(2.4)	98.9	44.0
1.0	250	176.4(18.4)	119.4	47.8
1.2	300	232.4(19.7)	158.9	53.0
1.6	400	272.1(7.6)	187.0	46.8
2.0	500	329.3(18.0)	227.3	45.5
		Mean Recovery (%) = 49.8±2.9		

[a]Calculated from standard calibration curve [Area = 1.42(pg) + 7.3; r^2 = 0.997]
[b]Calculated as (Actual Amount/Theoretical Amount) x 100
[c]( ) = standard deviation, N = 3
[d](-) = no standard deviation, N = 1
[e]ND = None Detected, <1.0 pg

Table 13. XAD-2 Extraction Efficiency-TBP

Spiked Water Sample TBP Concentration (ug/L)	Theoretical Amount TBP Injected (pg)	Spiked Water Sample GC Analysis (Relative Area)	Actual Amount TBP Injected (pg)[a]	Recovery (%)[b]
0.0	0	ND[d]	ND	--
0.1	25	12.2(7.2)[c]	27.4	109.6
0.3	75	31.4(4.6)	70.6	94.1
0.5	125	65.4(9.3)	147.0	117.6
0.7	175	86.7(8.7)	195.0	111.4
0.8	200	86.4(13.3)	194.3	97.2
0.9	225	118.3(13.8)	266.0	118.2
1.0	250	115.3(9.9)	259.3	103.7
1.2	300	162.4(24.3)	365.2	121.7
1.6	400	189.0(9.3)	425.0	106.3
2.0	500	229.9(19.4)	517.0	103.4
		Mean Recovery (%) = 108.3±6.5		

[a]Calculated from standard calibration curve [Area = 0.44(pg) + 0.0; r^2 = 0.999]
[b]Calculated as (Actual Amount/Theoretical Amount) x 100
[c]( ) = standard deviation, N = 3
[d]ND = None Detected, <1.0 pg

Table 14. XAD-4 Extraction Efficiency-FM680

Spiked Water Sample FM680 Concentration (ug/L)	Theoretical Amount FM680 Injected (pg)	Spiked Water Sample GC Analysis (Relative Area)	Actual Amount FM680 Injected (pg)[a]	Recovery (%)[b]
0.0	0	ND[e]	ND	--
0.1	25	32.8(-)[d]	8.3	33.2
0.3	75	66.3(6.6)[c]	30.7	40.9
0.5	125	108.0(0.8)	58.7	47.0
0.7	175	128.3(14.8)	72.3	41.3
0.8	200	142.4(6.3)	81.8	40.9
0.9	225	185.5(23.7)	110.7	49.2
1.0	250	171.5(16.6)	101.3	40.5
1.2	300	192.3(22.5)	115.2	38.4
1.6	400	257.6(21.6)	159.0	39.8
2.0	500	316.5(39.2)	198.5	39.7
		Mean Recovery (%) = 41.0±3.2		

[a]Calculated from standard calibration curve [Area = 1.49(pg) + 20.9; r^2 = 0.993]
[b]Calculated as (Actual Amount/Theoretical Amount) x 100
[c]( ) = standard deviation, N = 3
[d](-) = no standard deviation, N = 1
[e]ND = None Detected, <1.0 pg

Table 15. XAD-4 Extraction Efficiency-TBP

Spiked Water Sample TBP Concentration (ug/L)	Theoretical Amount TBP Injected (pg)	Spiked Water Sample GC Analysis (Relative Area)	Actual Amount TBP Injected (pg)[a]	Recovery (%)[b]
0.0	0	ND[d]	ND	--
0.1	25	6.4(0.4)[c]	20.1	80.4
0.3	75	24.4(6.2)	61.0	81.3
0.5	125	30.9(5.3)	75.8	60.6
0.7	175	68.4(11.5)	161.2	92.1
0.8	200	68.2(2.2)	160.7	80.4
0.9	225	105.2(14.8)	244.9	108.8
1.0	250	102.2(8.3)	238.1	95.2
1.2	300	113.9(9.5)	264.7	88.2
1.6	400	150.4(28.9)	347.8	87.0
2.0	500	238.2(24.3)	547.6	109.5
		Mean Recovery (%) = 88.4±10.3		

[a]Calculated from standard calibration curve [Area = 0.44(pg) -2.4; r^2 = 0.999]
[b]Calculated as (Actual Amount/Theoretical Amount) x 100
[c]( ) = standard deviation, N = 3
[d]ND = None Detected, <1.0 pg

Table 16. XAD-2/4 Extraction Efficiency-FM680

Spiked Water Sample FM680 Concentration (ug/L)	Theoretical Amount FM680 Injected (pg)	Spiked Water Sample GC Analysis (Relative Area)	Actual Amount FM680 Injected (pg)[a]	Recovery (%)[b]
0.0	0	ND[d]	ND	--
0.1	25	23.5(4.9)[c]	5.1	20.4
0.3	75	72.4(24.6)	29.7	39.6
0.5	125	117.8(3.2)	52.5	42.0
0.7	175	165.7(2.9)	76.6	43.8
0.8	200	197.3(8.0)	92.5	46.3
0.9	225	210.6(25.4)	99.2	44.1
1.0	250	237.0(40.8)	112.5	45.0
1.2	300	356.8(46.6)	172.7	57.6
1.6	400	394.9(11.9)	191.9	48.0
2.0	500	495.8(79.9)	242.6	48.5
		Mean Recovery (%) = 43.5±6.8		

[a]Calculated from standard calibration curve [Area = 1.99(pg) + 13.3; r^2 = 0.997]
[b]Calculated as (Actual Amount/Theoretical Amount) x 100
[c]( ) = standard deviation, N = 3
[d]ND = None Detected, <1.0 pg

Table 17. XAD-2/4 Extraction Efficiency-TBP

Spiked Water Sample TBP Concentration (ug/L)	Theoretical Amount TBP Injected (pg)	Spiked Water Sample GC Analysis (Relative Area)	Actual Amount TBP Injected (pg)[a]	Recovery (%)[b]
0.0	0	ND[e]	ND	--
0.1	25	24.9(-)[d]	33.4	133.6
0.3	75	31.7(5.3)[c]	45.8	61.1
0.5	125	66.7(1.7)	109.5	87.6
0.7	175	79.1(10.4)	132.0	75.4
0.8	200	94.1(22.0)	159.3	79.7
0.9	225	106.4(4.1)	181.7	80.8
1.0	250	127.9(5.1)	220.8	88.3
1.2	300	142.0(35.2)	246.5	82.2
1.6	400	187.1(7.1)	328.5	82.1
2.0	500	200.2(24.7)	352.4	70.5

Mean Recovery (%) = 84.1±13.7

[a]Calculated from standard calibration curve [Area = 0.55(pg) + 6.5; r^2 = 0.999]
[b]Calculated as (Actual Amount/Theoretical Amount) x 100
[c]( ) = standard deviation, N = 3
[d](-) = no standard deviation, N = 1
[e]ND = None Detected, <1.0 pg

680 and 88% of 2,4,6-tribromophenol. For the XAD-2/XAD-4 resin mixture, the mean recoveries were 43.5% and 84% for Firemaster 680 and 2,4,6-tribromophenol, respectively.

DISCUSSION

This study demonstrated that the XAD-2 system was the most efficient XAD resin system tested for the extraction of Firemaster 680 and 2,4,6-tribromophenol from water. The XAD-2 resin extraction procedure can be used to concentrate low levels of Firemaster 680 and 2,4,6-tribromophenol from aqueous media. However, because of the lower extraction efficiency for Firemaster 680, a correction factor should be incorporated into the XAD-2 analytical scheme. This can be accomplished best by using a concentration series of spiked water samples to develop a standard calibration curve. For example, a standard calibration curve for the Firemaster 680 spiked water samples which were subjected to the XAD-2 resin extraction procedure in this study is presented in Figure 15. These data show a linear relationship for this concentration range. The standard calibration curve is best described by the regression equation:

$$\text{Area} = 0.66\ X + 13.17 \qquad \text{(Equation 10)}$$

where X = amount (pg) of Firemaster 680 injected, assuming 100% recovery, and r = 0.993.

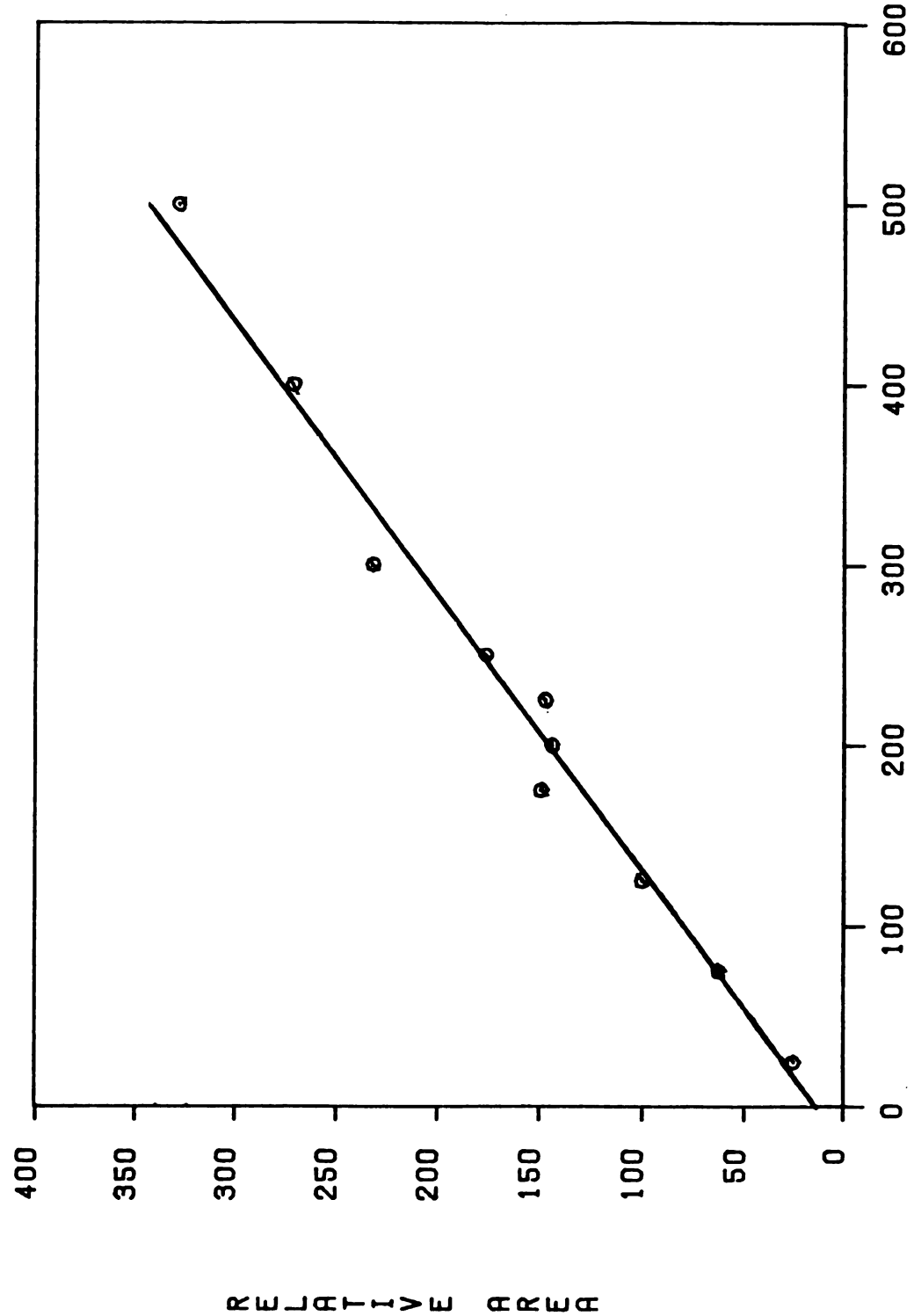


Figure 15. XAD-2 Analytical Scheme Standard Calibration Curve for Firemaster 680 (FM680)

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