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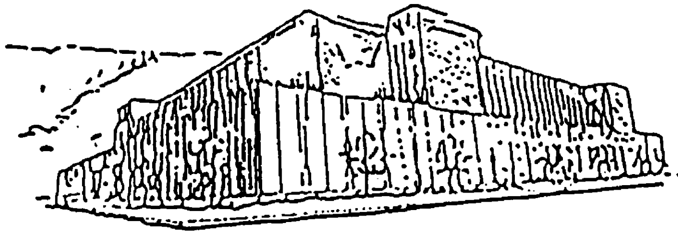

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ANALYSIS OF POLYCHLORINATED BIPHENYLS IN HONEY BEES

by

Ted J. Christian

B.A., University of Montana, Missoula, MT, 1992

Presented in partial fulfillment of the requirements

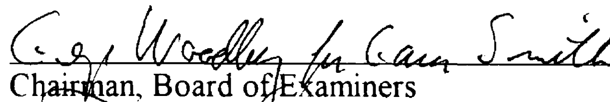
for the degree of

Master of Science in Chemistry

University of Montana

August, 1996

Approved by



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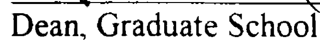

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Analysis of Polychlorinated Biphenyls in Honey Bees

Advisor: Garon C. Smith

ABSTRACT

An analytical method for the extraction and analysis of polychlorinated biphenyls (PCBs) from honey bees is described. Ten honey bee samples from three separate geographic locations (one each from Arizona, Idaho, and Montana) were studied. Whole bee samples from hives deployed either in pairs or in groups of six were extracted and analyzed. Samples were pooled from hives at each site. All extracts contained measurable amounts of PCB, with concentrations ranging from 0.5 to 3.2 μg/g as Aroclor 1260. Mean concentrations for sample sets varied between 1.1 and 1.9 μg/g. A duplicate analysis was performed to demonstrate the accuracy of the method. The difference between PCB concentration in this sample and its duplicate fell within the bounds of the standard error of the calibration curve. Additional duplicate analyses should be conducted in order to assure the accuracy of the method. No significant difference can be proven between bees collected from any of the sites, including the Arizona bees, which were considered "PCB free" prior to analysis.

To separate PCBs from a sample matrix, ground honey bee samples were subjected to Sohxlet extraction in hexane with Florisil cleanup, followed by extraction with concentrated sulphuric acid. It was necessary to employ all three techniques to adequately clean up honey bee samples, whose lipid content may approach 30% by weight. Sample extracts were analyzed by gas chromatography with ^{63}Ni electron capture detection (GC/ECD). Sample PCB concentrations were obtained by comparing the integration of 14 selected peaks in the samples to the same 14 peaks in Aroclor 1260 standard solutions. Peaks were selected to avoid the inclusion of early eluting, lighter congeners that were present in the standards but not in the bee extracts. The absence of several early eluting peaks is attributed to biodegradation, preferential uptake or retention, or to some unknown factor.

ACKNOWLEDGMENTS

I would like to thank my principal advisor and co-author, Dr. Garon Smith, for his expertise and patience, and for encouraging me to keep my family close to me in the face of all pressures. I also thank Dr. Charles Thompson for his sound advice and moral support, especially toward the end when all thesis projects seem overwhelming. His goodwill toward me will stand for a long while. Dr. Jerry Bromenshenk supplied to me many of the practical necessities at the outset, including instrumentation, glassware, samples, and financing, and I thank him heartily. His expertise in honey bees, the influence of which is apparent in the body of this work, encompasses a great many aspects.

I extend thanks to my friends in the Shafizadeh Center for Wood & Carbohydrate Chemistry for providing me with friendship and for improving my grasp of science, and to the University of Montana Chemistry Department for their professional handling of my academic career. I thank especially Gayle Zachariasen and Bonnie Gatewood for removing much of the drudgery that a graduate student must face. I thank also Joe Sinski, Chris Wrobel, and Bruce King, for their friendship and advice. Each of them enhanced my life in some subtle way.

Funding for this project was provided by the Environmental Science and Research Foundation, under Contract Number FOUND-94-IDAHO-01, and by the University of Montana - Missoula.

Finally, I thank my wife Peggy, and my sons J.C. and Dean Henry, without whom no endeavor would be worthwhile.

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CHAPTER 1: INTRODUCTION AND STATEMENT OF THE PROBLEM

The production of polychlorinated biphenyls (PCBs) in the United States was banned in 1979 by the U.S. Food and Drug Administration due to evidence of mutagenicity, carcinogenicity, and long biological half life. Environmental persistence and resistance to metabolic breakdown has caused PCBs to become almost ubiquitous, at low levels, throughout the United States.[1] Studies confirm the presence of PCBs in a variety of organisms and locations.[2-5] Although the focus of most published studies is upon aquatic systems, a number of them implicate terrestrial systems. Many areas in the U.S. have been subjected to industrial sources of contamination and many of these contain PCBs.[1]

At the Idaho National Engineering Laboratories (INEL) concern arose over the possibility of PCBs occurring in some of the area's water impoundments, after water fowl using these impoundments were found to contain elevated levels of PCBs. However, the source of the contamination could not be identified. In response to this concern, INEL requested PCB analysis be integrated into an existing terrestrial biomonitoring study to confirm or dismiss the presence of elevated PCB levels at the INEL site. This study was being conducted by Bromenshenk et al.,[6] who had deployed colonies of honey bees (*Apis mellifora*) at strategic locations throughout the INEL site and who had already obtained sample sets of honey bee foragers from which this work draws.

Several analytical methods exist for the identification and quantification of PCBs, the most common of which couple electron capture or mass spectral detection with capillary gas chromatography. The more complicated aspect lies in separating PCBs from the sample

matrix. Because other authors had determined PCB levels in honey bees, this appeared to be a straightforward task, but it proved to be difficult. PCBs are highly lipophilic and, after having been ingested or adsorbed by living organisms, tend to partition to adipose and other fat-rich tissues.[7] Any sample extraction technique designed to dissolve PCBs away from a biological matrix unfortunately carries unwanted lipid soluble compounds with it. Electron capture detection can differentiate between these compounds and halogenated species like PCBs, but only to a limited extent, since any non-halogenated compound that is present in high enough amounts can "flood" the detector, causing symptoms like elevated baseline and extraneous peaks. The focus of the following work, therefore, is to develop an acceptable method of extracting PCBs from honey bees, which boast an extraordinarily high lipid content. The success or failure of the method will then be demonstrated by applying it to four sets of honey bee samples that represent different geographic and environmental foraging histories.

1.1 History and Chemistry of PCBs

In 1881, Schmidt and Schultz [8] obtained a patent for the manufacture of polychlorinated biphenyls. Commercial manufacture in the United States began in 1930 and by 1971 PCBs were produced in nine countries.[9] The sole manufacturer in the U.S. was the Monsanto company, which marketed PCBs under the trade name Aroclor. Various other producers are listed in Table 1.1.

Table 1.1 Major Producers of PCBs [10]

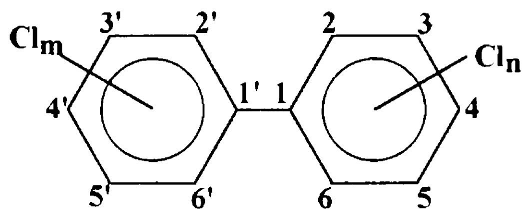
Producer	Country	Tradename
Monsanto	United States and Great Britain	Aroclor®
Bayer	Germany	Clophen®
Prodelec	France	Phenoclor® and Pyralene®
Kanegafuchi	Japan	Kanechlor®
Mitsubishi-Monsanto	Japan	Santotherm®
Caffaro	Italy	Fenclor®
Sovol	U.S.S.R.	
Chemko	Czechoslovakia	

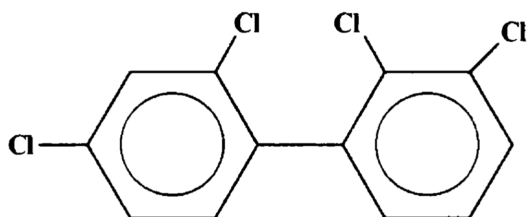
1.1.1 Physical and Chemical Properties

PCBs possess chemical and physical properties long sought after in various manufacturing settings. Their high dielectric constants, high heat capacities, high boiling points, low flammability, and resistance to chemical breakdown make them ideal for use in electrical capacitors, electrical transformers, vacuum pumps, heat transfer fluids, hydraulic fluids, lubricants, inks, and plasticizers.[10, 11] The major pathways for PCBs to enter the environment include vaporization during burning, leaks, disposal of industrial fluids, and disposal in dumps and landfills.[10]

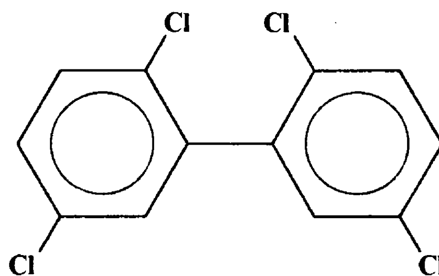
There are 209 possible PCB congeners, though many of these are rare or nonexistent in the environment.[9, 10] Figure 1.1 gives substitution positions and nomenclature for

Figure 1.1 PCB nomenclature

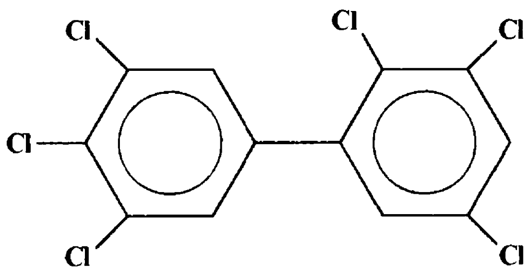




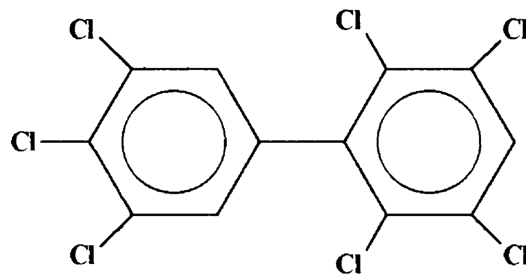
2,2',3,4'-Tetrachlorobiphenyl



2,2',5,5'-Tetrachlorobiphenyl



2,3,3',4',5,5'-Hexachlorobiphenyl



2,3,3',4',5,5',6-Heptachlorobiphenyl

representative PCB congeners; chlorine may be substituted in any of the 2-6 or 2'-6' positions. The Monsanto Company designated its Aroclor products according to chlorine content. The first two digits indicate the type of product - 12 for PCBs, 54 for polychlorinated terphenyls (PCTs), 25 and 44 for mixtures of PCBs and PCTs - while the last two digits indicate the chlorine content by weight percent. The World Health Organization [12] compiled a table of Aroclors by number of chlorine atoms and % weight (Table 1.2). Some physical properties

Table 1.2 Approximate Composition of Aroclors [12]

No. of Cl Atoms in molecule	% of Chlorine Weight	Aroclor				
		1221	1242	1248	1254	1260
0	0	12.7				
1	18.8	47.1	3			
2	31.8	32.3	13	2		
3	41.8		28	18		
4	48.6		30	40	11	
5	54.4		22	36	49	12
6	59.0		4	4	34	38
7	62.8				6	41
8	66.0					8
9	68.8					1

of three representative Aroclors appear in Table 1.3. Specific gravity and distillation temperature range increase with increasing chlorine content while aqueous solubility and vaporization rate decrease.

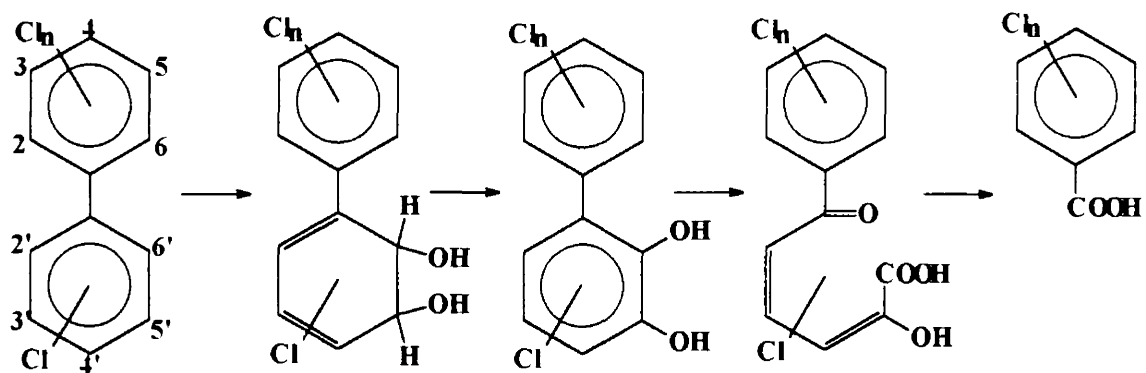
1.1.2 Uptake and Metabolism of PCBs

With low water solubilities and high lipophilicity, PCBs can be expected to partition into the fatty tissues of animals and plants. This process begins with adsorption of PCB congeners to cell membrane surfaces, where stereochemistry influences the strength with which each is absorbed.[9, 13] Once within the cells, PCBs are resistant to metabolic breakdown. In general, the greater the degree of chlorine substitution the less biodegradable the compound.[14, 15] Those congeners with chlorine distributed unequally between the rings are more readily metabolized, with attack directed at the least substituted ring (see Figure 1.2).[16] Also, ortho substituted congeners tend to be more bio-resistant.[16]

Table 1.3 Some Physical Properties of PCBs [10]

Property	Aroclor		
	1221	1248	1268
Appearance	Clear mobile oil	Clear mobile oil	White to off-white powder
Specific gravity	1.182-1.192	1.405-1.415	1.804-1.811
Distillation range (°C)	275-320	340-375	435-450
Solubility in nonpolar solvent	Very soluble	Very soluble	Very soluble
Solubility in water (mg L^{-1})	1.19-5.9	0.034-0.175	<0.007
Vaporization rate (at 100°C with 12.28 cm^2 surface area, in g cm^{-2} $hr^{-1}x10^5$)	174	15.2	<0.9

Figure 1.2 Metabolism of polychlorinated biphenyls by *Alcaligenes* sp. Y42 and *Acinetobacter* sp. P6.[16]



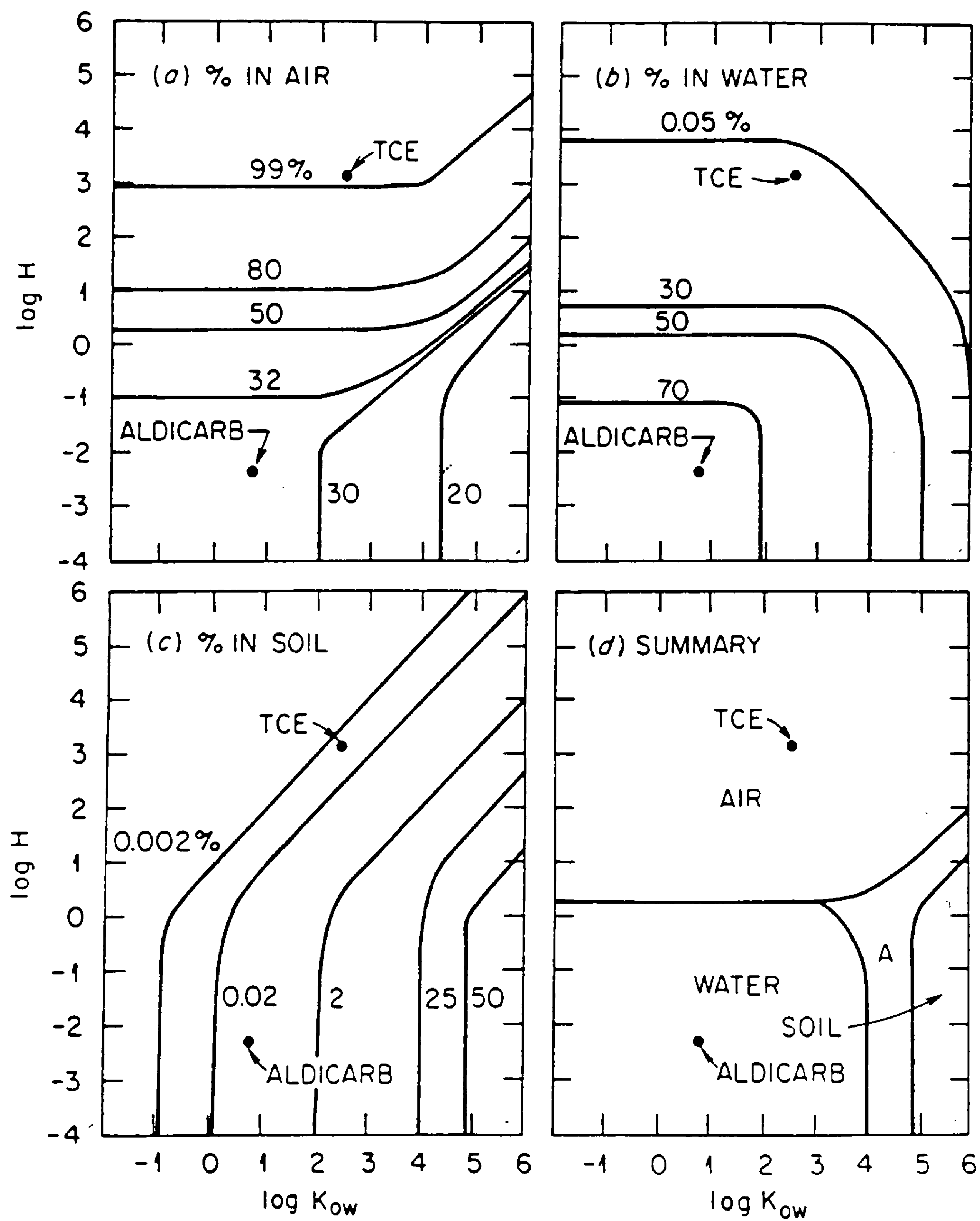
Bacterial degradation may occur as a result of one of two conditions. In some cases, bacteria have developed specific enzymes in response to chlorinated compounds.[17] In others, PCBs are fortuitously attacked by non-specific enzymes that accept chlorinated compounds as substrates.[9] Researchers have isolated bacterial strains that are capable of biodegrading the more highly substituted congeners [17, 18] and that have been used successfully in treatment of activated sludge contaminated with PCBs.[19]

1.1.3 Environmental Fate of PCBs

To address the topic of biological uptake and metabolism of PCBs, it is necessary to consider the probable fate of PCBs released into the air, the water, or the soil, since this, in turn, determines the exposure that any group of organisms will experience. Two commonly used physico-chemical properties for this purpose are the octanol-water partition coefficient (K_{OW}) and the Henry's law constant (H). The K_{OW} value has been used to estimate bioconcentration factors, soil and sediment organic carbon-water partition coefficients, and aqueous solubilities.[20-24] Henry's law constants have been used as integral parts of fate and transport models.[25, 26]

Cross-media partition profiles are useful tools in determining the fate of chemicals released into the environment. Using K_{OW} and H, these profiles predict the percentage of compound that will end up in air, water, and soil. Predictions are based on whether the contaminant is originally released into the air, the water, or the soil. Figure 1.3 shows the likely fates of trichloroethylene (TCE) and aldicarb when released into the air. TCE has a log

Figure 1.3 Cross-media partition profiles of a chemical released into air.[7]



K_{OW} of 2.3 and a log H of 3.1 while aldicarb has a log K_{OW} of 0.7 and log H of -3.4. As can be seen from Figure 1.3, 99% of TCE will partition to the air, less than 0.05% to the water, and a little more than 0.002% to the soil. Aldicarb will partition mainly between air (70%) and water (30%), with only 0.02% ending up in the soil. The regions of Figure 1.3.d represent 50% or more of the compound in each medium. Therefore, >50% of TCE would partition to the air and >50% of aldicarb would partition to the water.

Figures 1.4 and 1.5 depict the fate of chemicals released into the water and soil, respectively. All three of these figures (1.3 to 1.5) assume no further human intervention after release. For example, a sewage treatment plant might release a dissolved chemical into the atmosphere through aeration; a land farm engaged in microbial degradation might expose a chemical to greater dissolution or vaporization from the soil through cultivation and irrigation. Nevertheless, cross-media partition diagrams can be useful in predicting the initial fate of a compound.

Hawker and Connell [27] determined experimental values of K_{OW} for 13 congeners (log K_{OW} 5.5-7.5) and calculated K_{OW} for the remaining 196 (log K_{OW} 4.1-8.2). Dunnivant et al. [28] determined experimental H values for 17 PCB congeners, the logarithms of which range from -4.5 to -3.0. Using these values, the application of cross-media diagrams to PCBs places the majority of the contaminant in the soil component, regardless of mode of release. PCBs adsorb quickly to sediment particles [9] and may therefore be expected to be available to bees via contact with soil, with airborne particulates, or after ingestion or attachment of waterborne sediments.

Figure 1.4 Cross-media partition profiles of a chemical released into water.[7]

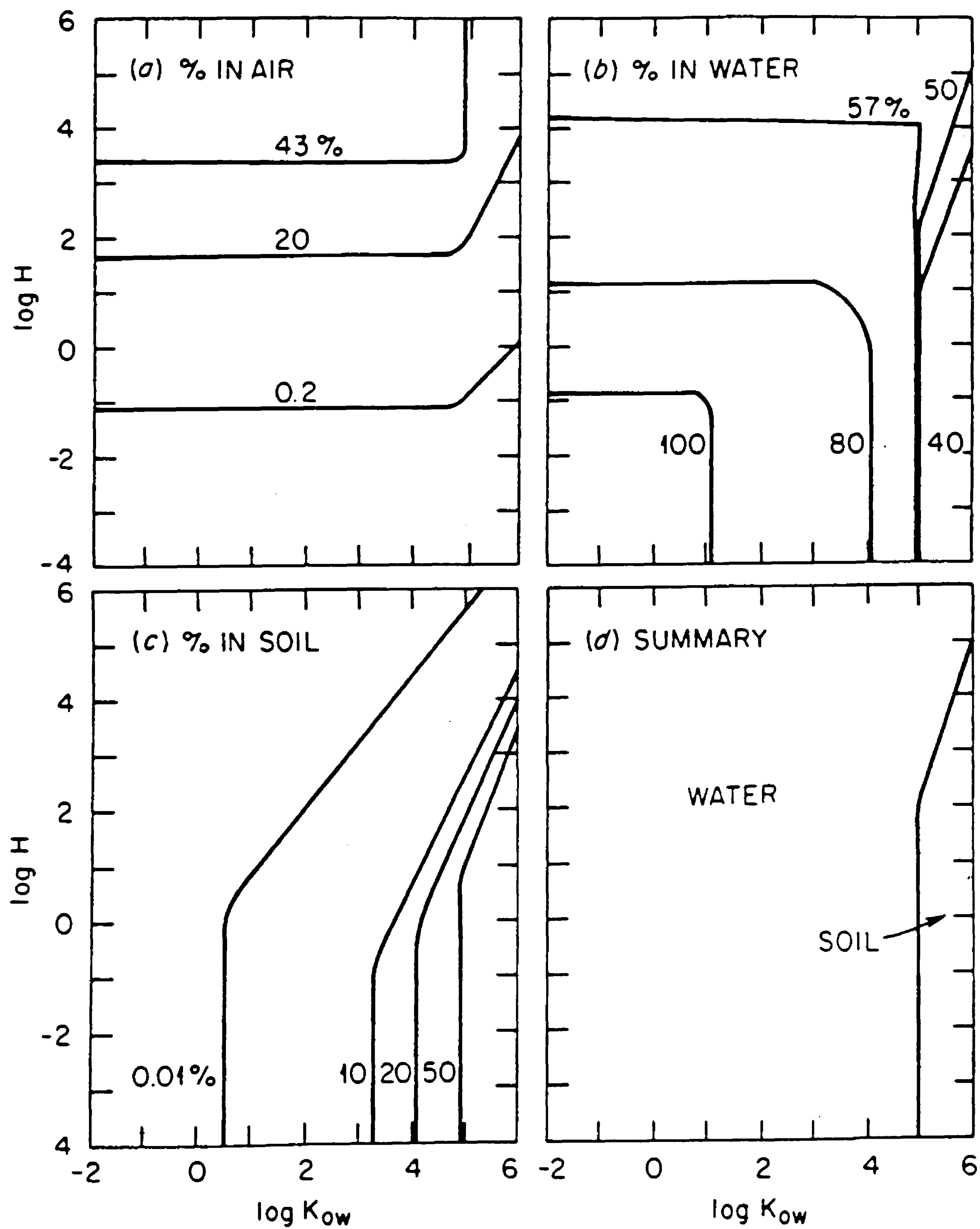
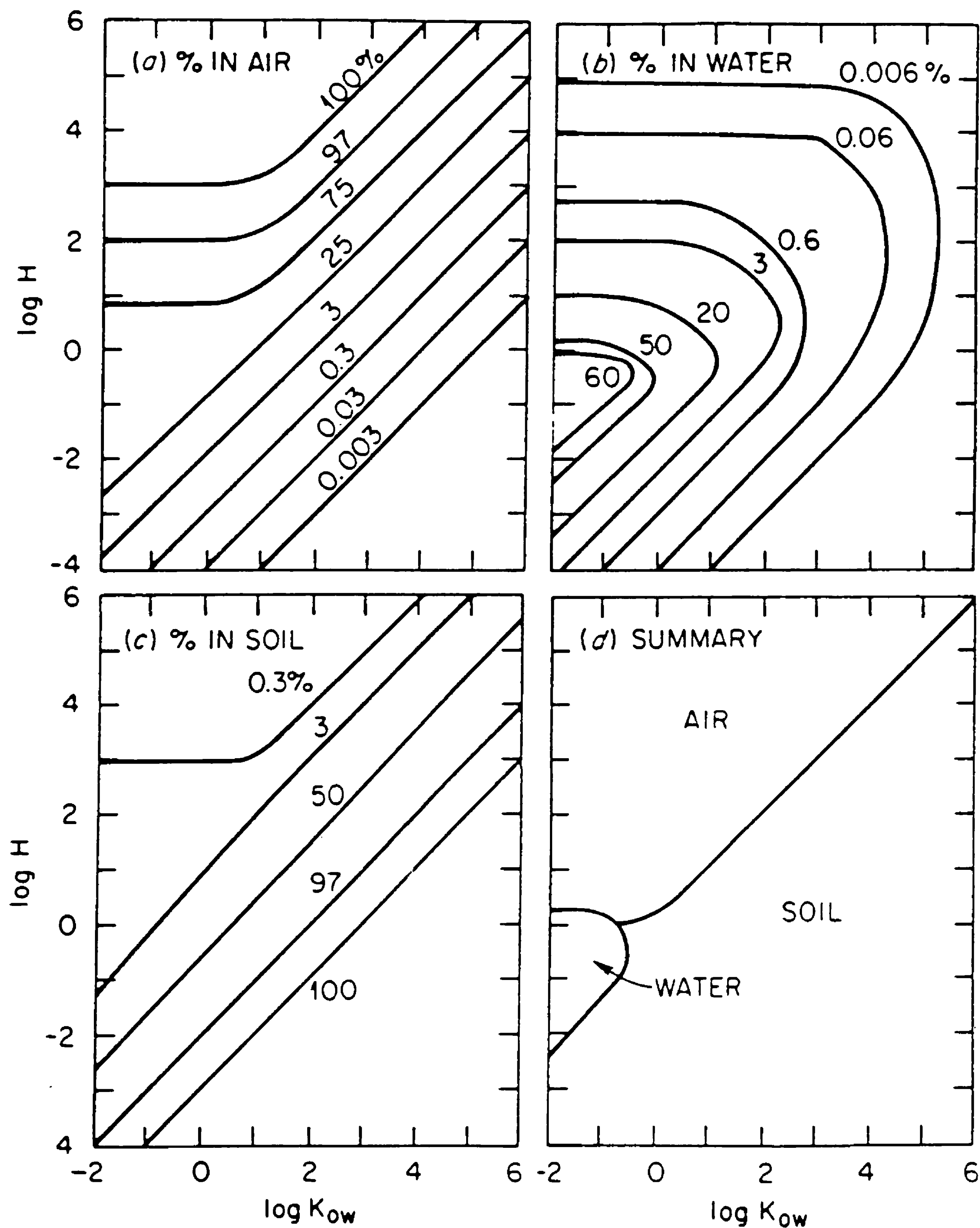


Figure 1.5 Cross-media partition profiles of a chemical released into soil.[7]



1.2 Honey Bees as Environmental Samplers

Honey bees can be used as an effective environmental sampling tool.[5,6,29-32] Their behavior, social structure and life span are predictable and it is relatively easy to establish and maintain colonies. Furthermore, they consistently return to the hive after foraging over large geographical areas, providing a fixed sampling point with a broad range. While foraging for nectar, pollen, and water, honey bees extensively contact all three media (air, soil, water) and may accumulate pollutants as gaseous or particulate matter by ingestion or by attachment to body parts.[33] The same species of honey bee (*Apis mellifora*) occurs globally.

Honey bees can indicate current contaminant levels at a particular site or they may be used as long term monitors. They have been used to detect various types of pollutants. Honey bees were among several species of insects found to contain elevated fluoride levels in the vicinity of an aluminum plant in Montana.[29] Bromenshenk et al.[30] defined the distribution and extent of emissions of arsenic, cadmium, and fluoride from a copper smelter in Tacoma, WA. The study took advantage of existing beekeepers to provide a convenient, in-place sampling network. In a more expansive study from 1986 to 1990, Bromenshenk et al.[33] monitored a suite of chemical contaminants including radionuclides, fluorine, and 17 trace elements. The results implicate honey bees as effective large scale exposure monitors of a wide variety of contaminants. Many other applications exist that incorporate honey bees, pollen, propolis, wax, and brood comb to monitor a host of environmental contaminants including metals and other toxic elements, radionuclides, PCBs, and pesticides.[5,31-33]

1.3 Thesis Organization

Chapter 2 covers a review of published studies relevant to this work. These studies were chosen to document both the variety of organisms that have been subjected to PCB analysis, and the relative complexity of applying conventional PCB extraction methods to biological matrices of high lipid content. Chapter 3 details the experimental methods employed to carry out PCB extraction from honey bees. It includes step by step extraction procedures, as well as site descriptions, analytical techniques, and quality assurance measures. Results and our interpretations of them appear in Chapter 4. Finally, conclusions and suggestions for further studies are presented in Chapter 5.

CHAPTER 2: LITERATURE REVIEW

The extraction of PCBs from a variety of matrices is commonplace, as the following review will illustrate. Complications arise, however, when the sample matrix contains a relatively high proportion of fat, as is the case with honey bees, whose fat content may approach 30% by weight.[34] In such instances, special measures need to be invoked to separate highly lipophilic PCBs from the equally lipophilic component of the sample matrix. The following references were chosen to illustrate both the variety of sample types that have been analyzed for PCBs in the past and the relative ease with which most extractions are carried out. Little mention is made of extraordinary difficulties in recovering PCBs from fatty samples; those studies dealing specifically with fatty samples demonstrate method efficiency using a relatively pure fat source rather than extracting all or part of an actual organism, which must necessarily contain a very complex matrix of biological materials. In the only two prominent works involving PCBs and honey bees,[5, 32] the sample preparation steps are briefly outlined with no apparent indication of trouble or evidence of efficiency.

2.1 Representative Investigations of PCB Contamination

Duke et al. [2] analyzed fish, crabs, oysters, and shrimp from estuarine areas near Pensacola, FL. PCB levels (as Aroclor 1254) in whole fish samples, as well as liver, muscle, and gill extracts, clustered around 12 ppm (μg/g) but in two instances flounder liver contained 76 and 184 ppm. Shrimp and crab whole samples ranged from 1 to 7 ppm; three sediment

samples showed 1.7, <0.03, and 486 ppm. The researchers also undertook a laboratory study of acute exposure of juvenile pinfish, juvenile pink shrimp, and oysters to aqueous concentrations of Aroclor 1254 at 1, 10, and 100 ppb (μg/L). The pinfish suffered no mortality, but whole body analysis of the high exposure (100 ppb) fish revealed 17 ppm Aroclor 1254, a concentration factor of 170. Pink shrimp exposed to the same level of contaminant all died after 48 hours, with body concentrations 39 times that of the tank in which they were held. Shell growth of oysters was severely inhibited while exposed to PCBs; after removing the contaminant source shell growth returned to normal. Oysters, as well, bioconcentrated the PCBs. Juvenile shrimp suffered high mortality (72%) after being chronically exposed (20 days) to 5 ppb aqueous concentration of Aroclor 1254. Tissue of the surviving shrimp showed 33 ppm PCB, a 6600-fold concentration.

Animal tissue for these analyses was extracted in petroleum ether, partitioned into acetonitrile to remove lipid solubles, and re-partitioned to petroleum ether after near complete evaporation. They were then cleaned on a Florisil column and Aroclor 1254 was eluted with 6% ethyl ether in petroleum ether. Quantitation was by averaging the height of five major peaks obtained via packed column GC and electron capture detection (ECD).

Pastel et al. [35] analyzed 52 female individuals of a migratory saltwater fish (American Shad) from two sites on the lower Hudson River. These fish were found to contain an average of 2.0 ± 1.0 and 6.1 ± 2.6 ppm total PCBs. Fish were scaled, beheaded, definned, deboned, gutted, and homogenized in a food processor. Aliquots of frozen homogenate were lyophilized, Sohxlet extracted with hexane, and cleaned up on deactivated Florisil. GC analysis was performed using both ECD and mass spectrometry (MS) on packed columns.

Response factors (RF) were calculated for 11 different homologs (i.e., the same number of chlorine atoms) that appear in a mixture of 4 µg each of Aroclors 1016, 1221, 1254, and 1260. The remaining 17 response factors were extrapolated from a plot of the log of the average RF for a group of homologs versus the chlorine number. These RF values were then used to calculate the actual concentrations of each of the 28 homologs in fish tissue extracts.

The focus of a study by McKone et al. [36] was to demonstrate the advantages of using SEP-PAK Florisil cartridges (Waters Associates, Milford, MA) in place of standard drying columns in the clean up of tissue samples extracted in petroleum ether. The study showed comparable results for both methods with a considerable reduction in waste solvent and time invested using the SEP-PAK cartridges. Analysis was carried out on seven species of freshwater fish, whose fat content ranged from 1-4% and whose PCB content ranged from ca. 2 to 178 µg/g. Interestingly, two cartridges were needed to clean samples with fat concentrations in excess of 4%. It is doubtful that these cartridges would be effective as purification tools if higher organism fat content were analyzed; their capacity is too low.

Fish and aquatic insects are known to bioaccumulate PCBs and other organochlorine contaminants.[9, 20, 24] For this reason, many of these organisms have been used or proposed as environmental monitor species in lieu of sampling the medium in which they grow. In a unique study by Tarradellas and Diercxens,[4] PCB concentrations in the tissue of earthworms showed a much smaller relative divergence from the mean (8.5%) than actual soil samples (60.0%), implicating earthworms as a valid soil contaminant monitoring species. Mean PCB concentrations were 0.65 ± 0.05 for tissues and 0.08 ± 0.05 for soils. Sample preparation began with a 24 hour digestion of whole worms in a mixture of perchloric and acetic acid,

followed by extraction with hexane in a separatory funnel, concentration, shaking with concentrated sulfuric acid, and centrifuging for 10 minutes at 4,000 rpm. To quantify PCB, peak areas for 45 peaks were compared with a mixture of equal quantities of Aroclors 1242, 1254, and 1260, using capillary GC with ECD.

Several papers exist that address explicitly the separation of PCBs from fat, two of which are reviewed here. Swift and Settle [37] employed an elaborate series of treatments and elutions from both Florisil and silica absorption columns to isolate PCBs (Aroclors 1248, 1254, 1262) and organochlorine pesticides (DDD, DDE, DDT, HCB, lindane, dieldrin) from either beef fat or cheese. Recoveries for spiked samples ranged from 81 to 107 percent. Fat samples were spiked with contaminants, mixed with deactivated Florisil, and chromatographed on activated Florisil. The eluate was then extracted with hexane in a separatory funnel, washed with saturated NaCl solution, dried with sodium sulphate, and concentrated. This concentrate was then cleaned on deactivated Florisil and eluted twice with 20% methylene chloride in hexane. The first fraction (A) contained PCBs, HCB, lindane, and DDT analogs; the second (B) contained dieldrin. Fraction A was then chromatographed on silica and eluted first with hexane to recover HCB and PCBs, and second with 20% methylene chloride to recover lindane and the DDT analogs. Confirmation was carried out using TLC on alumina/silica gel/silver nitrate plates developed with n-heptane and corrected with standards.

Seidl and Ballschmiter [38] also addressed PCB extraction from fat. Their paper compares the performance of two liquid/liquid extraction methods (partition in hexane/acetonitrile or hexane/dimethylformamide), one adsorption method (Florisil), and

saponification followed by acid extraction. The test material was olive oil spiked with either Clophen A-30 (Bayer, Germany) or a ^{14}C-labeled mixture of PCB isomers. The liquid/liquid extractions both rely on a hexane/acetonitrile or hexane/dimethylformamide solvent extraction to remove PCBs from the fat and a "back" extraction to re-partition PCBs into hexane. Recoveries for the acetonitrile and dimethylformamide procedures were 45-60% and >95%, respectively, as determined by capillary GC/ECD and comparison of four peaks. For the saponification procedure, fat samples were refluxed in 2.5% ethanolic potassium hydroxide for 2 hours, diluted with water, extracted with hexane, and the hexane extracts shaken with concentrated sulfuric acid. Recovery for this procedure was 80% as determined by liquid scintillation counter. For the fourth procedure, 0.5-1 g fat was dissolved in 5 ml hexane, added to deactivated Florisil, and eluted with 20% dichloromethane (DCM) in hexane. The eluate was repeatedly rotary evaporated and re-diluted with hexane to remove DCM. Recovery was 92-97% as determined by capillary GC/ECD and liquid scintillation counter. All but one of these methods showed 80% or greater recovery of PCBs.

The presence of even very small amounts of organochlorine compounds other than PCBs can seriously interfere with the analysis. For this reason, Ahnoff and Josefsson [39] investigated five separate procedures for cleaning river water samples. The five procedures included the use of sulphuric acid, Florisil, and Raney nickel in various combinations. Since PCBs were present only at low levels, initial water samples needed to be concentrated from 800 L to 500 ml, then each of five 100 ml aliquots concentrated to 1 ml, an overall concentration factor of 1.6×10^5. Qualitative comparison of chromatograms generated from packed column GC/ECD (using tritium as the electron source instead of the more common

^{63}Ni) showed the Florisil-Raney nickel cleanup to be the most effective, DDE being the sole interference peak. PCB was quantified at 0.34 ppt in the sampled water. Sulfuric acid as a stand alone treatment was not acceptable.

2.2 PCBs in Honey Bees

Anderson and Wojtas [5] quantified a host of pesticide and PCB contaminations of honey bees in Connecticut. Although the major focus of the study was the inclusion of multiple pesticides in honey bee analyses initiated over concern about colony health, this was the first report of the presence of PCBs in honey bees, brood comb, or honey. PCB levels generally remained below ca. 4.5 ppm, though one bee sample contained 56 ppm. The source of this contamination was not known. Dead bees and brood comb were macerated in anhydrous sodium sulfate and blended with benzene, then concentrated to dryness and redissolved in petroleum ether. Samples were then partitioned with acetonitrile, washed with water, and eluted from a Florisil column, first with a methylene chloride/hexane mixture, and then with a methylene chloride/hexane/acetonitrile mixture. Propolis, pollen, and honey were extracted with water/acetonitrile and partitioned to petroleum ether. Analysis was via GC/ECD; PCB was Aroclor 1260 in all but one sample, which contained Aroclor 1248.

Morse et al. [32] collected honey bees, honey, pollen, propolis, wax, and other insects from various locations within New York, Florida, and Vermont. They found detectable levels (0.2 to 1.5 ppm) of PCB as Aroclor 1254 in all but one of 20 samples, including the Vermont bees, which were not located close to any known source of PCB contamination. None of the

honey samples contained measurable amounts of PCB. Five of six pollen samples, eight of twelve propolis samples, and both wax samples - all collected in New York - contained measurable PCB levels. The study also investigated yellow jackets, wasps, horse flies, gypsy moth larvae, deerflies, and crickets. All but one yellow jacket sample and one horse fly sample contained PCBs. All samples except honey were freeze-dried, ground, and mixed. Sub-samples were Sohxlet extracted for 6 hours with hexane, partitioned with acetonitrile, diluted with water, and extracted with hexane. The hexane extract was chromatographed on Florisil and analyzed with packed column GC/ECD.

2.3 Summary

Obviously, a variety of extraction and cleanup techniques exist for PCB analysis of animal tissue. The solvents and reagents for some involve potentially dangerous chemicals, i.e., acetonitrile, benzene, methylene chloride, potassium hydroxide, and concentrated sulfuric acid. Most of the methods also generate considerable gaseous, liquid, and solid waste, an unavoidable consequence for this type of analysis. One paper reports the use of SEP-PAK Florisil cartridges to reduce time and waste, but this method is clearly not capable of handling the excess fat that occurs in honey bees.

The extraction and analysis procedures used in the current work were derived from the above citations with a focus on those techniques that could be employed without incurring major expenses. Funding that was available at the onset was necessarily applied toward purchasing reagents, standards, and a limited stock of consumable supplies, i.e., injector septa, inlet sleeves, glass wool, etc. As a consequence, there was very little leeway for experimenting with different techniques; I was compelled to make choices based on literature reviews and personal communications. The resultant analytical method does not revolutionize the body of existing procedures, but instead augments it.

CHAPTER 3: EXPERIMENTAL METHODS

This chapter details experimental methods and materials used in my PCB analysis. *Sample Handling and Preparation* reviews briefly the sites from which bees were collected and includes descriptions of hive deployment, collecting regimen, and transfer and storage of whole bees. This section also explains the grinding process which yields a homogenous powder of bee tissue. *Extraction of PCBs from Samples* explains the three step procedure to selectively remove PCBs from bee tissue while excluding other lipid soluble compounds. This procedure combines three well known techniques - liquid-liquid extraction, solid phase extraction, and acid catalyzed hydrolysis. *GC Analysis of Sample Extracts* lists information regarding quality control, instrument parameters, and equipment.

3.1 Sample Handling and Preparation

3.1.1 Site Descriptions

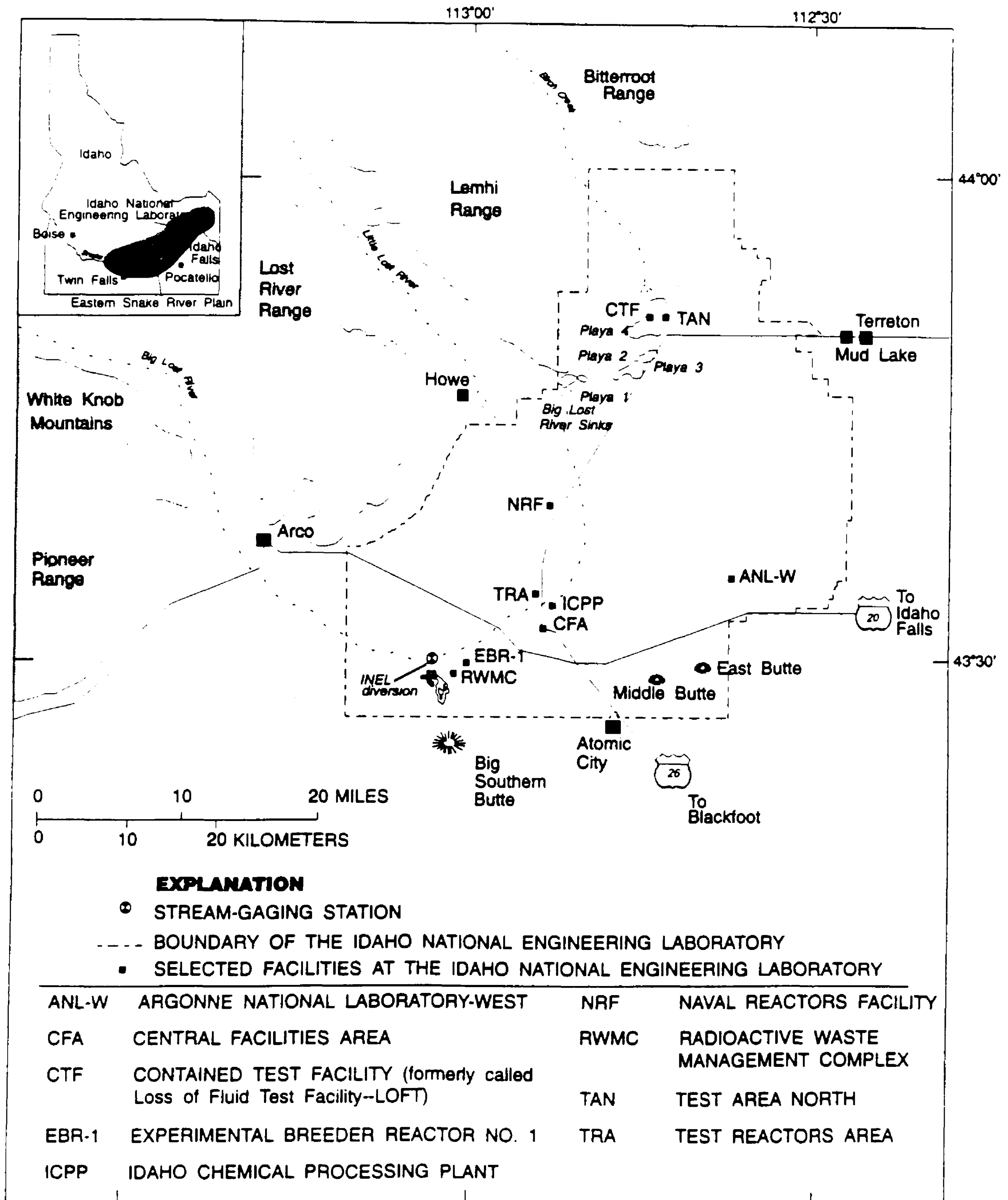
A collection of honey bees was compiled from three separate geographical regions - one each in Arizona, Idaho, and Montana. The Arizona bees were acquired from the Carl Hayden Bee Research Center, a cooperative facility of the United States Department of Agriculture and the University of Arizona in Tucson. These bees were raised in a "clean" environment, with no known exposure to organochlorine compounds.[40] They arrived frozen under dry ice and were kept frozen until use, away from any other chlorinated hydrocarbon

source. This Arizona sample served as a control group from which to establish expected levels of PCBs in bees that were not raised on sites of known PCB contamination.

The Idaho bees were collected in conjunction with a research project headed by Professor Bromenshenk [6] that was prompted by the discovery of PCBs in migratory water fowl that had foraged on the holdings of the INEL (Figure 3.1). To address the question of whether or not PCB contamination in the birds was being accrued at the INEL site or elsewhere, honey bees were deployed on selected sites and their tissue analyzed for PCBs. Two to four hives were placed near ponds at facilities within the holdings of INEL. These are TRA (Test Reactors Area) and LOFT (Loss of Fluid Test facility).

The INEL complex covers about 890 mi^2 in southeastern Idaho on the upper Snake River Plain. The area is a cool desert with predominantly big sagebrush-grass vegetation types and flat to rolling topography.[41] Several tributary streams flow through the area from mountains to the north. The TRA site, tens of acres in size, surrounds a plutonium fuel rod recovery facility and contains a number of water impoundments - a warm waste leach pond, a chemical waste pond, a cold waste pond, and several sewage leach ponds. Hives were deployed in pairs in three separate locations within TRA to allow bees to forage, as nearly as possible, the entire area. Samples for the current work were comprised of bees from these hives and were labeled TRA-1A, -2A, -2B, -3A, and -3B. The LOFT site within INEL is situated adjacent to a remote test area and is geographically removed from any other facility. LOFT was recently renamed CTF (Contained Test Facility) and contained at least one waste disposal pond. Two hives were placed in close proximity to LOFT and a sample from each (LOFT-A, -B) is included in this work.

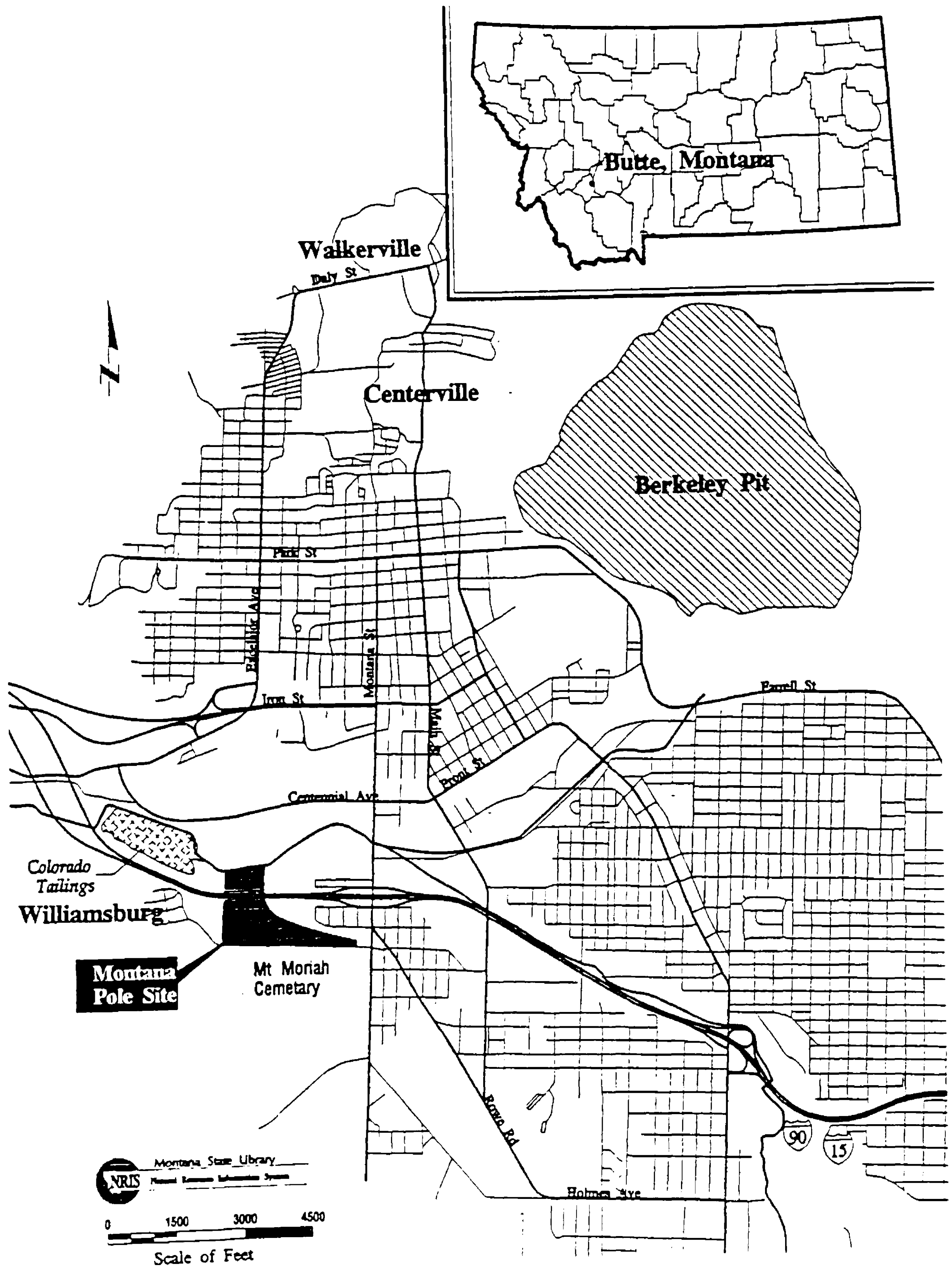
Figure 3.1 Location of the Idaho National Engineering Laboratory.[41]



Bees flown on the Idaho sites were collected using an aspirator. Bees from the fronts of hives were swept into sealable foil pouches, which were immediately placed on dry ice, transported to the University of Montana, and stored at -80°C until use. These samples comprised the target population that was suspected of PCB contamination.

The Montana bees were collected from the vicinity of the now defunct Montana Pole and Treatment Plant (Figure 3.2). The plant used pentachlorophenol (PCP), diesel oil and, on occasion creosote, as a preservative treatment for wood products. It operated from 1946 to 1984, at which time the owner(s) forfeited all holdings to Miners Bank of Montana as a result of financial loss and impending remediation liabilities.[42] Preliminary testing in 1983 by the Montana Department of Health and Environmental Sciences (MDHES) revealed oil and sludge seepage into a drainage ditch connected directly to Silver Bow Creek, one of the main source streams to the Clark Fork River. Since then, the site has been designated a U.S. EPA Superfund Site, with attendant remedial activities. The primary contaminants of concern are PCP, polynuclear aromatic hydrocarbons (PAHs), and polychlorinated dibenzo-*p*-dioxins and dibenzofurans.[43] PCBs have not been documented at this site.

Figure 3.2 Location of Montana Pole and Treatment Plant.[43]



3.1.2 Grinding of Whole Bees

Frozen whole bees were spread on sterilized metal sheets, covered with aluminum foil, and oven dried at 40°C for 5-7 days, with twice-daily stirring. They were then ground in a Wiley mill to pass a 20 mesh sieve. Samples were immediately placed in closed glass bottles and stored in a desiccator at room temperature.

3.2 Extraction of PCBs from Samples

Extraction techniques commonly rely on dissolution of PCBs in either hexane or petroleum ether.[5, 32, 36, 44, 45] The extract will then also contain other lipid soluble materials that complicate chromatographic interpretations. Since solubility is the predominant chemical property upon which extractions of this type are based, it was prudent to accept the performance of hexane and to rely on subsequent procedures to clean the sample. Florisil chromatography is simple, relatively inexpensive, and reasonably specific for PCBs.[36, 46] Many researchers have relied upon Florisil at some stage to isolate PCBs from other chlorinated and non-chlorinated hydrocarbons.[2, 4, 5, 32, 35-39, 45] Dieldrin and organophosphate pesticides can be effectively removed from the extract using concentrated sulfuric acid.[47]

The following extraction procedure incorporates these recognized techniques in three phases: 1) extraction of lipid soluble compounds in hexane, 2) separation of PCBs from other lipid solubles using Florisil, and 3) separation of PCBs from acid hydrolyzable compounds using concentrated sulfuric acid.

3.2.1 Liquid-Liquid Extraction

The initial liquid/liquid extraction took advantage of the high K_{OW} value and low volatility of polychlorinated biphenyls compared to many other organic species. A Sohxlet extractor fitted with a Snyder condensing column was employed to repeatedly wash the sample with distilled hexane (Baker Ultra Resi-Analyzed, 95% n-hexane). The exact procedure was as follows:

i) Using a suitable balance (Metler P163), weigh 5.0 g ground sample into an extraction thimble (Whatman single thickness cellulose, 25 mm x 100 mm) and cover with sufficient glass wool (Supelco, silane treated) to prevent bee tissue from escaping.

ii) Place the thimble into a Sohxlet extractor (30 mm ID), add 150 ml (±5 ml) hexane to the reservoir, heat to boiling, and allow to cycle for three hours.

iii) Remove heat, allow to cool enough to handle, and transfer extract to a 250 ml Kadurna-Danish (KD) flask fitted with a Snyder condensing column and a 10 ml receiving vessel.

iv) Heat the extract to a gentle boil and maintain until approximately 10 ml remains. Remove from heat.

3.2.2 Solid Phase Extraction

The second phase of the extraction utilized Florisil (Fischer Scientific, 60-100 mesh), a highly active, polar sorbent (magnesium silicon oxide, Mg_2SiO_3) to preferentially retain PCBs over other lipid soluble materials. Polychlorinated biphenyls were subsequently eluted with a hexane/diethyl ether solvent. All extractions were carried out using the same batch of Florisil, eliminating the need to standardize absorptive capacity with lauric acid.[48] The procedures was as follows:

v) Place 8.0 g Florisil into a drying column with a 60 ml reservoir and a 19 mm x 10 cm bed volume, settle by tapping lightly, and add 1-2 cm anhydrous sodium sulfate (Fischer Scientific, ACS grade).

vi) Wet the column with 50 ml (±2 ml) hexane, treating the eluate as waste solvent.

vii) Place a suitable collection vessel below the outlet and add bee extract to the column. The extract is added slowly to the surface as the hexane falls to within one or two centimeters of the level of solids in the column.

viii) When the surface of the liquid again approaches the top of the solid bed, add 60 ml hexane/ether (94:6 v/v, Fischer Scientific ACS grade diethyl ether) to elute PCBs.

ix) Continue to collect until the column stops draining.

x) Concentrate the eluate carefully (using KD apparatus) to just less than 5 ml and bring to 5.0 ml with hexane.

3.2.3 Acid Hydrolysis

The third phase of the extraction ensured that any acid hydrolyzable materials were also removed. It was as follows:

xi) Transfer the 5.0 ml of extract to a centrifuge tube.

xii) Add 3 ml concentrated sulfuric acid (Fischer Scientific ACS grade) and shake vigorously for one minute.

xiii) Centrifuge five minutes at 2000 rpm (Adams Compact II bench top centrifuge), pipette upper hexane phase into a vial with teflon lined cap and store at 0-5 °C for later analysis by GC/ECD.

3.3 GC Analysis of Sample Extracts

3.3.1 Equipment and Parameters

Analysis for PCBs was carried out on a Perkin-Elmer Series 300 capillary gas chromatograph equipped with electron capture detector (ECD) and an S & W Scientific split/splitless inlet flow controller in splitless mode. The analog signal was digitized and integrated using a Perkin-Elmer 3600 Data Station. Real time chromatograms were recorded on a Beckman strip chart recorder and peak areas assigned based on retention times, which were consistent to ±.05 minutes throughout all experiments. Table 3.1 presents run time parameters used for the analysis. Figure 3.3 depicts the optimal temperature program for

Table 3.1 Chromatographic conditions

Column	HP-5 MS low-bleed 5% phenyl methyl siloxane (30m x 0.25mm ID x 0.25μm film thickness, Hewlett Packard, 19091S-433)
Detector	^{63}Ni Electron Capture Detector (ECD), Temp. 250°C
Injector	Split/splitless in splitless mode, 0.75 min. purge delay, temp. 150°C
Gasses	Helium carrier @ 1.3 ml/min, Nitrogen moderator @ 36.0 ml/min

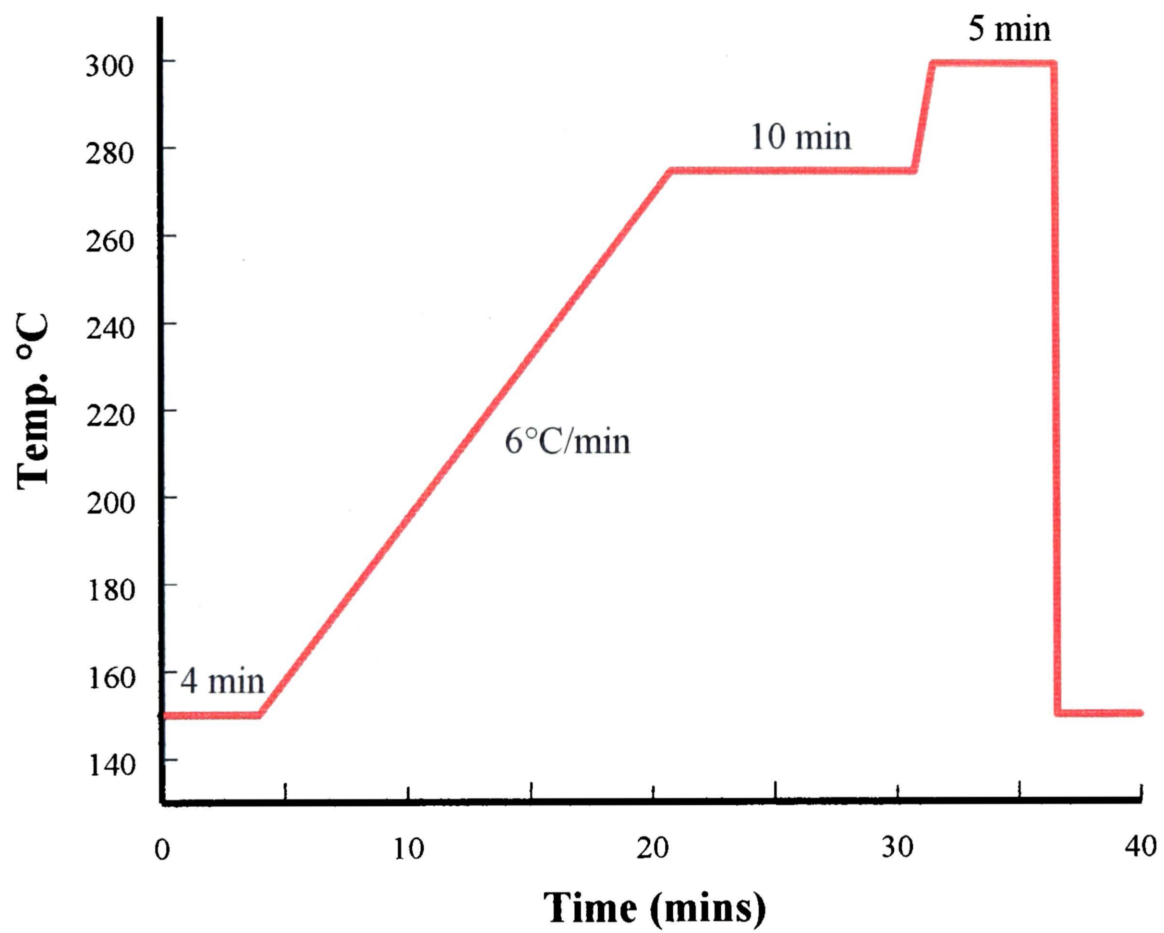
resolving Aroclors on the capillary column used.

The detection limit for this instrumental configuration approached 0.1 μg/g (ppm) total Aroclor 1260. A 1 ppm Aroclor 1260 standard yielded over 35 detectable peaks, placing the detection limit for individual congeners of this mixture at 30 parts per billion (ppb).

3.3.2 Preparation of Standards

Calibration standards were purchased from Supelco Inc. (Bellefonte, PA) as individual Aroclor mixtures. Each standard (Aroclors 1016, 1221, 1232, 1242, 1248, 1254, and 1260) was assayed by Supelco to 1000 μg/ml in methanol, with guaranteed purity of at least 96%. Successive dilutions of these standards were carried out using microliter syringes of appropriate capacity (Hamilton Series 1700 gas tight) to dispense aliquots of Aroclor standard into a known volume of hexane. The hexane had been previously dispensed into screw capped vials, with Teflon lined septa, using an Eppendorf pipetter. Dilutions were prepared for each Aroclor to span a range of 0.1-5.0 μg/ml.

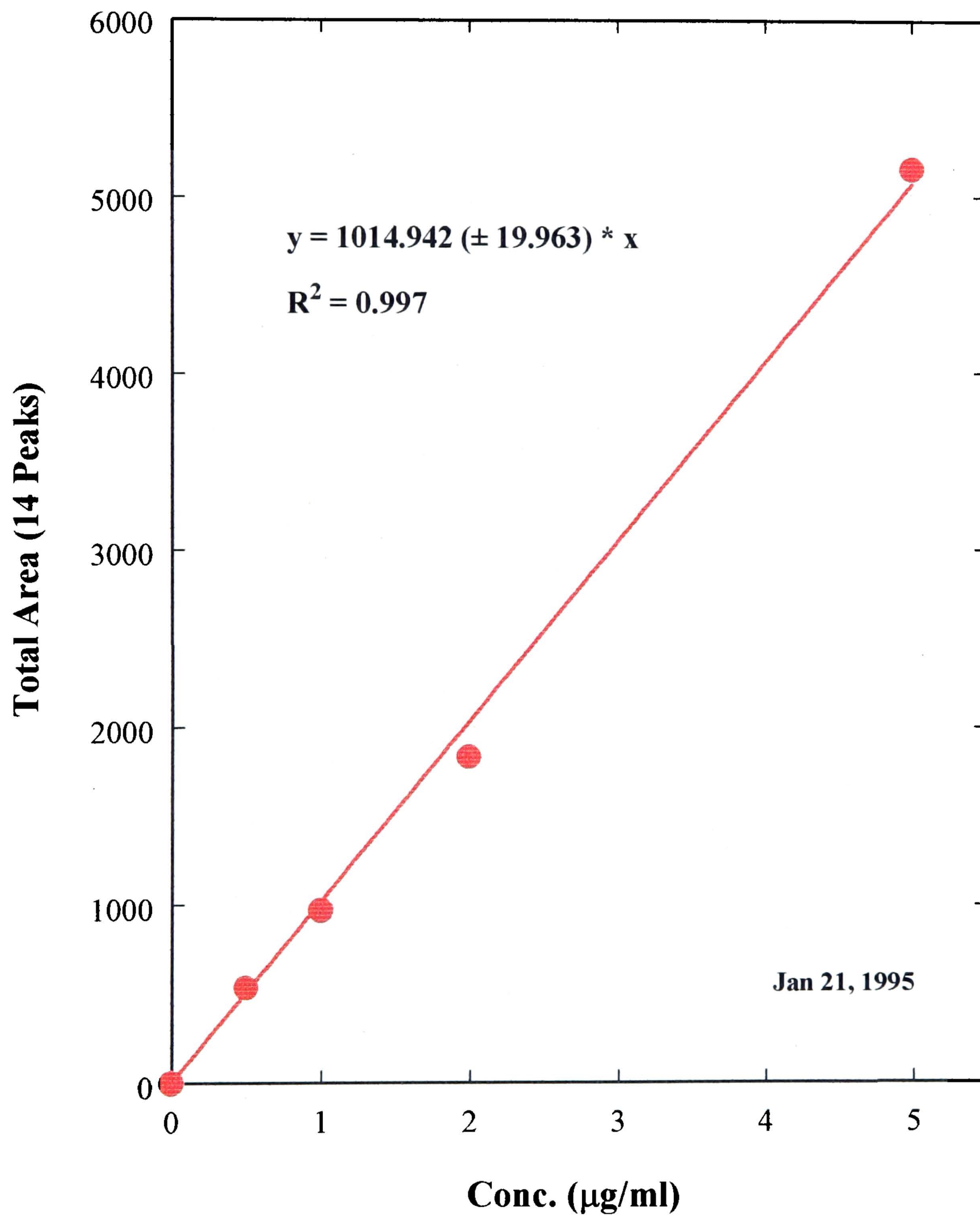
Figure 3.3 GC temperature program



3.3.3 Quantitation

The accuracy of any quantitation scheme for PCBs is dependent upon the nature of the contaminant source and upon the degree to which biological degradation has occurred prior to sampling. A single PCB mixture (i.e., Aroclor 1248 or 1254, etc.) yields a chromatogram with characteristic peak areas and retention times - a "fingerprint" pattern. A common quantitation method, therefore, is to compare the areas of selected peaks in the sample with the same peaks from a standard PCB mixture. This was the technique employed for the current work. A standard curve based on 1 μl injection volume was generated using a series of dilutions of Aroclor 1260. (Inspection of retention times and relative peak areas of preliminary sample chromatograms confirmed the presence of Aroclor 1260. Other halogenated compounds were present that may have been contributed by a different Aroclor, e.g. 1254, or by some other halogenated compounds, e.g. organochlorine pesticides.) Fourteen peaks were chosen that spanned the entire retention time range (approx. 15 min.) and that were cleanly resolved from neighboring peaks. The sum of the absolute areas of these 14 peaks in each standard dilution was plotted against known PCB concentrations in μg/ml (Figure 3.4). A least squares linear regression was used to fit a curve to these points. The resulting equation was then used to calculate sample PCB concentrations, as Aroclor 1260, based on peak area. For example, if a sample injection yielded a total integration for the specified 14 peaks of 1467, the PCB concentration for this injection, based on the calibration curve equation, would then be 1.45 μg/ml, as follows:

Figure 3.4 Calibration curve



$$(1467 / 1014.9) = 1.45\ \mu g/ml$$

Since the sample consists of 5.0 g bee tissue extracted and concentrated to a final volume of 5.0 ml (equivalent to 1.0 g/ml), then μg/ml extract is directly equivalent to μg/g bee tissue and the bees from which this sample was extracted contained 1.45 μg/g PCB as Aroclor 1260.

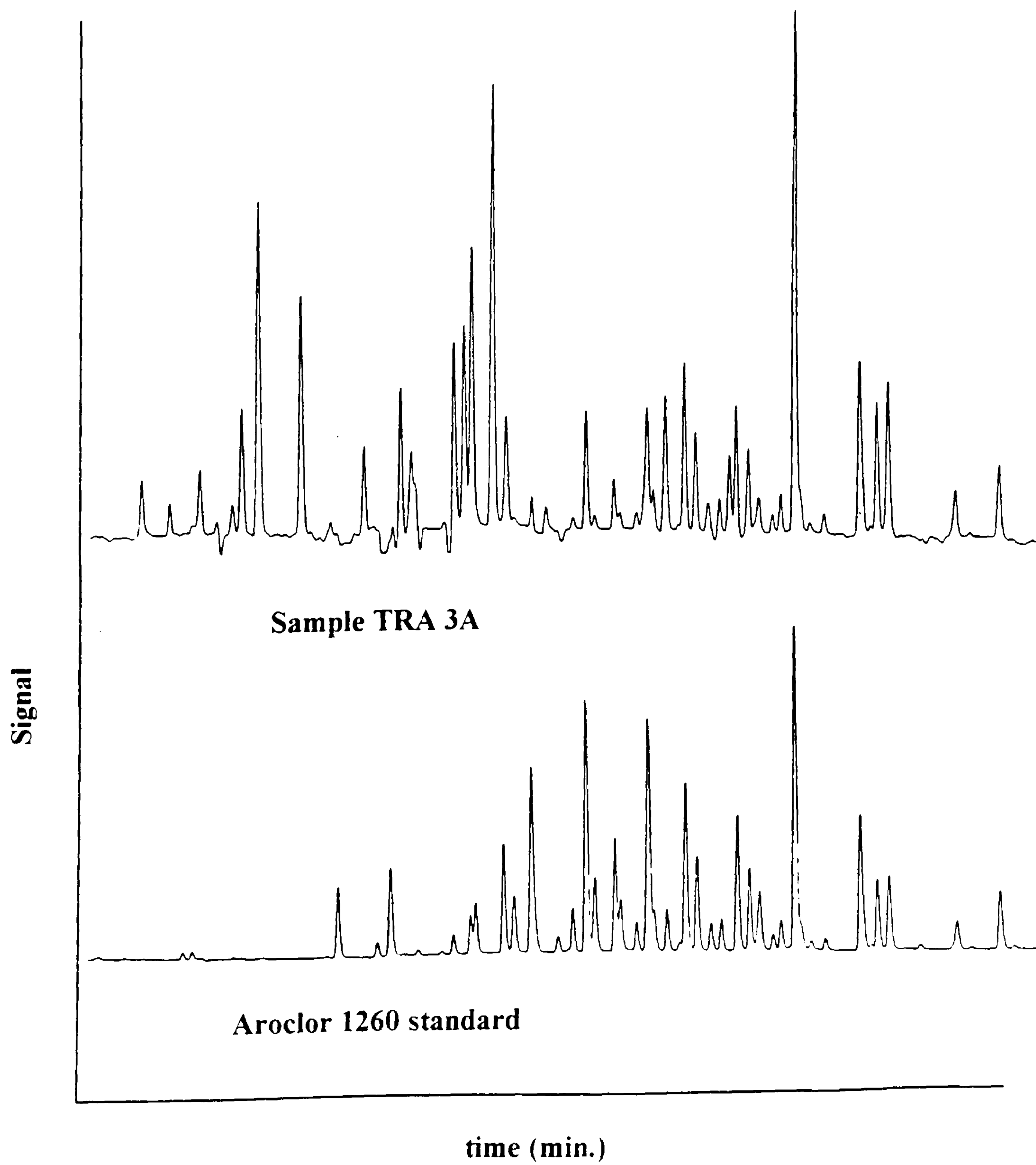
An external standard method of quantitation is suitable for complex chromatograms that elute over an extended period. It is difficult, when using standard additions, to choose internal standards that do not co-elute with any other compound; more than 40 peaks occur in the honey bee chromatograms. Also, more than one internal standard would be needed, since the use of response factors is most accurate when used with compounds that display similar retention times. The accuracy and precision of any external standard method rely, in large part, on the ability of the analyst to make injections of consistent volume. To this end, all injections were carried out with the same type of syringe using an established procedure for rinsing the syringe before and after each use and for drawing and injecting the sample.

The differences between relative peak areas (or heights) of different Aroclors is quite distinctive. It would be difficult to mistake one for the other, provided the sample in question was contaminated with only one Aroclor mixture. When more than one Aroclor occurs in the sample, it may be necessary to resort to analysis based on individual congeners or to report PCB levels based on the commercial mixture that most closely resembles the sample. For this analysis, PCB is reported as Aroclor 1260.

As stated, the lighter, less substituted biphenyls are more easily biodegraded. This condition manifests itself as diminished or missing peaks for the early eluting congeners. Some degree of degradation can be expected from any biological sample. A typical sample chromatogram is compared to a 1 ppm Aroclor 1260 standard in Figure 3.5. Two or three early peaks are present in the Aroclor 1260 standard but not in the sample. On the other hand, all of the remaining 30 or so Aroclor 1260 peaks were present in each bee sample, an indication that relatively little biological degradation of the heavier congeners had occurred. Additionally, there is a strong correlation between retention times and relative intensities of peaks in the standard to those in the samples.

In addition to the above quantitative procedures, it is customary to perform percent recovery experiments. In order to document the extent to which the analytes are lost during the extraction procedures, 1 μg/g Aroclor 1260 was added to three separate samples (TRA-2B, TRA-3B, and Tucson). This was accomplished by injecting 5 μl of concentrated Aroclor 1260 standard solution (1000 μg/ml in methanol) onto 5.0 g ground tissue just after placement in the extraction thimble. Admittedly, this technique does not account for any biological influence on the degree to which PCBs can be extracted from tissue, since the PCBs have not been assimilated into the organism, but simply poured on top of prepared tissue. One alternative is to feed PCB-laden food stuffs to a subset of living organisms. This was not possible in this instance. On the other hand, the method used should provide some insight into the degree to which PCBs are lost during the numerous extraction steps.

Figure 3.5 Chromatograms of Aroclor 1260 and sample TRA-2A



3.3.4 Quality Assurance

Sample handling before, during, and after collection, grinding, and storage was stringently carried out as stated earlier. These procedures ensured minimum opportunity for contamination from airborne polyhalogenated substances that may have caused interferences. Individual halogenated contaminants manifest themselves as extraneous peaks that may or may not co-elute with PCB congeners. Those which do not co-elute are easily recognized through comparison with chromatograms of PCB standards. Co-elution may cause peaks of extraordinary relative intensity provided the detector is sensitive to the contaminant and the contaminant is present in high enough quantities. Small contributions through co-elution are difficult to distinguish without the use of some confirmatory technique such as resolution on a different capillary column or analysis by some other instrumentation, i.e., mass spectrometry.

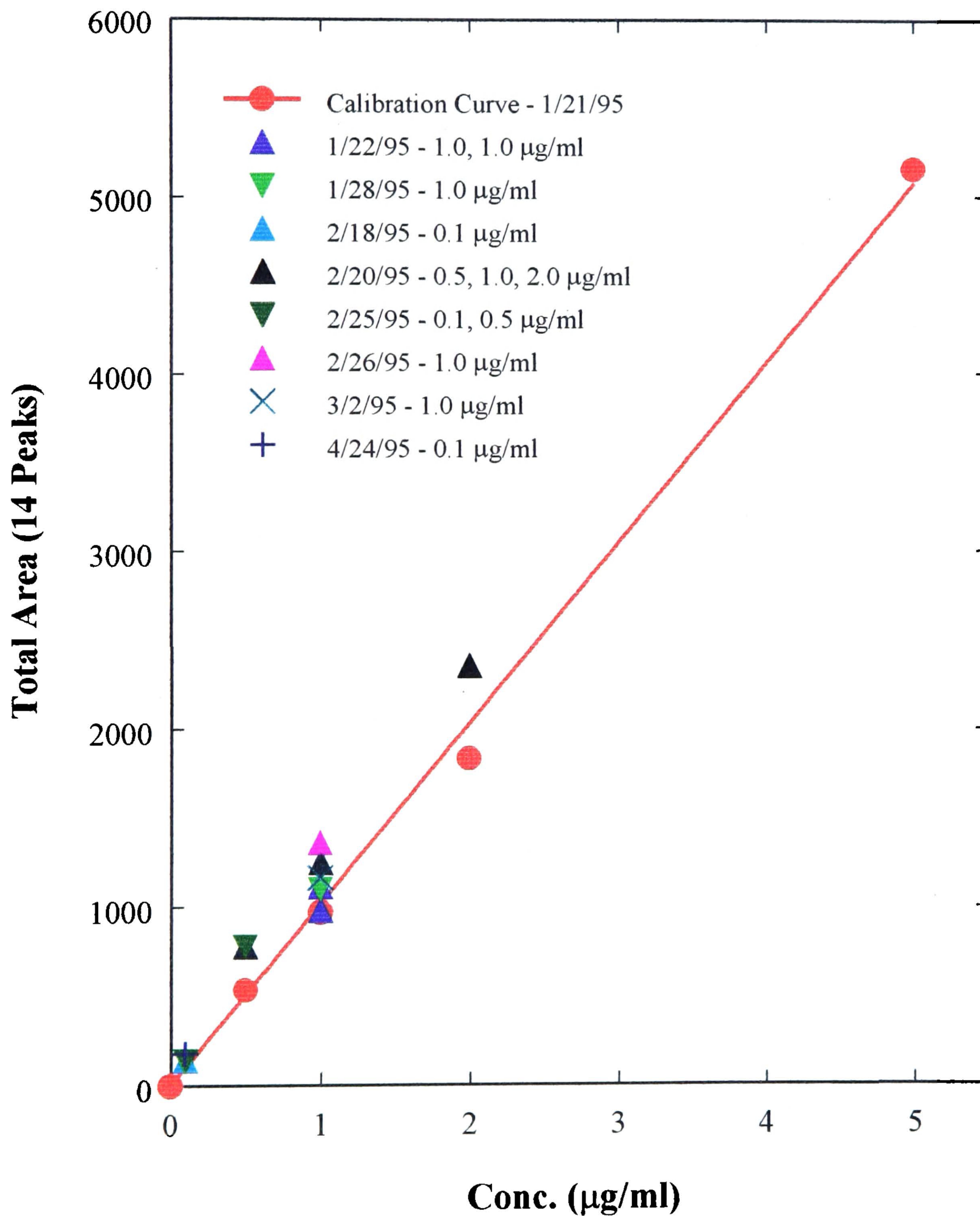
To rule out contamination introduced by laboratory reagents and supplies with which samples came into contact, solvent and method blanks were included in the analysis. Solvent blanks are simply daily injections of hexane, straight from the bottle. This is also a convenient method of tracking the integrity of the column, the carrier gasses, and the injector components. Method blanks consist of solvent which has undergone the entire extraction procedure in the absence of actual bee tissue, all other factors remaining the same. Method blanks for this analysis consistently showed a flat or mildly convex baseline with no detectable peaks.

CHAPTER 4: PCB ANALYSIS OF HONEY BEE SAMPLES

4.1 Calibration

The calibration curve for the bee samples was based upon four separate 1 μl injections of Aroclor 1260 standards plus one method blank. Exact concentrations were 0.0, 0.5, 1.0, 2.0, and 5.0 μg/ml. The R^2 for the linear fit to these points was 0.997. The curve-fit equation appears in Figure 3.4. The constant was omitted from the least squares determination of the best linear fit because solvent blanks consistently showed no detectable levels of halogenated compounds of any kind; a zero level calibration standard gives a zero peak area. Periodic calibration runs, with at least one calibration run on each day that analyses were carried out, were included to insure that the instrument response fell within the calibration curve range (Figure 4.1). Sample analysis took place on February 25 and 26, 1995, and on March 2, 1995. Instrument responses for these dates were 29%, 34%, and 15% higher than expected, respectively, based on the calibration curve of Figure 3.4.

Figure 4.1 Comparison of standard checks to calibration curve



4.2 Results of PCB Measurements

All samples, including the Tucson bees, which were thought to be "PCB free", showed measurable amounts of PCB as Aroclor 1260. A graphical representation of the results of analyses performed on actual samples appears as Figure 4.2. Levels ranged from 0.48 μg/g in TRA-2A to 3.17 μg/g in Butte A. Analytical methods of this type may easily rely on recoveries of 70% or less, provided they can be consistently duplicated.[57] Recoveries were 69% for TRA-2B, 122% for TRA-3B, and 91% for Tucson (Figure 4.3). These recoveries were calculated by dividing the experimental PCB level, in μg/g by the expected, or theoretical level, in μg/g. For example, the TRA-2B sample extract contained 1.59 μg/g. The TRA-2B spiked extract contained 1.79 μg/g. With a 1.0 μg/g spike, this extract should have contained 2.59 μg/g (1.59 μg/g + 1.0 μg/g). Therefore the actual spike recovery was (1.79 μg/g / 2.59 μg/g) x 100 = 69%. Similar calculations for TRA-3B and Tucson yielded recoveries of 122% and 91%, respectively.

One possible explanation for such wide variation in spike recovery is that the method employed to transfer the spike to the bee tissue was faulty. The use of a less concentrated Aroclor solution - perhaps 10 μg/ml instead of 1000 μg/ml - would have allowed a more efficient transfer. Assuming the volume of solution lost to the outside of the syringe or to other causes remains relatively constant, the less concentrated this solution, the more quantitative the transfer. Also, it may have been prudent to transfer the spike directly to the extraction solvent rather than to the ground bee tissue. This would have insured, at least, that

Figure 4.2 PCB levels, as Aroclor 1260, in honey bee extracts

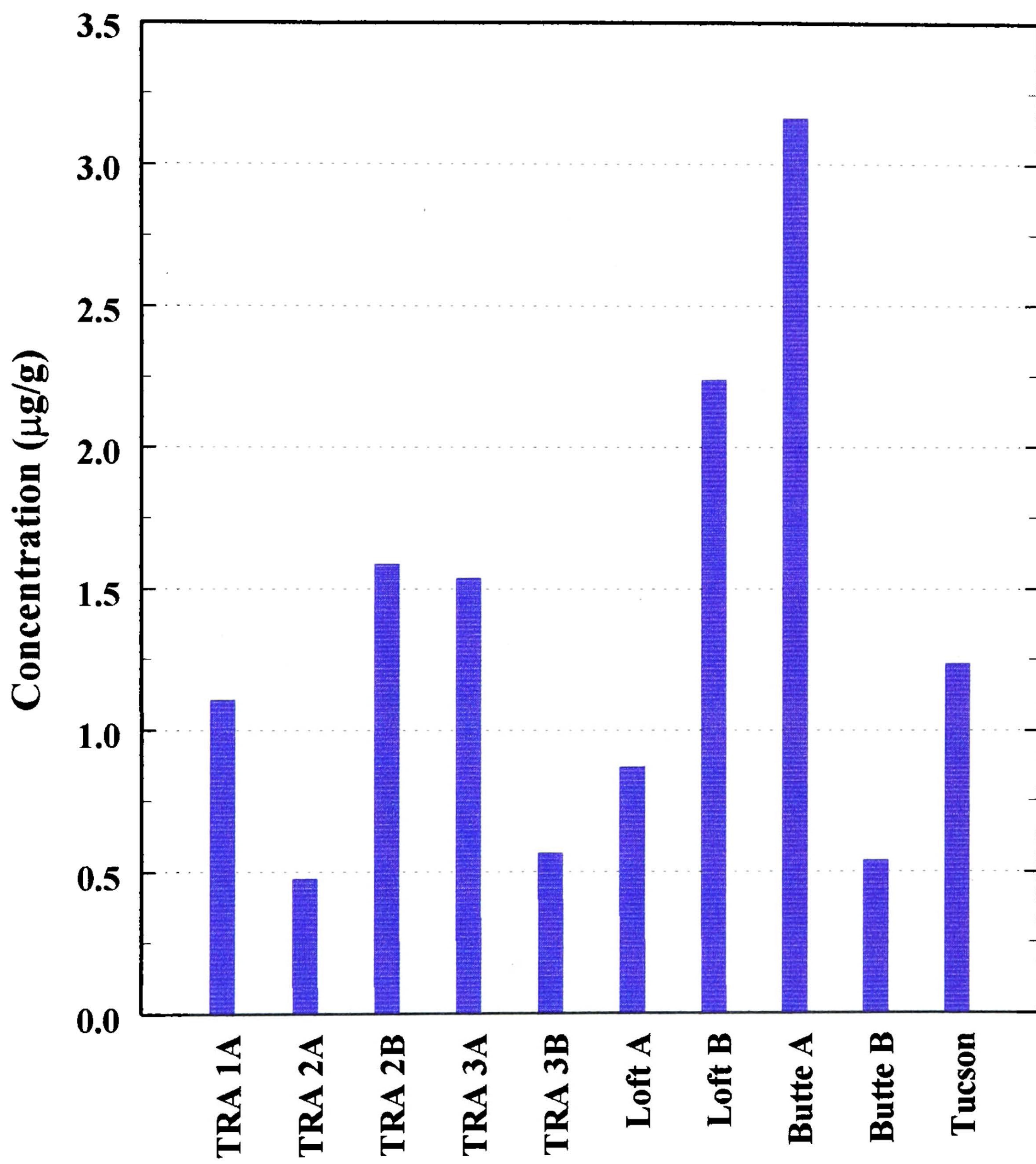
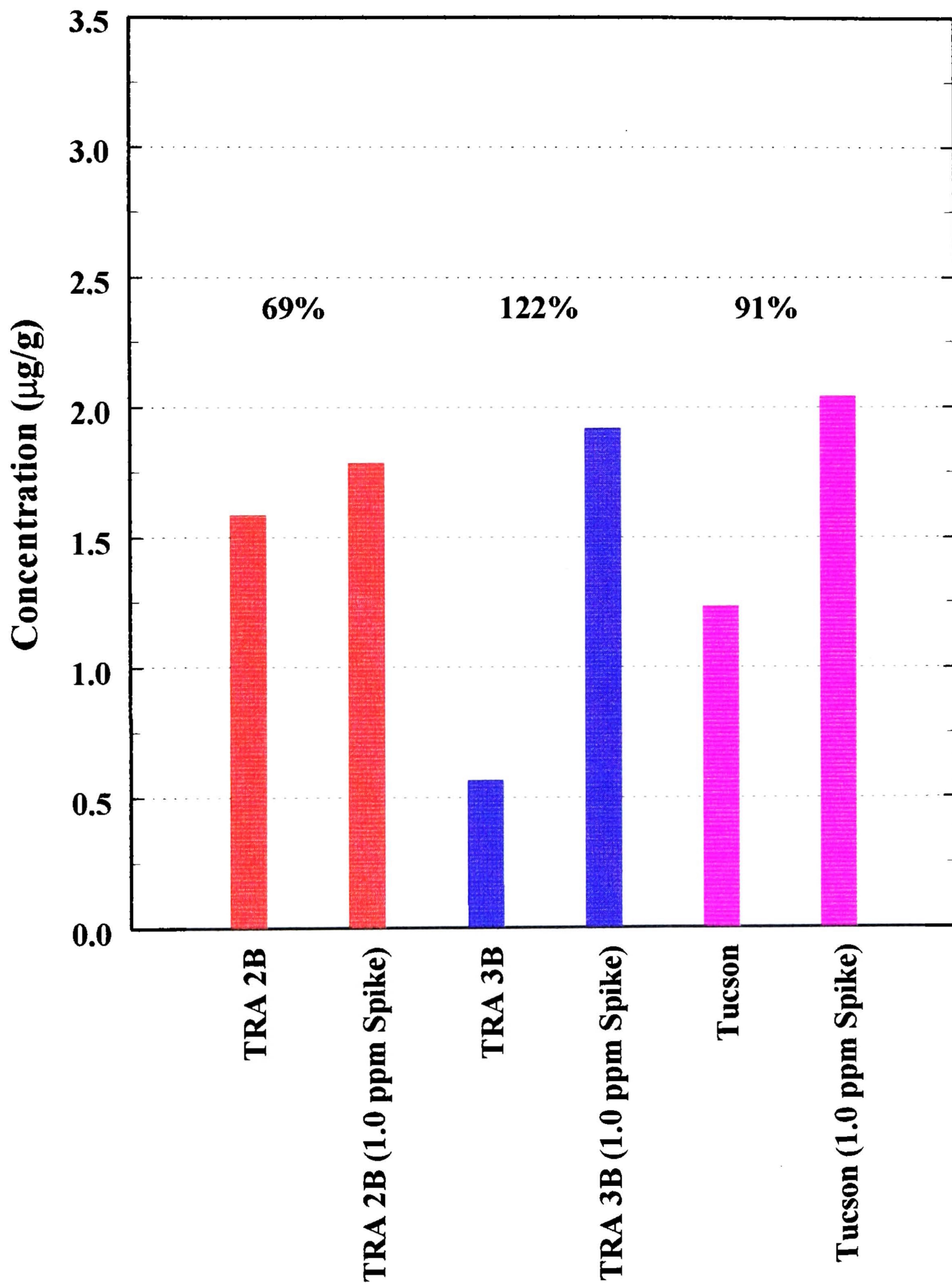


Figure 4.3 Percent recovery of Aroclor 1260 from three honey bee samples



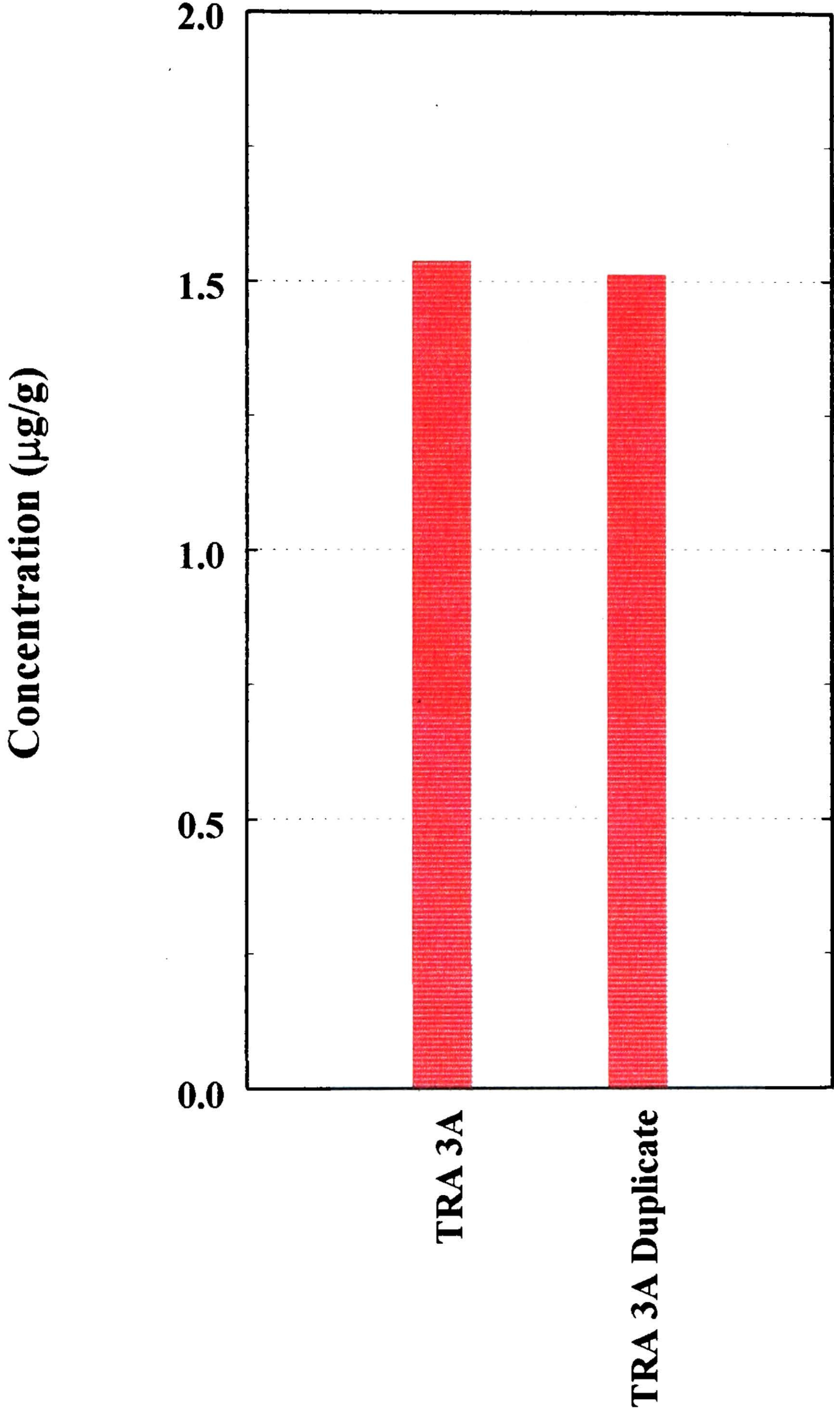
all of the spike started out within the extraction apparatus. Spike recoveries were not skewed in any one direction, implying some type of non-systematic error.

Duplicate extractions of the same sample can reveal inconsistencies in the extraction procedure itself. A duplicate trial of sample TRA-3A confirmed that the results for this method fell within the expected margin of error from the regression (Figure 4.4), as follows. The standard error for the x coefficient was ±19.963. Sample TRA-3A yielded a peak integration of 1561. The concentration range, therefore, for this value is 1.57-1.51 μg/g, i.e.

$$1561 \;/\; (1014.942 + 19.963) = 1.57 \;\mu g/g$$
$$1561 \;/\; (1014.942 - 19.963) = 1.51 \;\mu g/g$$

The duplicate shows a PCB level of 1.51 μg/g, which lies at the lower end of the acceptable margin of error.

Figure 4.4 Duplicate sample analysis



CHAPTER 5: CONCLUSIONS

This chapter begins with a discussion of the results and some of their implications. The main theme is to address briefly the following topics:

- Instrument calibration
- Method consistency
- Spike recovery
- Average PCB levels among sites
- Hive orientation

The chapter finishes with suggestions for further studies.

5.1 Discussion of Results

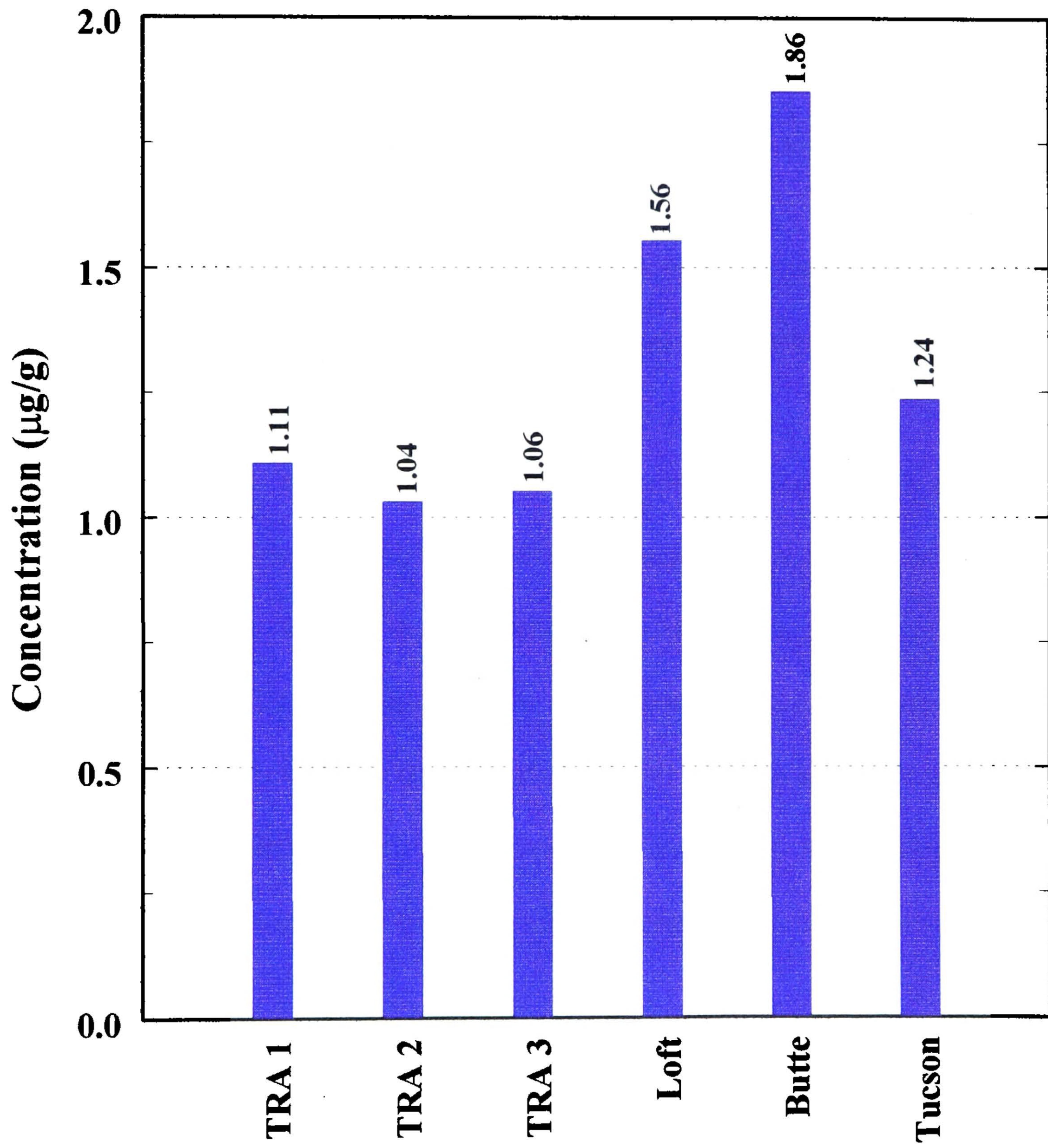
Certain EPA methods for the analysis of organochlorine pesticides and PCBs by GC (i.e. methods 8080, 508, 608, and 6633) require recalibration if daily checks fall outside a certain range from predicted response.[49] Typically, this range is ±15 to 25%, depending on the method. Periodic calibration checks for the current work consistently showed higher PCB concentrations than expected from the calibration curve, some of which fall outside the acceptable range (Figure 4.1). A regression curve fitted to a different set of calibration solutions, for example those from February 20, 1995, might alleviate some or all of this discrepancy. One could simply recalculate sample concentrations based on this new calibration curve. A more appropriate approach, however, would be to re-analyze the samples under a tighter time frame to insure compliance.

The fact that the instrument response was linear within the necessary concentration range for these samples, and was relatively consistent with the calibration curve (albeit high) supports the premise that differences in PCB levels among bee samples was due to either real differences in samples or in differences in extraction efficiencies between samples, and not to inconsistencies in the analytical method. Since only one duplicate analysis appears in this data set, though, it is premature to draw final conclusions concerning analytical precision.

The disparity between spike recoveries raises serious concerns about the accuracy of the method. Nevertheless, if one accepts, temporarily at least, the premise that the method determines the PCB level to within a factor of two, then a basic conclusion can be drawn. No significant difference appears to have existed between bees from different geographical sites. Figure 5.1 presents the average PCB levels for each site, all of which fell between 1 and 2 μg/g. It is particularly surprising to find the PCB level in the Tucson bees within this range, since they were raised without any known exposure to PCBs. In this context, the results suggest that neither the Butte bees nor the INEL bees contained PCB levels appreciably higher than that expected in "uncontaminated" bees.

Each of the sample pairs in TRA-2, TRA-3, and Loft consistently showed lower PCB level for one hive in the pair while the concentration of the bees from the other hive was approximately three times greater (3.31 times for TRA-2, 2.70 times for TRA-3, 2.57 times for Loft, see figure 4.2). The increase for the Butte samples was nearly six times (5.87). This may indicate a correlation between hive foraging patterns and PCB accumulation. The analytical method as it stands, however, is not unequivocally able to differentiate between one

Figure 5.1 Average PCB levels for each site



and three ppm, as already implied. Therefore, the question of whether observed differences were due to the varying extraction efficiencies or to bee behavior cannot be reasonably addressed by this study. Although most of their samples showed measurable PCB levels, neither Anderson and Wojtas [5] nor Morse et al. determined the source of PCB contamination in their bees.

5.2 Suggestions for Further Studies

As mentioned in the previous section, it is advisable to re-analyze the sample set under more optimal analytical conditions, specifically performing a complete set of analyses. This should eliminate calibration errors.

The lack of evidence for the precision of the method could be easily avoided by performing a more complete set of duplicate analyses. A more intuitive conclusion could then be drawn concerning the basis for any differences in PCB levels among samples. Provided duplicates survived an error analysis similar to the one presented here, it may then be possible to design experiments to resolve the question of whether or not PCB extraction efficiency is different for different samples from the same species of honey bee. If duplicates failed the error analysis, it may be necessary to review the extraction procedure for inconsistencies before continuing.

Spike recoveries for these experiments were unacceptable, ranging from 69% to 122%. Some different technique for determining the ability of the method to recover a known amount of analyte must be developed. It is my suggestion that injection of a known amount of a dilute solution of Aroclor directly into the extraction solvent would be more appropriate

than the procedure used. Once it is established that the method can show consistency in spike recovery, it may prove useful to design an experiment with bees that are given PCBs as part of their diet. This would lead to such inquiries as to what degree bees biodegrade and bioaccumulate PCBs.

The extraction procedure itself should come under scrutiny only if one or more of the above suggestions fails to remedy a specific problem. For instance, if a significant number of duplicate or triplicate analyses from several samples fall outside of acceptable tolerance limits, a step by step review of the extraction would be appropriate. Additionally, if spike recoveries cannot be brought under control, the same review process should be instituted.

Assuming the method is capable of determining PCB concentrations to within a factor of two, there is no real concentration difference between any of the samples. Also, both Anderson and Wojtas [5] and Morse et al. [32] report PCBs in almost all samples at levels similar to those reported here. This leads us to suspect that neither the INEL bees nor the Butte bees contained PCBs above what can reasonably be assumed to be background levels.

APPENDIX A: Peak integration data for calibration curve

ABSOLUTE AREAS

Date	peak - / time - / Conc.	A 19.27	B 19.67	C 20.45	D 21.89	E 22.05	F 22.64	G 22.82	H 22.97	I 23.51	J 24.44	K 24.69	L 24.86	M 25.85	N 26.50	Total Area
21-Jan-95	5.0	242	476	735	448	233	329	192	186	1257	406	178	203	95	179	**5159**
21-Jan-95	2.0	102	193	251	173	94	130	80	74	340	151	65	78	36	68	**1835**
21-Jan-95	1.0	56	105	131	95	49	71	42	40	174	80	36	40	18	34	**971**
21-Jan-95	0.5	30	63	76	45	26	41	24	22	97	45	19	23	8	18	**537**

APPENDIX B: Peak integration data for Aroclor 1260 standard solutions

ABSOLUTE AREAS

Date	peak -	A	B	C	D	E	F	G	H	I	J	K	L	M	N	Total
	time -	19.27	19.67	20.45	21.89	22.05	22.64	22.82	22.97	23.51	24.44	24.69	24.86	25.85	26.50	Area
	Conc.															
22-Jan-95	1.0	62	120	149	106	57	80	48	42	206	93	41	50	17	44	**1115**
22-Jan-95	1.0	55	104	133	94	50	71	43	39	180	79	36	43	15	39	**981**
28-Jan-95	1.0	60	117	150	105	53	80	48	46	201	93	39	47	21	39	**1099**
18-Feb-95	0.1	9	17	21	13	7	11	5	5	26	14	6	6	0	5	**145**
20-Feb-95	0.5	44	84	107	73	40	56	32	29	143	67	28	34	16	27	**780**
20-Feb-95	1.0	70	132	170	116	61	90	53	48	231	107	46	57	24	45	**1250**
20-Feb-95	2.0	124	237	318	217	118	171	102	95	447	192	95	103	48	87	**2354**
25-Feb-95	0.1	8	19	23	13	7	11	6	5	26	13	6	5	0	0	**142**
25-Feb-95	0.5	44	83	107	66	37	58	35	31	143	65	29	35	16	29	**778**
26-Feb-95	1.0	74	141	185	123	66	98	59	54	256	119	50	62	25	52	**1364**
02-Mar-95	1.0	73	143	174	107	58	85	48	42	199	80	36	44	26	49	**1164**
24-Apr-95	0.1	12	25	28	17	10	15	8	0	32	16	7	6	0	4	**180**

APPENDIX C: Peak integration data for bee samples

ABSOLUTE AREAS

	peak -	A	B	C	D	E	F	G	H	I	J	K	L	M	N	Total
	time -	19.27	19.67	20.45	21.89	22.05	22.64	22.82	22.97	23.51	24.44	24.69	24.86	25.85	26.50	Area
Date	Sample															
26-Feb-95	TRA 1A	68	14	67	90	51	65	53	33	305	133	76	96	30	44	**1125**
25-Feb-95	TRA 2A	28	5	32	37	33	25	29	0	132	49	28	46	20	20	**484**
26-Feb-95	TRA 2B	100	19	98	122	75	91	72	34	492	167	100	140	44	56	**1610**
26-Feb-95	TRA 2B Spike	116	89	120	176	101	109	80	53	477	165	87	117	48	75	**1813**
25-Feb-95	TRA 3A	80	17	95	138	85	86	70	33	452	157	96	137	48	67	**1561**
26-Feb-95	TRA 3A Dup	78	17	90	129	84	82	67	30	448	160	115	140	46	53	**1539**
26-Feb-95	TRA 3B	38	7	36	48	36	33	34	6	161	58	36	49	14	19	**575**
26-Feb-95	TRA 3B Spike	116	120	187	186	107	112	86	49	448	180	107	123	52	77	**1950**
26-Feb-95	Loft A	51	12	62	58	39	50	42	17	253	91	56	79	38	36	**884**
26-Feb-95	Loft B	129	26	129	194	114	118	91	52	688	227	158	192	69	85	**2272**
02-Mar-95	Butte A	159	41	173	242	140	165	114	81	1121	300	189	253	104	131	**3213**
25-Feb-95	Butte B	32	8	35	45	32	28	32	4	151	54	32	49	27	21	**550**
02-Mar-95	Tucson	75	13	87	100	63	74	56	33	355	123	78	109	35	54	**1255**
02-Mar-95	Tucson Spike	108	67	163	165	108	110	87	51	572	205	135	159	59	84	**2073**

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