AD_____

Award Number: DAMD17-97-C-5072

TITLE: New and Improved Methods for Monitoring Air Quality and the Terrestrial Environment

PRINCIPAL INVESTIGATOR: Jerry J. Bromenshenk, Ph.D.
Dr. Garon C. Smith

CONTRACTING ORGANIZATION: University of Montana
Missoula, Montana 59812-1329

REPORT DATE: June 1999

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1. AGENCY USE ONLY *(leave blank)*.	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	June 1999	Annual (15 May 98 - 14 May 99)

4. TITLE AND SUBTITLE New and Improved Methods for Monitoring Air Quality and the Terrestrial Environment

5. FUNDING NUMBERS

DAMD17-95-C-5072

6. AUTHOR(S) Jerry J. Bromenshenk, Ph.D.
Dr. Garon C. Smith

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Montana
Missoula, Montana 59812-1329

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, MD 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT Honey bees (*Apis mellifera* L.) are multi-media monitors of chemical exposures and biotic effects. This five-year project has developed an automated system to assess in real-time colony behavioral responses to stressors, both anthropogenic and natural, including inclement weather. 1998 field trials at the Aberdeen Proving Ground— Edgewood area included the Old O Field and J Field landfills, D Field and Boundary Areas, and a Churchville, MD reference site. Preliminary results indicate that in general the levels of organic contaminants seen at APG sites are no better or worse than those seen regionally Off-Base. Industrial solvents in ambient air and in the air inside beehives exhibited the greatest between site differences, with the highest levels occurring in hives at Old O Field, J Field, some D Field sites, and Boundary area sites. Compared to 1996, both in 1997 and in 1998 the levels of organic solvents in Old O Field hives decreased by an order of magnitude, while colony performance improved, probably as a consequence of capping the landfill. There was no evidence of acute bee toxicity at most APG sites, although four colonies slowly failed at the Boundary sites. Hives will be re-deployed at ten Boundary sites in 1999 to further investigate these locations. A major objective of proposed 1999 studies will be to develop a framework for incorporating exposure characterizations of ambient air and hive atmospheres along with colony behavioral metrics into a risk assessment framework.

14. SUBJECT TERMS Biomonitoring, real-time monitoring, hazard assessment, acute toxicity, chronic toxicity, honey bee colony populations, environmental exposures, air quality, terrestrial environment, aliphatic hydrocarbons, heavy metals, military unique chemicals.

15. NUMBER OF PAGES 108

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	**Unclassified**	**Unclassified**	**Unlimited**

FOREWORD

Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

✔ Where copyrighted material is quoted, permission has been obtained to use such material.

✔ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

✔ Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

N/A In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985)

N/A For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules

N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories

6/30/99

PI - Signature **Date**

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ACRONYMS

AIA	Absolute Ion Abundance
ANL	Adjusted Net Loss (of bees)
ANN	Artificial Neural Network
APG	Aberdeen Proving Ground
As	Arsenic
Be	Beryllium
Ba	Barium
Benz	Benzene
BP	Beach Point Site
BR	Bush River Site
BTEX	Benzene, Toluene, Ethybenzene, Xylene
BRSA	Bush River Study Area
CD-ROM	Compact Disk - Read Only Memory
CGI	Common Gateway Interface
CC	Canal Creek Site
Cu	Copper
CV	Churchville Reference Site
C.V.	Coefficient of Variation
DARPA	Defense Advanced Research Projects Agency
DCB	Dichlorobenzene
DF	D Field Site
DER* DIFF	Derivative-Differential Analysis
Ethbenz	Ethylbenzene
FM	Fort Missoula Site
GS	G Street Site
Hz	Hertz
ipl	ions per liter
JF	J Field Site
JFN	J Field North Site
LC	Lauderick Creek Site (Cluster 13)
mg	milligram
Mg	Magnesium
Napth	Napthalene
ng/mg^3	nanogram per cubic meter
Ni	Nickel
NL	Net Loss of Bees
OF	Old O Field Site
PCR	Percentage Return of Bees
ppt	parts per trillion
QA	Quality Assurance
QC	Quality Control
Pb	Lead
PCE	Perchloroethylene

Rb	**Rubidium**
r^2	**Regression Coefficient**
SSE	**Summed Square of Error**
SOPs	**Standard Operating Procedures**
SSYY MMDD	**File Naming Format: Site, Year, Month, Day**
Sr	**Strontium**
SVOCs	**Semi-volatile Organic Chemical**
TCE	**Trichlorethylene**
TCM	**Tetrachloromethane**
TD/GC/ MS	**Thermal Desorption/Gas Chromatograph/Mass Spectrometry**
TFA	**Tetracholormethane**
Tolu	**Toluene**
USA CEHR	**U.S. Army Center for Environmental Health Research**
VOCs	**Volatile Organic Chemicals**
WP	**Work Plan**
WWW	**World Wide Web**
YC	**Youth Center**
Zn	**Zinc**

EXECUTIVE SUMMARY

The objective of this five-year study is to develop and apply real-time biomonitoring using honey bee colonies to assess toxic chemical contaminants in military-unique, terrestrial ecosystems. The Edgewood Area of Aberdeen Proving Ground (APG) provided appropriate test locations for conducting top down (field to laboratory, colony to individual, effects to exposures) testing.

This report covers activities and goals of the 1998 field season project, including: 1) continued monitoring of behavioral responses of bees to contaminants, weather and other environmental factors at J Field, Old O Field, and a Churchville reference site, 2) improvements of the sampling and calibration methods for exposure characterizations for volatile and semi-volatile organic chemicals (VOC's and SVOC's), 3) initial surveys to characterize bioavailable (i.e., available for uptake by bees - see Glossary) chemicals in the ambient air and in beehives at D Field, 4) initial surveys to examine bioavailable chemicals at sites near the Boundary of the Aberdeen Proving Ground (APG) and along transects extending more than 20 miles into the communities surrounding APG, and 5) proposed activities for 1999, with an emphasis on developing a framework for incorporating colony behavioral metrics (i.e., measures of effects) and exposures to bioavailable chemicals into a risk assessment framework.

Biomonitoring at APG and in the Surrounding Communities

The overall biomonitoring objectives of this study were to:

- Conduct a comparison of conditions at J Field, Old O Field, and Churchville
- Perform a broad brush survey of D Field and of the Boundary areas.

Real Time Monitoring of Colony Dynamics and Behavioral Responses

Measurements of colony response metrics were guided by two overall objectives:

- Real-time monitoring of colony population dynamics to establish the dose-response relationship between chronic and acute effects and short and prolonged exposures to specific chemical agents and measures of individual (bee) and population (colony) effects,
- Site-to site comparisons of honey bee colony populations with respect to the effects of chronic as well as acute ecosystem exposures to bioavailable chemical agents.

Since 1996, a tiered numerical analysis approach has been applied to the biomonitoring data collected at APG and at the Churchville, MD and Missoula, MT reference sites. The greatest variability in flight activity at specific sites was seen in 1996. Regionally, the least amount of variability in flight activity (by site) was documented for 1997. Flight variability increased somewhat in 1998 compared to 1997. Overall, 1998 results more closely reflected those for 1997 than 1996. Coefficients of variation for flight activity for Maryland sites ranged from 15-130% in 1996, 15-75% in 1997, and 20-100% in 1998, with the highest variability seen at the Churchville reference site. The APG sites ranged from 20-80% in 1998. For all years, the

percent return rates for bees leaving and returning to the hives exceeded 90%. The values for this metric ranged from 90-108% in 1996, 92-104% for 1997, and 92-112% for 1998. Values in excess of 100% were due to bees drifting from one hive to another or from bees that sometimes managed to bypass the hive-mounted counters when exiting the hives. A modification of a shield on the counter assemblies should eliminate this bypassing problem for 1999. Overall, there was no evidence of any acute toxicity in any of the electronically monitored hives on the APG post or the reference sites in Maryland and Montana over the three year period.

In general, all of the measured hive metrics indicated similar, but slightly degraded, behaviors for 1998 compared to 1997. Both years displayed an improvement in comparison to the sites monitored in 1996 when the Old O Field cap was being installed. Because we had to feed colonies, especially at the Churchville reference site in 1998, we think that it is probable that the slight reduction in colony condition observed in 1998 was more a consequence of limited food shortages than anthropogenic stress.

Calibration trials conducted at the University of Montana, that these real time measures of colony performance would clearly and identify exposure to a known toxic chemical such as methyl parathion. In addition, changes in colony flight and thermoregulation in response to this chemical correlated well with the number of bees found in traps installed to capture dead and dying bees. However, the real time system showed that colony foraging continued to exhibit the effects of the toxic event long after bees stopped dying.

In 1998, the only losses of bee colonies occurred among the survey hives at three of the Off-post Boundary sites and at Carroll Island. The cause of these losses is unknown. Therefore, in 1999, bee colonies were re-deployed at most of the Boundary sites to further investigate conditions at these locations.

Monitoring of Bioavailable Contaminant Concentrations in Ambient Air, Hive Air, Bees, and Pollen

The primary objectives of the chemicals sampling and analysis portion of this project were to:

- Measure chemical agents in the ambient air as well as those that are bioavailable to honey bees from multiple sources,

- Make site-to-site comparisons with respect to these chemical exposures—at sites on the Aberdeen Proving Ground (mainly Edgewood area) and in the communities surrounding APG,

- Provide chemical exposure data needed to characterize relationships between exposures (to specific chemical agents) and effects (lethal to sublethal, acute to chronic, dying bees to behavioral changes in flight activity or thermoregulation) as measured in honey bee colony populations.

The introduction of a multi-bed sampling train in a sealed box, termed a JAG box, virtually eliminated sample degradation or loss due to excessive moisture (from humidity or rain) wetting the Carbotrap desorption beds, and tube breakage in the field. A Carbotrap 150 also removed

terpenes that in the past had produced a shellac-like coating on the inside of the analysis instrument.

In 1998, both the ambient air and hive air samples contained the same general types of volatile and semi-volatile organic chemicals as seen at APG sites in 1996 and 1997. Chlorinated hydrocarbon solvents were detected, including perchloroethylene (PCE), trichloroethylene (TCE) and tetrachlormethane (TCM). TCE was highest at J Field. Dichlorobenzene (DCB) was seen at most locations, but only at low levels. The BTEX (benzene, toluene, ethylbenzene) group was almost ubiquitously present. In September, Old O Field had unusually high ethylbenzene levels in ambient air and hive air samples; especially those closest to the water treatment plant. Napthalene was present at most sites. Acetophenone, a tear gas degradation product, occurred at some APG sites. Methenamine, a principal reagent from which RDX explosive is manufactured, as well as other uses such as in adhesives and the vulcanization of rubber, appeared at some APG sites. It also appeared in high levels at some of the agricultural locations along the Off-Post, Boundary Study Transects. PCE and TCE in the air inside beehives were often at levels significantly in excess of ambient air. TCM, BTEX, and napthalene occurred in ambient air at levels comparable to those in hive atmospheres. With the exception of TCE, D Field samples contained the highest levels of every VOC and SVOC contaminant. High levels of BTEX were detected at Boundary sites nearest to Baltimore or major highways, which suggested vehicles as contributing sources. Taking all of the organic chemical exposure results into consideration, in general the levels of VOC and SVOC contaminants at APG sites were no better nor worse than those seen regionally Off-post.

Biomonitoring and Risk Assessment

Over the last three years, the emphasis has been on monitoring colony condition and chemical exposure levels in the ambient air and in beehives. The overall objective of the honey bee biomonitoring applications at APG can be summed up by the National Center for Environmental Assessment (NCEA)'s final statement in its ecological assessment guidelines: "If the decision is to mitigate risks through exposure reduction, for example, monitoring could help determine whether the desired reduction in exposure (and effects) is achieved." A major objective of the 1999 APG tasks are to work toward more fully incorporating the results of the honey bee chemical exposure and colony effects characterizations into a risk assessment framework that will meet APG needs and be accepted by decision makers, regulatory agencies, and the local community.

PROPOSED ACTIVITIES FOR 1999

Proposed activities for 1999 include:

- An intensified investigation of the potential transfer of VOC/SVOCs to bees in the phytoremediation grove of trees at J Field — using 15 standard-size colonies of bees, placed on site March 1, 1999, and sampled before, during, and after bud break of the hybrid popular trees in early April

- Continued biomonitoring of the installation restoration activities at J Field

- Verification of the 1998 VOC/SVOC findings for the off-post (i.e., in the communities surrounding APG) Boundary Sites by re-deploying and re-sampling hives at the 10 transect sites tested in 1998. Hives will be deployed by end of June and sampled at end-of-summer for VOCs, SVOCs, and heavy metals.

- Initiation of biomonitoring with a system of electronic hives at Bush River Cluster 3 near the Skipper Point housing area—prior to initiation of capping of this site.

- Provision of on-line access to colony response behavioral data at Churchville, J Field, and BR Cluster 3 via the World Wide Web, with the option of using automatic or remote manual triggering of pumps to sample for VOCs and SVOCs

- Continued calibration of colony dose-response relationships with an emphasis on determining the effects of exposures to VOCs and SVOCs that are commonly found at APG

- Testing (at J Field) of improved chemical sampling and analysis methods for the detection of explosives (under a related DARPA sponsored project)

- Development of a protocol for incorporating real-time colony behavioral responses and measures of exposures to bioavailable chemicals in hives and in ambient air into a risk assessment framework.

SUBJECT TERMS

Biomonitoring, real-time monitoring, hazard assessment, automated monitoring, acute toxicity, chronic toxicity, honey bee colony populations, environmental exposures, exposure characterization, effects characterization, air quality, terrestrial environment, chlorinated hydrocarbons, BTEX, heavy metals, military unique chemicals.

ACKNOWLEDGMENTS

We would like to thank Cindy Powels, John Wrobel, Don Green, Ruth Golden, Allison O'Brien, Chris Jacobs, and Tim Rodeffer of the Directorate for Safety, Health, and the Environment for their support during the study. Alan Rudolph from DARPA helped us provide new dimensions to the study. Mr. David Simmons and Mr. James Zink provided their expertise concerning proper management of honey bee colonies in east coast habitats as well as providing queens, bees, and off-post reference sites. Jason Volkman, Jeremy Hamma, Lenny Hahn, and Kenny Morris managed the Maryland and Montana bees and coordinated more than a dozen undergraduate students who helped design, build, and test these methods and equipment. We also wish to thank Mr. Alan Rosencrance of the U.S. Army Center for Environmental Health Research (USACEHR)(provisional, formerly U.S. Army Biomedical Research and Development Laboratory), Fort Detrick, Maryland, for conducting the heavy metal and other inorganics analysis. Mr. Tommy Shedd of USACERH provided critical guidance as the contracting officer representative. We acknowledge USACEHR for supporting the study through U.S. Army Contract No. DAMD17-95-C-5072

SECTION 1 OVERVIEW AND WORK PLAN FOR 1998

1.1 PREVIOUS INVESTIGATIONS

Previous investigations conducted at Aberdeen were covered in the annual reports for 1996 and 1997 (Bromenshenk *et. al.*, March, 1997 and March, 1998). A summary of these activities follows:

- In 1995, a pilot test and demonstration of the honey bee biomonitoring technology was conducted by deploying six electronic hives for two weeks in August at West Branch Canal Creek. The trial concluded that bees could be maintained at this location and that trace amounts of volatile (VOCs) and semi-volatile (SVOCs) could be found inside the colonies. No acute toxicity was observed during the test.

- In 1996, electronic hives were deployed in June/July at Old O Field, West Branch Canal Creek, and at a Churchville reference site. Additional survey hives were deployed at Old O Field and several locations across the Canal Creek Study area. The colonies remained on site until late fall. Capping of the landfill at Old O Field was undertaken while the colonies were on-site. The study found that a wide array of VOCs and SVOCs occurred in hive atmospheres and in ambient air at many of the APG Edgewood locations. In general, the levels of VOC and SVOC contaminants were highest at Old O Field. The queens disappeared from half of the electronic and of the survey hives at Old O Field in August. The colonies lacking queens also recorded the highest exposures to bioavailable chemicals, namely organic solvents. Colonies at West Branch Canal Creek, where a removal action had been completed in 1995, performed as well as or better than the colonies at the Churchville reference site and exposures to bioavailable organic chemicals was usually low compared to other sites. Survey colonies at the Youth Center, Beach Point, and Lauderick Creek locations of the Canal Creek Study Area recorded higher levels of several VOCs and SVOCs than did other colonies on the upper post area of APG Edgewood, although the levels of organics at these three sites usually were considerably lower than those at Old O Field.

- In 1997, a full year of biomonitoring was completed at the Old O Field and Canal Creek Study areas. At the end of the growing season, the electronic hives at West Branch Canal Creek were relocated to J Field, where preliminary background data was obtained prior to initiation of an installation restoration removal activity. Twelve additional survey sites were established across the Bush River Study area to characterize that part of APG Edgewood. As in 1996, the highest levels of VOC and SVOCs usually were observed at Old O Field, but the concentrations tended to be lower by an order of magnitude than during the previous year. None of the Old Field colonies lost their queens; nor was any acute toxicity observed in any of the colonies. However, overall colony performance was more variable and colonies were weaker at Old O Field than at either West Branch Canal Creek or the Churchville reference site. The capping of the Old O Field landfill begun in 1996 was completed during the 1997 biomonitoring period. As in 1996, the highest contaminant levels in the Canal Creek area were again observed at sites known to

have some exposure to VOCs and SVOCs. The Youth Center again ranked among the three highest sites in terms of exposure concentrations. Prior to the 1996 biomonitoring study, this site had not been reported as exposed to these contaminants. Although the chemical levels were low (parts per trillion in hive atmospheres), this site continued to rank among those known to have exposure sources. As in the previous year, the bees at West Branch Canal Creek did as well as or better than the reference site in terms of overall colony condition and recorded exposures to organic contaminants. Not surprisingly, several of the Bush River sites, which contained several chemical storage facilities and old landfills, also displayed higher than background exposure concentrations to some VOCs and SVOCs .

- In both 1996 and 1998, slightly elevated levels of a few trace elements and heavy metals were observed at APG sites, although the levels of bioavailable inorganic chemicals were low compared to those observed in bees from industrial regions near copper and lead smelters.

- In 1998, we continued the biomonitoring at Old O Field and J Field, began a survey of D Field, and initiated a Boundary Site survey using locations near the APG boundary and along transects extending into the communities surrounding the Army Post. Continuing the Old O Field study provided a post-capping evaluation of the effectiveness of the restoration project in terms of reducing bee exposures to VOC and SVOCs and improving colony performance. The J Field study provided ongoing monitoring of the removal project began in the summer of 1998. Again, the J Field bees can provide a means of assessing exposures and colony condition during the restoration activities as well as the information needed for a post-removal action assessment.

For 1998, the objective of the Boundary Study was to assess locations near the APG boundary that have not been previously examined *and* to provide information needed to begin to assess whether contaminants of concern are migrating off of the APG facilities and into the surrounding communities. The D Field study examined the utility of using bees to survey an APG area that had previously not been well characterized with respect to bioavailable chemicals in the terrestrial environment.

1.2 DATA GAPS

The technical approach and investigative activities planned to address existing data gaps for APG sites is detailed later in this report. Some major data gaps regarding the characterization of APG sites are as follows:

- During the removal action at J Field, potential releases of bioavailable chemicals warrant monitoring. In addition, there is a need to further assess the effectiveness of the trees being used for phytoremediation of subsurface VOCs and SVOCs and to address whether the trees might act as a conduit for VOCs and SVOCs, transporting these chemicals from the subsurface water into the air or into bee colonies. Whether these uptake, transport, and fate pathways occur is considered to

be unlikely, but prior to 1998 this potential route of transport into bee colonies had not been measured.

- Although bee colonies at Old O Field performed better in 1997 than in 1996 with respect to improved colony condition and reduced levels of contaminant exposure, in 1997 capping of the landfill was being completed and the summer was unusually dry. Data from 1996 at APG and from 1997 field tests conducted in Montana suggested that the high levels of some of the VOCs and SVOCs observed in hives at Old O Field during 1996 may have originated from a water source. In 1998, the capping of the dump had been completed. Additional trials were conducted at UM to further examine chemical uptake into hive atmospheres from water. Most importantly, 1998 also provided a second year of chemical exposure and colony response data which could be used to judge the effectiveness of the capping of the Old O Field landfill in reducing chemicals in the terrestrial environment.

- The nature and extent of bioavailable contaminants in the air and terrestrial ecosystems at D Field has not previously been investigated. Most of the prior investigations had focused on water quality as measured by a system of monitoring wells. An objective of the 1998 honey bee biomonitoring was to compare the speed and reliability of using bees as an initial survey tool compared to the results of longer-term studies (years) of water quality requiring the drilling of wells and electricity to operate pumps.

- Many areas of APG have not been monitored for VOC and SVOC contaminants in the ambient air nor have many off-post areas been monitored for chemicals commonly found in APG environments. The potential for migration of these chemicals into the surrounding communities warranted investigation. Similarly, sources in these communities (i.e., non-APG sources) of a diverse array of contaminants may impact the communities in which they are located. These sources also could be dispersing materials that might impact APG. Previous honey bee biomonitoring at three Harford county locations suggest the existence of off-post sources of several of the same VOCs and SVOCs observed at locations at APG-Edgewood.

1.3 SCOPE OF WORK

This subsection describes the work that was completed during the 1998 Study. Also included in this section is a description of the specific sites at which hives were placed. The following work was conducted during this Project:

Task 1. J Field Real-Time Behavioral Monitoring and Chemical Exposure Survey

Subtask 1: Conduct real-time monitoring of colony behavioral responses to further document assessment metrics. Colony response investigations begun in 1997 were continued in order to monitor the removal actions conducted at J Field in 1998.

Subtask 2: Conduct chemical exposure surveys -- hive air, hive components, bees, pollen, and ambient air to link real-time behavioral responses to exposures to toxic agents. Continue the pre-capping chemical exposure investigations begun in 1997. Determine the potential for uptake and transfer of VOCs to bees from trees used for phytoremediation.

Subtask 3: Provide an expanded database for use in a post-removal action assessment of exposures and the effectiveness of reducing potential risks to natural systems (i.e., bees and pollinators).

Task 2. Old O Field Post-Capping Assessment

Conduct both real-time monitoring of colony behavioral responses and chemical exposure surveys to document in a second year the degree to which capping the landfill reduced exposures or risks to bees and pollination systems.

Task 3. D Field Area Surveys: Chemical Exposure and Acute Toxicity of Colonies Placed along a Transect through the Area.

Conduct early, mid-, and late season sampling to document exposure levels to a variety of VOC and SVOC chemical contaminants in hive air and ambient air. Conduct periodic sampling to characterize the concentrations of trace elements and heavy metals that may accumulate through time in hive components, bees, pollen, and ambient air. Determine acute toxicity, if any. Develop analytical methods to detect possible unique contaminants at D Field, such as explosives. Conduct additional mid- to late- season sampling at additional D Field sites based on the outcome of early season chemical monitoring characterizations.

Task 4. Boundary Area Surveys: Chemical Exposure and Acute Toxicity Assessments of Colonies Deployed Along Transects Extending outward from APG

Deploy chemical exposure survey hives at four locations along each of three transects extending from the Youth Center on the APG-Edgewood Post area into Harford and Baltimore Counties. Place additional test colonies at the Lauderick Creek, Westwood, Graces Quarters, Carroll Island, and Aberdeen near the fragmentation pits. Conduct early, mid-, and late season sampling to document exposure levels to a variety of VOC and SVOC chemical contaminants in hive air and ambient air. Conduct periodic sampling to assess the levels of trace elements , heavy metals, and radionuclides that may accumulate through time in hive components, bees, pollen, and ambient air. Determine acute toxicity, if any.

1.4 GENERAL SCHEDULE

Colonies established from packages brought from Moultrie, Georgia, April 6, 1998. Hives were deployed at APG sites in April and May and at Boundary sites in May and June. Real-time monitoring began in late April, with chemical exposure pre-sampling started in April. Routine

sampling began in May. The1998 field study was completed in October. VOC and SVOC analyses were completed by December, 1998. Sample analysis verification and interpretation was completed spring, 1999. Samples for inorganic analysis were prepared (i.e., dried and ground) in winter, 1999, and then submitted to USA CEHR for analysis. All of the colony behavioral response data was processed by late fall. The colony data from sites with a telephone or wireless data link (e.g., Churchville or Fort Missoula, MT) could be accessed and processed the same day as collected.

SECTION 2 EDGEWOOD AREA DESCRIPTION

2.1 ABERDEEN PROVING GROUND—HISTORY

Aberdeen Proving Ground (APG) encompasses 79,000 acres of land and water near the head of Chesapeake Bay and north of Baltimore, Maryland. The post consists of two primary areas: the Edgewood area and the Aberdeen area, separated by the Bush River. Both parts of the post contain large natural habitats, being composed of approximately 50% hardwood forests, 13% marsh or marsh shrub, 2% bare earth, 1% natural shrub, and 34% mowed/grassy areas.

The Edgewood area includes Gunpowder Neck, Pooles Island, Carroll Island, and Graces Quarters. Since 1917, Edgewood has been used for the development and testing of chemical agent munitions, where the Army conducted chemical research programs as well as manufactured, stored, and disposed of toxic agents. Numerous buildings and large areas of land and water have been contaminated or are suspected of being contaminated, with significant quantities of napalm, white phosphorous, and other chemical agents. On-site sampling has identified various metals, phosphorus, and VOCs in groundwater and soils, with unexploded ordinance in surface and subsurface soils.

Preliminary sampling at the Aberdeen area has identified various heavy metals, phosphorus, and volatile organic (VOCs) compounds in groundwater and surface water. Soil is contaminated with pesticides and polychlorinated biphenyls (PCBs), VOCs, and petroleum hydrocarbons. Cleanup activities focused on Michaelesville Landfill Source Control, Michaelsville Landfill Groundwater, Phillips Field Disposal Area, White Phosphorus Underwater Munitions Burial Site, Aberdeen Fire Training Area, and Other Michaelsville Areas were undertaken in the early 1990s.

On both the Edgewood and Aberdeen areas of the Proving Ground, removal of contaminated soil, capping of landfills, and installation of water treatment systems have reduced immediate threats at the site while additional studies and removal activities are underway. The wetlands areas are designated as habitat for eagles. There is a possible risk of bioaccumulation of contaminants in food chains in the natural habitats. People who accidentally ingest or come into contact with contaminated soils, sediments, or water may be at risk. Over 3000 military personnel are assigned to the post and about 2900 military families reside on-site. An additional 7,600 civilian employees and 3,000 contractors work on the post. In addition, approximately 38,600 people live within 3 miles of the Edgewood site boundary.

2.2 SITE LOCATIONS AND DESCRIPTIONS

Assuming that 50 percent of the foraging honey bees in an eastern hardwood forest concentrate their efforts within 600 feet of the hive, nucleus hives can be placed at strategic locations for monitoring exposures where bioavailable chemicals may be found. This study has separated the Aberdeen Proving Ground area into four areas of concentration: J Field, Old O Field, D Field, and Boundary Areas. Within each of these areas are specific sites with distinctly different histories, origins, and contaminants. The overall biomonitoring objectives of this study were to:

- Conduct a comparison of conditions at J Field, Old O Field, and Churchville
- Perform a broad brush survey of D Field and the Boundary areas.

If contaminants were found in specific hives at any of these sites, then additional investigations could be performed to determine the possible sources and extent of distribution of the bioavailable contamination.

The placement of hives was determined by: the probability of encountering contaminants (on APG sites), sufficient amounts of foraging vegetation available in the surrounding area (for maintenance of the colonies), and reviews of historical information and sampling data and analysis reports found in Remedial Investigation Reports for APG and RCRA Facility Agreements.

The following sections describe specific sites, hive locations, and overall Materials and Methods for the honey bee population behavioral response monitoring as well as the sampling and analysis for VOCs, SVOCs, heavy metals and other trace elements, and radionuclides.

2.3 ELECTRONIC/SURVEY HIVE STUDY AREAS

Seven electronic hives supplemented by additional chemical survey hives were established at J Field, Old O Field, and the Churchville reference site in 1998. An additional set of seven electronic hives (condos) and more than 50 survey hives were maintained in Montana to gather additional reference information. Primarily, these colonies served as a means of determining what chemical contaminants are commonly found in bee colonies in less industrialized/ urbanized areas of the U.S. for comparison to those found in colonies in Maryland, both On- and Off- the military post. The Montana colonies also provided opportunities for testing the effects of specific volatile organic contaminants on colonies via controlled dose-response trials. The Montana studies are part of ongoing studies for the U.S. Army's Center for Environmental Health Research. These studies compliment the APG applications described in this work plan.

2.3.1 J Field, APG Edgewood: This site is at the southern tip of the Edgewood peninsula. An open, grassy area is surrounded by hardwood forests and marsh. The Army has detonated munitions in trenches throughout J Field. Erosion control efforts to prevent toxic substances from being eroded into Chesapeake Bay have been undertaken. A test grove of hybrid popular trees has been planted to investigate the possibility of using phytoremediation to remove VOCs and SVOCs from groundwater. Seven honey bee condos were placed near the phytoremediaton trees on the southwest side of the grove. An additional 4 survey hives were placed on the southeast and northwest sides of the J Field area, near the margins of the clearing. A removal activity was carried out by a contractor throughout the spring and summer of 1998. This effort was focused on a location near the middle of the J Field clearing that is more or less centered among the hives. This work was continued in 1999.

2.3.2 Old O Field, APG Edgewood: This is a heavily wooded site located on the lower west side of the Edgewood peninsula next to Watson Creek. Large quantities of munitions were disposed of by the Army in a variety of ways at Old O Field's landfill and other areas. Long-term cleanup activities undertaken by the Army included construction of a permeable cap over the landfill and a water treatment plant to address groundwater that has become contaminated as

a result of seepage from the landfill and other O Field areas, including contaminated sediments in nearby Watson creek. The water treatment plant sits on a grassy strip between the landfill and the Gun Powder River.

Seven condos were situated on the east side of the landfill with an additional two survey hives placed in the woods south of the water treatment plant. These same sites were monitored in 1996 and 1997. The Old O Field study for 1998 was part of the ongoing research and development studies funded by other sponsors through the U.S. Army's Center for Environmental Health Research and were provided to APG at no cost for 1998.

2.3.3 D Field, Edgewood Peninsula, APG: A series of monitoring wells have been installed to assess water quality at D Field. The site is located on the west side of the Edgewood Peninsula more or less opposite of Old O Field. With the exception of two open areas facing Bush River, the site is heavily wooded and bisected by an unpaved road. A recent report tentatively identified Saran as a possible contaminant at this site. Survey hives were deployed in pairs at regular intervals along this road. Initially five pairs of hives were placed at D Field. Based on initial chemical exposure results, additional pairs of hives were deployed at D Field to intensify the sampling at a location showing evidence of significant contamination.

2.3.4 Churchville, APG Edgewood: This is a rural site consisting of a grassy clearing bounded by hardwood forests. It is privately owned and is not part of the APG area. There are no known reports of any landfills or other industrial activities occurring at this site. The area provides a reasonable approximation of the vegetation and habitats found across much of the APG areas and as such serves as a reference site. A set of seven electronic hives has been maintained on this site since 1996.

2.3.5 Missoula, Montana: The University of Montana's Research Test site is located on the west end of the Missoula valley. Missoula is in the western part of Montana and is surrounded by mountains. The beeyard location is situated on UM's College of Technology campus. It is in an open area at the end of a parking lot, near the banks of a river, and surrounded by grassy/weedy old fields and alfalfa hay fields. The research site is near agricultural and residential areas, similar in makeup to the reference site near the Churchville, MD. Seven electronic hives are secured behind a locked chain-link fence at this location. Additional survey hives are kept at this apiary and at three other locations in the Missoula valley. A wireless modem transmits real-time monitoring data to the Honey Bee Monitoring Offices on UM's main campus on the east side of the valley.

The Missoula area is much less industrialized and urbanized than Edgewood, Maryland. No military installation occurs in this part of western Montana. Therefore, many of the chemicals commonly found in bee colonies at both On- and off-post sites near APG in Maryland do not appear in colonies at Missoula. The Missoula location provided a useful place to examine the toxic effects of some of the specific contaminants found in Maryland. This site also was used to conduct controlled uptake, transfer, and fate studies of these chemicals.

2.3.6 On- and Off-Post Survey Hive Study Areas: Historically, characterizations of contaminant distributions at APG have focused on groundwater and surface waters, soils, and sediments. Much less is known about the quality of the air. Some sites like Old O Field and J

Field have been extensively investigated and have or are being remediated, whereas others have received less attention. Also, except for monitoring water quality in wells, comparatively few investigations of air quality and the terrestrial environment have been conducted in the communities surrounding the APG facilities.

Honey bees were used in 1998 to provide a first-cut survey of some of these On- and off-post areas under an investigation that was termed the Boundary Study. In addition, an initial survey of D Field was conducted. Maps 1-3 provide an overview of the sampling locations. Specific locations for the off-post Boundary locations appear in Table 2.1. The following sites comprise the Boundary and D Field studies:

2.3.7 Youth Center, APG Edgewood: Investigations along approximately 700 acres of Canal Creek have been conducted where the Army disposed of large quantities of munitions. Removal actions have been completed along West Branch Canal Creek. Biomonitoring studies conducted at seven sites in the Canal Creek area in 1996 and 1997 identified three locations with consistently elevated levels of several VOCs and SVOCs: Youth Center, Beach Point, and Lauderick Creek. Although technically part of the Nike Study Area, the Lauderick Creek Site was included as part of the Canal Creek Biomonitoring because the hive location was just outside of the fence north of the APG/Edgewood golf course. Both Lauderick Creek and Beach Point have been the subject of previous investigations, but the Youth Center area has not been the subject of any study of suspected or known contaminant sources. Preliminary trials in 1997 failed to discern the source of these organic chemicals at Youth Center. For these reasons, Youth Center was chosen as the starting point for the Boundary Area study which is focused on identifying the potential for migration of chemicals on to or off of the APG/Edgewood post. Three survey hives were placed at Youth Center and sampled during 1998.

2.3.8 Westwood Area, APG Edgewood: This site is located in a small clearing against the APG boundary fence near the northwest corner of the APG/Edgewood post. It was used as a bomb-drop test area. It has an active solid waste landfill which is licensed to receive only rubble and asbestos materials. A spill of radioactive material occurred at the Westwood Area. Removal of soils contaminated by the spill was completed in the spring of 1998. Because this site is very near off-post homes, a pair of survey hives was placed just inside the fence between the houses and the cleanup area to investigate the potential occurrence of any remaining, bioavailable radioactive contaminants.

2.3.9 Cluster 13, Lauderick Creek, APG/Edgewood: The Cluster 13 location is in the woods east of the building complex at the Nike site. It is farther east than the sampling location used in 1996 and 1997. This site was included because it is near the north boundary of APG. The Army used the Nike area in the early 1900s to dismantle and remove several anti-ballistic missile silos from this area and filled the silos with concrete. The area has been extensively investigated for VOCs and SVOCs in groundwater and surface waters, and some of these organic chemicals were found to be slightly elevated in colonies at the previous Lauderick Creek location. There has been some concern expressed by local community groups about the possibility of finding elevated levels of radionuclides at the Cluster 13 location, although the presence of radionuclides at this site has not documented by the Army. Because of these public citizen concerns, a pair of survey hives was placed at Cluster 13 as part of the Boundary study. These hives was sampled for radionuclides in addition to VOCs, SVOCs, and heavy metals.

2.3.10 Graces Quarters, APG Edgewood: A largely wooded area located on a small peninsula below Gunpowder State Park and separated from the main Edgewood area by the Gun Powder River. The Army has removed munitions waste from this area. Monitoring wells continue to provide information about VOCs and SVOCs in groundwater. At the current time, no further removal actions are anticipated to be necessary at this site. A pair of survey hives was placed in a grassy clearing in the middle area to assess current conditions in terms of the bioavailability of contaminants.

2.3.11 Carroll Island, APG Edgewood: The Army conducted open-air testing of chemical agents on this island, which like Graces Quarters, is separated from the main Edgewood area by the Gun Powder river. Several structures and toxic pits containing munitions have been removed, and another removal action was being conducted during the summer of 1998. The island is next to a coal-fired power plant complex. Like other APG locations, the habitat is a mixture of hardwood forests, grassy areas, and marshes. A pair of survey hives was placed close to the area where the removal activity was taking place.

2.3.12 Aberdeen Peninsula, APG: As on the Edgewood peninsula, the Aberdeen peninsula contains large areas not covered by specific study areas. In addition, previous honey bee biomonitoring activities have been limited to the Edgewood portion of the post. As an initial survey in the Aberdeen area, a pair of survey hives was placed near the Fragmentation Pits. The hives were placed on an old tower in the woods near the pits. Other nearby facilities/activities of interest include a munitions loading facility and a firing range.

2.3.13 On- and Off-Post Boundary Study Areas: In 1998, pairs of survey hives were deployed along three transects beginning at Youth Center in the West Branch Canal Creek Area and extending over 20 miles into the adjacent communities to Cecil County, Shawsville in Harford County, and the Clyburn Arboretum in Baltimore County. The locations of this set of 10 pairs of hives along with extra locations near the Transects on the Aberdeen Post, both at the Edgewood and Aberdeen areas. Unlike the APG sites, there is no readily available history of land usage for the Off-Site locations. Present day uses are noted in Table 2.1. Several of these locations were near heavily traveled highways.

Table 2.1

1998 APG Boundary Study Sites

Transect Hub - Youth Center
Hives: 98100 & 98101
Location: Youth Center, APG
Map coord: HC29D06

Transect 1 - Site 1(extra)
Hives: 98126 & 98127
Location: APG Cluster 13
Map coord: HC29F05

Transect 1 - Site 2 (3 mi)
Hives: 98104 & 98105 (was 98051)
Location: Estuary/State Park
Map coord: HC24H11

Transect 1 - Site 3 (extra)
Hives: 98128 & 98129
Location: APG Aberdeen Post (Fragmentation Pit)
Map coord: HC30H05

Transect 1 - Site 4 (6 mi)
Hives: 98108 & 98109
Location: Orchard
Map coord: HC19B08

Transect 1 - Site 5 (12 mi)
Hives: 98110 & 98111
Location: Orchard
Map coord: Harford County Index Map, 48mm from right edge, 39mm from top edge (about the "w" in Rowlandsville Rd)

Transect 2 - Site 1 (3 mi)
Hives: 98102 & 98103
Location: Fruit Stand/Truck Garden
Map coord: HC24A12

Transect 2 - Site 2 (6 mi)
Hives: 98112 & 98113
Location: Farm
Map coord: HC22H03

Transect 2 - Site 3 (12 mi)
Hives: 98114 & 98115
Location: Landscaping Firm
Map coord: HC07E02

Transect 3 - Site 1 (Extra)
Hives: 98124 & 98125
Location: APG Westwood
Map coord: HC28J07

Transect 3 - Site 2 (3 mi)
Hives: 98106 & 98107
Location: Private Residence
Map coord: HC28C06

Transect 3 - Site 3 (Extra)
Hives: 98122 & 98123
Location: Graces Quarters Island (APG)
Map coord: BC39B07

Transect 3 - Site 4 (Extra)
Hives: 98120 & 98121
Location: Carroll Island (APG)
Map coord: BC39B13

Transect 3 - Site 5 (6 mi)
Hives: 98116 & 98117
Location: Private Residence
Map coord: BC29G09

Transect 3 - Site 6 (12 mi)
Hives: 98118 & 98119
Location: Clyburn Arboretum
Map coord: BC34F01

Figure 2.1 Edgewood Peninsula Study Area Map Showing the Locations of Old O Field, D Field, and J Field.

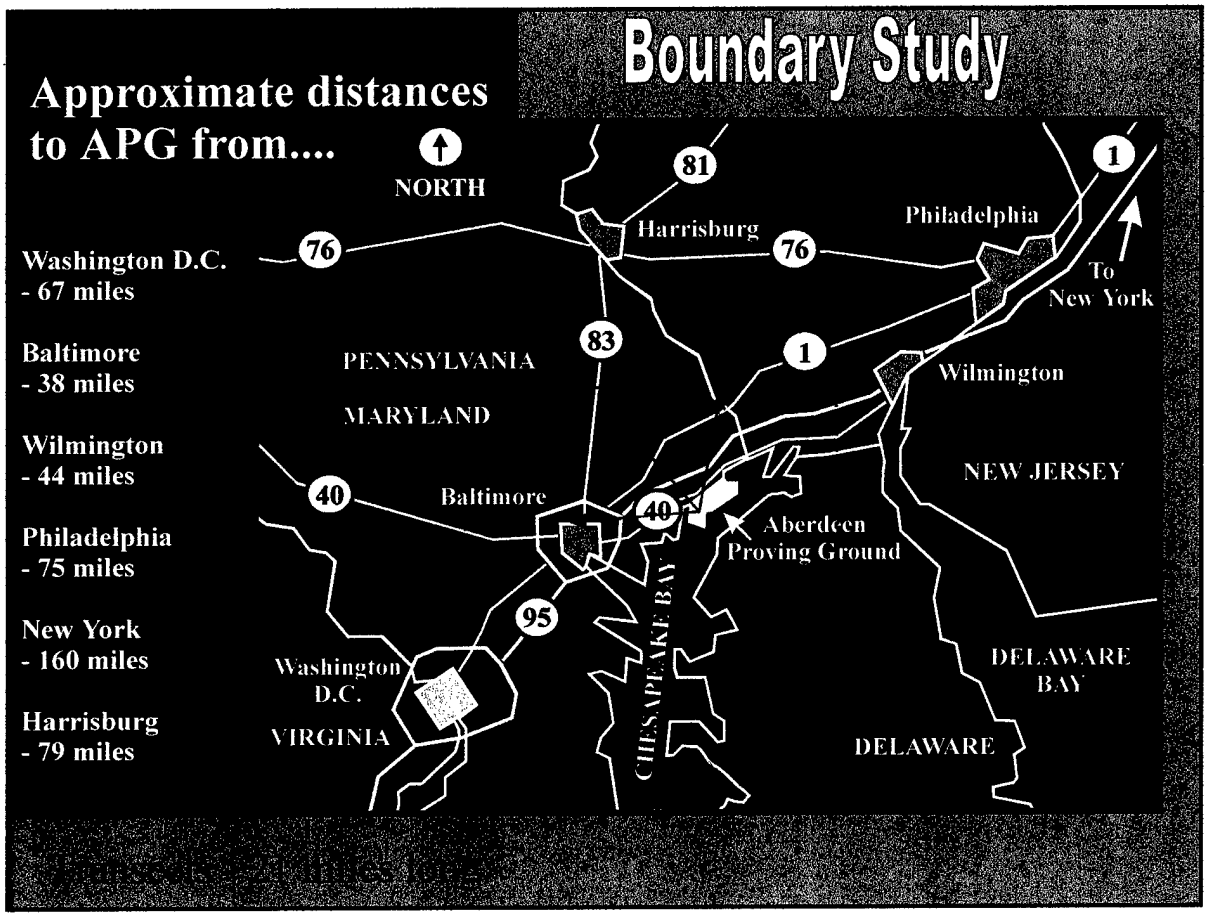


Figure 2.2 Boundary Study Transects, 1998

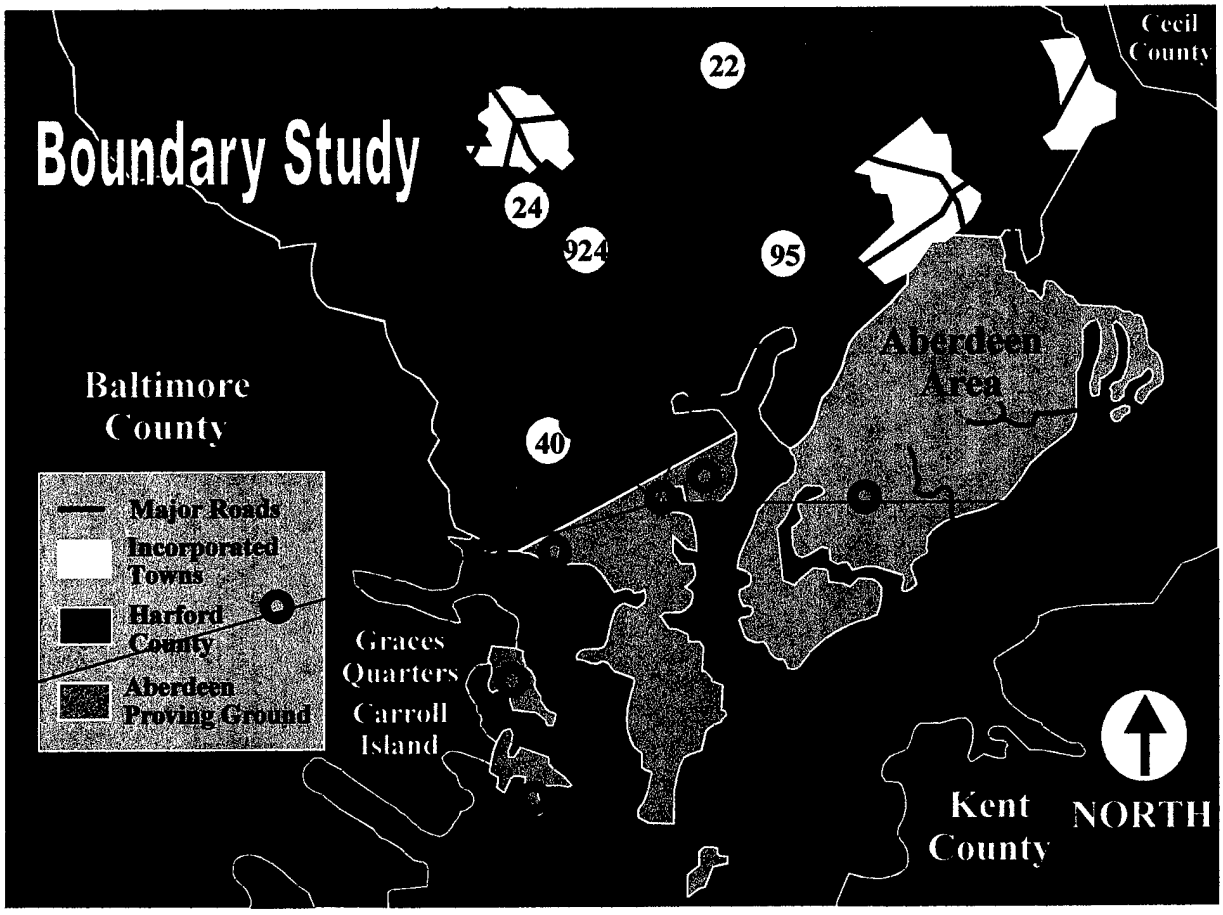


Figure 2.3 Closeup map of Boundary Sites, including APG sites. The terminal site for each transect does not appear on this map, refer to Figure 2.2 for an expanded view of the area.

2.4 SAMPLING FREQUENCY

Chemical sampling frequencies for VOCs and SVOCs varied from biweekly to longer periods, depending on individual locations and specific study objectives. Sampling for trace elements, heavy metals, and radionuclides was conducted twice—in the early summer following the spring rains and nectar flow, and again in late summer or early fall. Based on the results of the survey for bioavailable VOCs and SVOCs, hives at the ten primary Boundary Sites will be deployed and sampled again in 1999. Similarly, additional survey hives were added in midsummer to the sampling array at D Field. At both the Boundary locations and the D Field locations, hives were fitted with traps to periodically collect dead and dying bees to monitor for acute toxicity. Colonies were also being inspected and monitored for any symptoms of disease or other stress-related responses on a periodic basis. In all cases, inspection and sampling schedules were subject to weather—bee colonies can not be worked and sampled when it is raining.

Because VOCs and SVOCs are a principal focus of this biomontoring effort, the schedule of when samples were taken for these organic chemicals appears in the following Table 2.2.

TABLE 2.2 Hive- and Ambient-Air Sampling
1998 APG Field Applications

Site (Colony ID)	Sampling Dates
Old O Field	
OF1	@ 4/13, 5/18, 6/2, 7/15, 8/7, 9/3, 9/29
OF2	@ 4/13, 5/18, 6/2, 7/15, 8/7, 9/3, 9/29
OF3	@ 4/13, 5/18, 6/2, 7/15, 8/7, 9/3, 9/29
OF4	@ 4/13, 5/18, 6/2, 7/15, 8/7, 9/3, 9/29
OF5	@ 4/13, 5/18, 6/2, 7/15, 8/7, 9/3, 9/29
OF6	@ 4/13, 5/18, 6/2, 7/15, 8/7, 9/3, 9/29
OF7	@ 4/13, 5/18, 6/2, 7/15, 8/7, 9/3, 9/29
OFE1	@ 4/13, 6/2, 7/15, 8/7, 9/3, 9/29
OFE2	@ 4/13, 6/2, 7/15, 8/7, 9/3, 9/29
OF air	5/18, 6/2, 7/15, 8/7, 9/3, 9/29
OFE air	@ 4/13, 6/2, 7/15, 8/7, 9/3, 9/29

Site (Colony ID)	Sampling Dates
J Field	
JF1	@ 4/14, 5/18, 6/5, 6/27, 7/12, 8/8, 8/11, 8/29, 10/12
JF2	@ 4/14, 5/18, 6/5, 6/27, 7/12, 8/8, 8/11, 8/29, 10/12
JF3	@ 4/14, 5/18, 6/5, 6/27, 7/12, 8/8, 8/11, 8/29, 10/12
JF4	@ 4/14, 5/18, 6/5, 6/27, 7/12, 8/8, 8/11, 8/29, 10/12
JF5	@ 4/14, 5/18, 6/5, 6/27, 7/12, 8/8, 8/11, 8/29, 10/12
JF6	@ 4/14, 5/18, 6/5, 6/27, 7/12, 8/8, 8/11, 8/29, 10/12

Site (Colony ID)	Sampling Dates
JF7	@ 4/14, 5/18, 6/5, 6/27, 7/12, 8/8, 8/11, 8/29, 10/12
JFS1	@ 4/14, 5/18, 6/5, 6/27, 7/12, 8/8, 8/11, 8/29, 10/12
JFS2	5/18, 6/5, 6/27, 7/12, 8/8, 8/11, 8/29, 10/12
JFNL	5/18, 6/5, 6/27, 7/12, 8/8, 8/11, 8/29, 10/12
JFNM	5/18, 6/5, 6/27, 7/12, 8/8, 8/11, 8/29, 10/12
JFNR	5/18, 6/5, 6/27, 7/12, 8/8, 8/11, 8/29, 10/12
JF air	5/18, 6/5, 6/27, 7/12, 8/8, 8/11, 8/29, 10/12
JFS air	5/18, 6/5, 6/27, 7/12, 8/8, 8/11, 8/29, 10/12
JFN air	5/18, 6/5, 6/27, 7/12, 8/8, 8/11, 8/29, 10/12
D Field	
DF1.1	@ 4/13, 6/3, 8/9, 8/12, 9/30
DF1.2	@ 4/13, 6/3, 8/9, 8/12, 9/30
DF1.3	8/9, 9/30
DF1.4	8/9, 9/30
DF1.5	8/9, 9/30
DF1.6	8/9, 9/30
DF1 air	6/3, 8/9, 8/12, 9/30
DF2.1	@ 4/13, 6/3, 8/12, 9/30
DF2.2	6/3, 8/12, 9/30
DF2 air	6/3, 8/12, 9/30
DF3.1	@ 4/13, 6/3, 8/12, 9/30
DF3.2	6/3, 8/12, 9/30
DF3 air	6/3, 8/12, 9/30
DF4.1	@ 4/13, 6/3, 8/9, 8/12, 9/30
DF4.2	6/3, 8/9, 9/30
DF4.3	8/9, 9/30
DF4.4	8/9, 9/30
DF4.5	8/9, 9/30
DF4.6	8/9, 9/30
DF4 air	6/3, 8/9, 8/12, 9/30
DF4 air 2	8/9, 9/30
DF5.1	@ 4/13, 6/3, 8/12, 9/30
DF5.2	@ 4/13, 6/3, 8/12, 9/30
DF5 air	6/3, 8/12, 9/30
Boundary Survey Sites	
North Transect	
Youth Center 1 (#303)	6/9, 8/13, 10/22
Youth Center 2 (#388)	6/9, 8/13, 10/22
Youth Center 3	8/13, 10/22
Youth Center air	6/9, 8/13, 10/22

Site (Colony ID)	Sampling Dates
North Transect (cont.)	
Cluster 13 Hive 1	6/9, 8/11, 10/22
Cluster 13 Hive 2	6/9, 8/11, 10/22
Cluster 13 air	6/9, 8/11, 10/22
Otter Point Creek 1	6/9, 8/10, 10/22
Otter Point Creek 2	@ 4/13, 6/9, 8/10, 10/22
Otter Point Creek air	6/9, 8/10, 10/22
Fragmentation Pit 1	6/9, 8/10, 10/22
Fragmentation Pit 2	6/9, 8/10, 10/22
Fragmentation Pit air	6/9, 8/10, 10/22
Lohr's Orchard 1	6/9, 8/10, 10/22
Lohr's Orchard 2	6/9, 8/10, 10/22
Lohr's Orchard air	6/9, 8/10, 10/22
Conowingo Orchard 1	6/9, 8/10, 10/22
Conowingo Orchard 2	6/9, 8/10, 10/22
Conowingo Orchard air	6/9, 8/10, 10/22
Northwest Transect	
Jones Farm 1	7/7, 8/13, 10/22
Jones Farm 2	7/7, 8/13, 10/22
Jones Farm air	7/7, 8/13, 10/22
Tower Hill Farm 1	7/7, 8/13, 10/29
Tower Hill Farm 2	7/7, 8/13, 10/29
Tower Hill air	7/7, 8/13
Farview Manor Nursery 1	7/7, 8/13, 10/29
Farview Manor Nursery 2	7/7, 8/13, 10/29
Farview Manor Nursery air	7/7, 8/13
Southwest Transect	
Westwood Road 1	7/7, 8/11, 9/29
Westwood Road 2	7/7, 8/11, 9/29
Westwood Road air	7/7, 8/11, 9/29
Rumsey Mansion 1	6/18, 8/11, 10/22
Rumsey Mansion 2	6/18, 8/11, 10/22
Rumsey Mansion air	6/18, 8/11, 10/22

Site (Colony ID)	Sampling Dates
Southwest Transect (Cont.)	
Graces Quarters 1	6/18, 8/11, 10/22
Graces Quarters 2	6/18, 8/11, 10/22
Graces Quarter air	6/18, 8/11, 10/22
Carroll Island 1	6/18, 8/7, 8/10, 10/22
Carroll Island 2	6/18, 8/7, 8/10, 10/22
Carroll Island air	6/18, 8/7, 8/10, 10/22
Silver Lake Drive 1	6/18, 8/14, 10/29
Silver Lake Drive 2	6/18, 8/14, 10/29
Silver Lake Drive air	6/18, 8/14
Cylburn Arboretum 1	6/18, 8/14, 10/29
Cylburn Arboretum 2	6/18, 8/14, 10/29
Cylburn Arboretum air	6/18, 8/14
Churchville Reference Site	
CV1	4/25, 7/7, 8/13, 10/27
CV2	4/25, 7/7, 8/13, 10/27
CV3	4/25, 7/7, 8/13, 10/27
CV4	4/25, 7/7, 8/13, 10/27
CV5	4/25, 7/7, 8/13, 10/27
CV6	4/25, 7/7, 8/13, 10/27
CV7	4/25, 7/7, 8/13, 10/27
CV Air	4/25, 7/7, 8/13, 10/27

2.5 MEASUREMENT AND ANALYTICAL METHODS

Colony behavioral assays and chemical sampling and analysis were used to assess the bioavailability and hazard of chemical agents to honey bees for site-to-site comparisons with respect to characterizing ecosystem exposures.

Measurement, sampling, and analytical methods are fully described in the previous annual reports (Bromenshenk *et al.*, March, 1996 and March, 1997). These measurement and assessment methods can be divided into two categories:

- Real-time colony behavioral response measurements
- Chemical exposure sampling and analysis.

2.6 COLONY BEHAVIORAL ASSAYS

This study uses honey bee "condos" that bear little resemblance to conventional beehives. Condos are sets of tilt-top boxes outfitted with electronic sensors and chemical probes, provided

with electric fans and water misters, equipped with a digital weather station, connected to a bank of computers, and transportable on a tandem-axle trailer (Figures 2.4a and 2.4b). These systems, each containing a small beehive, offer rapidly deployable, automated, biological units for continuously assessing air quality and terrestrial environments. The bee colonies monitor environmental hazards (i.e., effects associated with exposures to hazardous chemicals, if any), collecting bioavailable contaminants, and respond by their behavior to changes in their environment.

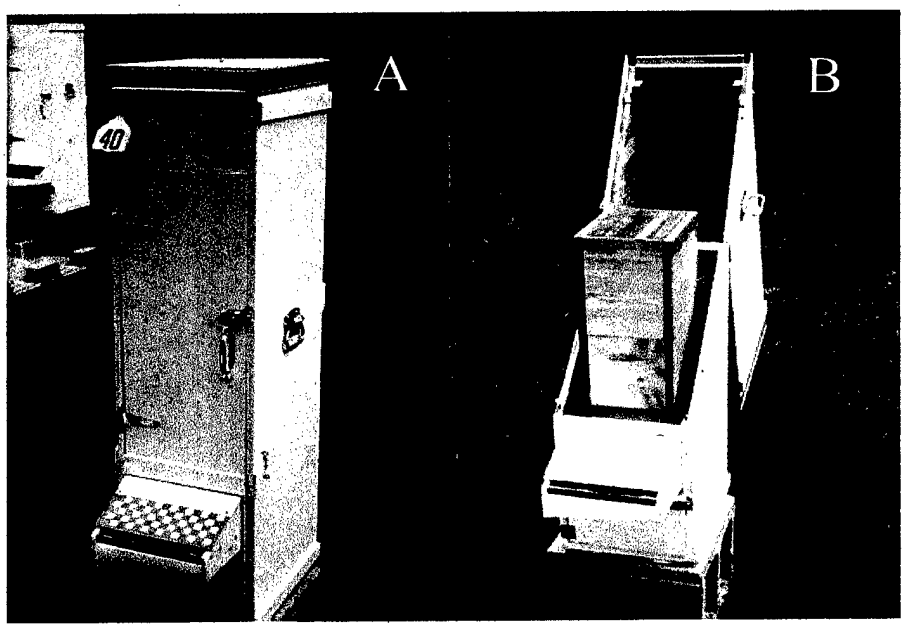


Figure 2.4a and 2.4b. Honey bee condo. A. Closed. B. Flip-top was opened to reveal internal hive components.

Each bee leaving or entering a condo passes through an infra-red counter (Figure 2.5) mounted on the front of the condo box (Figures 2.4a and b). Integrated circuit-based sensors continuously monitor temperatures in the brood nest and other parts of the hive. A trap under each hive collects any dead or dying bees cast out by undertaker bees. Digital pumps pull samples of air from inside the hive. Small numbers of returning forager bees are periodically vacuumed from the hive entrance and frozen for chemical analysis. A plastic screen scrapes pollen from incoming bees and drops the sample into a clock-driven tray.

For the purposes of this study, the term survey hive is applied to a hive lacking electronics. Survey hives can be fitted with pollen collectors, traps to collect dead bees, and probes to sample the air inside the hive.

2.6.1 Real Time Data Delivery: The honey bee condos continuously log colony behavioral response data. For the present study, electronic hives were deployed at J Field, Old O Field, and at the Churchville and Montana reference sites. Real-time data delivery via the Internet was accomplished in the fall of 1997 and was utilized in 1998. The bee counter data acquisition software can communicate via the RS-232 serial port to a computer equipped to receive data

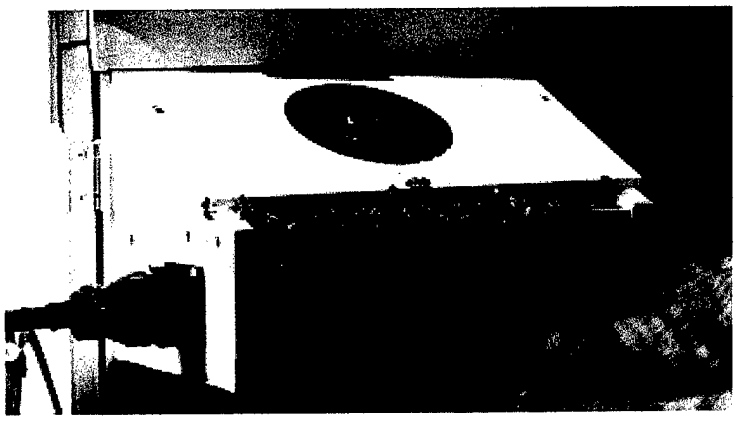


Figure 2.5 Bidirectional forager bee counter.

summaries on a 15 minute interval. This time interval was chosen to minimize the bandwidth needed to transfer the field data. At each field location, a PC running a Linux operating system acted as a server to transfer data from all other computers at the site (i.e., weather conditions, hive parameters such as temperature) to a central server in the USA CEHR Administrative Trailer at the APG/Edgewood site (using wireless modems). Due to radio noise at APG, the wireless system was not dependable and data had to be manually transferred from the Old O Field and J Field PCs to the Administrative Trailer server. By July 1, 1999, direct access to J Field via telephone or a fiber optics connection should be feasible. The central server in the Admin Trailer transferred the field data to the data processing and storage facilities at The University of Montana in Missoula. A telephone connection between the Churchville site in Maryland and the UM server facilitated direct access to the Maryland reference site.

An on-line observation hive at The University using this same technology can be accessed via the Internet:

(http://www.umt.edu/biology/bees)

The observation hive has been on-line for over a year. The only down time has been when the system was intentionally taken off-line to update software. This observation hive provides a working example of the real-time data delivery capability and the data formats.

All APG data from this study and ongoing applications is secured and restricted to access by authorized users only.

2.6.2 Numeric Data Processing: The honey bee flight data provides a large number of behavioral metrics that reflect the immediate and longer-term condition of each colony and of the colonies at each site. Short-term changes in the rate of outgoing or incoming bees occur in response to disturbances ranging from chemical exposures, weather, inspection of the hives, or natural processes such as swarming by a colony. Longer term trends may reflect growth or

decline of a colony or of the colonies at a site. A key metric is the percent of bees that return to the hive each day. Although a few bees will die of old age, fall victim to predation, get lost, or join another colony, a significant failure of bees to return to the hive usually only occurs if:

- The colony swarms (which can be identified by a decrease in colony core temperatures, loss of the original queen from a strong colony, and queen cells and workers left in the hive to re-start the colony). This is the normal way in which a colony reproduces.

- The colony absconds (all of the bees leave with the queen). This situation usually occurs in response to severe stress (i.e., the colony gets too hot or is fleeing from some other event that could threaten the viability of the colony such as heavy infestations of mites or exposures to specific chemicals.

- The forager bees encounter and accumulate toxic doses of a poisonous substance such as a pesticide or environmental contaminant. In this case, bees in the dead bee trap can be analyzed for body residues and the hive sampled to investigate and identify the harmful agent.

Whereas events such as emergence of a swarm or exposure to an acutely toxic substance can easily be seen on the computer display or printouts of the flight activity charts, many events of interest may be of short duration or difficult to discern among all of the data points generated by real-time monitoring. To effectively process and flag these events, a set of custom numerical processing software utilities named "Siteview" was utilized. Siteview was developed at The University of Montana and is distributed on CD ROM with the real-time data sets as part of the Reporting Methods employed for this study.

2.6.3 Artificial Neural Networks (ANNs): Because bee colonies behaviorally respond to weather conditions and other events such as inspection of the hive, specific tools are needed to distinguish possible exposure events from these more routine events. ANNs were used to accomplish this task. A recently completed M.S. thesis project conducted at the University of Montana has provided a customized ANN that has proven successful in evaluating bee flight activity and flagging atypical behavior.

At its most basic level, an ANN is a computer program designed to recognize patterns. For example, a speech recognition ANN can be trained to learn the wave form of the word "hello." Later, when the ANN finds a wave form similar to "hello," it may produce a match with the word hello.

In application, the ANN is trained on the flight activity of the hives deployed at each of the study sites. Weather data and other hive information such as colony core temperatures are used as part of the training set. Within a few days, the ANN can begin to pick out deviations from expected activity patterns for each colony and for each site.

2.7 VOLATILE/SEMI-VOLATILE ORGANIC CONTAMINANTS IN HIVE ATMOSPHERES

Heavy metals, other inorganic elements of concern (Be, As and Se), radionuclides and polychlorinated biphenyls (PCB's) were assayed from whole bees and pollen samples. Heavy metals and other inorganics were analyzed by inductively coupled plasma mass spectrometry (ICP/MS) by the chemical analysis laboratory at USA CEHR. Radionuclide analysis were performed by gamma spectroscopy.

2.7.1 Hive Atmospheres: All of the electronic and survey colonies of honey bees were monitored for volatile and semi-volatile organic compound residues by pulling hive atmospheres through chemical traps and, subsequently, thermally desorbing them into a gas chromatograph/ mass spectrometer (TD/GC/MS). Most of the electronic hives were sampled for these chemicals as often as every two weeks. Survey hives were sampled two to three times during the growing season. Detailed methods for sampling and analysis have been presented in our previous reports (Bromenshenk *et al.*, 1996 and 1997). Recent modifications and upgrades of these methods are detailed in Section 4. An important change instituted in early summer of 1998 was the use of a multi-bed sampling system to remove excess moisture and high molecular weight compounds such as terpenes, resulting in fewer lost samples, reduced interferences, and markedly less wear-and-tear on the GC/MS instrument.

2.7.2 Trace Element and Metals Sampling and Analysis: Two rounds of whole bees and pollen were analyzed for their heavy metal and inorganic content. Live bees samples with 100 to 150 individuals were collected via a hand-held vacuum with a PVC nozzle that trapped returning forager bees in a plastic bag (Whirl-Pak). Bee samples and pollen were kept on ice (i.e., frozen gels) in the field and then frozen and stored at -4°C until analysis. Dead bees were obtained from passive traps placed under hives. Pollen samples were taken by inserting a plastic scrapper screen under each hive and trapping pollen pellets knocked off the legs of incoming forager bees.

Whole bees and pollen were analyzed for Be, V, Cr, Mn, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Cd, Cs, Ba, Tl, Pb, Bi and U. Bee and pollen samples were oven-dried in Acid-washed, covered glassware at 45°C for about 10 days. Dried bees and pollen were then ground in a Wiley mill to pass a 20-mesh screen.

For sample preparation, a 0.5 gram portion of each sample was transferred to a digestion vessel with 10 mL of trace metal grade nitric acid (Fisher) and processed in a CEM MDS-2000 microwave digestion oven (40% power to 20 psi in 10 min, 5 min at 20 psi, 50% to 40 psi in 10 min, 5 min at 40 psi, 50% to 80 psi in 10 min, 5 min at 80 psi, 40% to 100 psi in 10 min, 5 min at 100 psi). Following digestion, deionized water was added to bring total volume to 50 mL.

A Hewlett Packard 4500 Inductively Coupled Plasma-Mass Spectrometer was used to determine the trace metal concentrations. Peak intensities were measured against internal standards. A 1 ppm multi-element standard was used as a laboratory control spike. Recovery was evaluated from 1-mL additions of the standard to 0.5 gram dried samples prior to the acid digestion step.

Blind duplicates, blanks, spiked samples and knowns were interspersed with unmodified bee and pollen digests to provide quality assurance. Metal (Ba, Bi, Cd, Co, Cs, Cr, Cu, Ga, Mn, Ni, Pb, Rb, Sr, Tl, U, V and Zn) and inorganic element (Be, As and Se) concentrations were quantified with the inductively coupled plasma mass spectrometer.

We also required that QA/AC data relevant to instrument calibration be supplied to us along with the sample results.

2.7.3 Radionuclides: Two to five grams of finely ground and compressed whole bee tissue or pollen were analyzed by gamma spectroscopy by a subcontracting laboratory following a protocol developed for radionuclide analysis (in bees and pollen) by the U.S. Department of Energy's Idaho Operations Office Radiological and Environmental Sciences Laboratory. The estimated random uncertainty reported was one standard deviation (1 SD). Small negative and other results less than or equal to 2 SD were interpreted as including "zero" or as not detected. For results greater than 2 SD but less than or equal to 3 SD, detection was considered to be questionable. Results greater than 3 SD indicated detection. 0 was the estimated overall uncertainty.

SECTION 3 MONITORING OF COLONY BEHAVIORAL RESPONSES TO CONTAMINANTS, WEATHER, AND OTHER ENVIRONMENTAL FACTORS

3.1 Colony Dynamics and Behavioral Responses

For this project, colony response metrics were measured in real-time at a variety of field sites. These measurements were guided by two overall objectives:

- Real-time monitoring of colony population dynamics to establish the relationship between acute exposures to specific chemical agents and measured behavioral endpoints; and

- Site-to-site comparisons of honey bee colony populations with respect to the effects of chronic as well as acute ecosystem exposures to bioavailable chemical agents.

We have made considerable progress toward accomplishing automated, real-time monitoring of honey bee colony performance. During the summer of 1996, 21 electronically-equipped, mini-hives containing nucleus (small) colonies of bees were deployed at two Aberdeen Proving Ground (APG) sites (i.e., West Branch Canal Creek and Old O Field) and at a rural Maryland reference site near Churchville. Another set of seven electronic hives were established in 1997 at UM's Montana reference site. In 1998, colonies were deployed at Old O Field, J Field, and Churchville in MD and UM's Montana site. Distance delivery via the Internet of the data from electronic hives deployed in Maryland and Montana was accomplished in 1998. Distance or automated (i.e., controlled by computer feedback from colony performance data) sampling of volatile (VOC) and semi-volatile (SVOC) chemicals became possible in 1999. Also, in 1998, exposure of colonies to methyl parathion in different forms and concentrations was used to characterize acute toxicity as reflected by electronic, real-time monitoring, of colony response metrics.

All of these electronic hives were equipped with: (1) sensors that continuously measured several colony performance parameters, (2) sorption traps that sampled hive atmospheres over 8-10 hour periods, and (3) in-hive traps that continuously collected pollen and dead bees. The field trials produced an extensive data set needed to: (1) determine the sensitivity, variability, and usefulness of several population-level, toxicity assessment endpoints, (2) further develop and refine models of honey bee population dynamics, and (3) conduct ongoing hazard assessments at APG.

3.2 Materials and Methods

Design and construction of the electronic bee-counters and other hive sensors was described in detail in previous reports submitted to the Army (Bromenshenk *et. al.*, 1997, 1998). The 1998 report added the methods for data delivery via the Internet and for the use of Artificial Neural Networks and numeric processing software to process and interpret colony response data. Two recent publications cover these procedures in detail (Seccomb, 1998 and King, 1998). An example of Internet data delivery can be seen by logging into the on-line observation hive at The University of Montana at http://www.umt.edu/biology/bees.

3.3 Results of Tier 1 Evaluations of Flight Activity Data

Tier 1 flight activity analysis included total flight activity (TFA), inter-colony coefficient of variation (C.V.) of total activity, the percentage of bees returning to the colonies at each site at the end of the day (PRC), the net loss (NL) of bees at the end of the day, and the adjusted net loss (ANL) of bees at the end of each day.

Flight activity data collected during the Spring, Summer and Fall of 1998 was summarized and compared with data from the 1996 and 1997 field monitoring seasons. Figure 3.1 presents Tier 1 summaries comparing the corrected (i.e., adjusted for the number of sampling periods and number of bee-counters being monitored) total daily flight activity throughout the season for each of the test sites. A detailed summary of events that occurred during the 1996 and 1997 field monitoring seasons was presented in previous annual reports (Bromenshenk *et al.,* 1997, 1998).

In general, flight activity at all of the APG sites displayed similar day to day seasonal trends, believed to a consequence of colony responses to local weather conditions. All sites showed a similar seasonal trend characterized by high activity during the summer, a slow decline in activity as summer progressed into fall, and then very little activity after the middle of October.

Coefficients of variation of flight activity among the colonies at a site for the 1998 monitoring season also were plotted and compared. As seen in Figure 3.2, the C.V. for J Field and Old O Field sites remained near or below 50% until mid-July and then slowly crept up to 75%. The spike on July 8th for all of the APG sites was due to a rain event. The large hump visible in the C.V. from mid-May to early June at the Churchville reference site is due to a single colony having unusually high flight activity compared to the other colonies at the site.

From mid-May on, the total flight activity (Figure 3.3) at Churchville was lower than at the other Maryland sites, probably as a consequence of limited food resources. Although one might expect the colonies to increase flight activity in response to food shortages, in previous studies we found that colony's conserve energy and don't fly, if little or no food is available. In addition, heavy rain stops bee flight. The Churchville hives grew rapidly in size from April through early May, then fell off quickly in size during the rainy period and had to be fed to prevent starvation.

Power failures, usually following thunderstorms, continued to plague all of the Maryland sites as evidenced by data gaps when the electronic systems were off-line. Storms also suppress flight activity. The decrease in C.V. at Fort Missoula from mid-April to mid-May is due to seasonal changes, i.e. increased flight activity as ambient temperature increases. The large humps in C.V. at Fort Missoula in late-July and late-August were due to dose/response experiments conducted with methyl parathion, a chemical that is acutely toxic to bees.

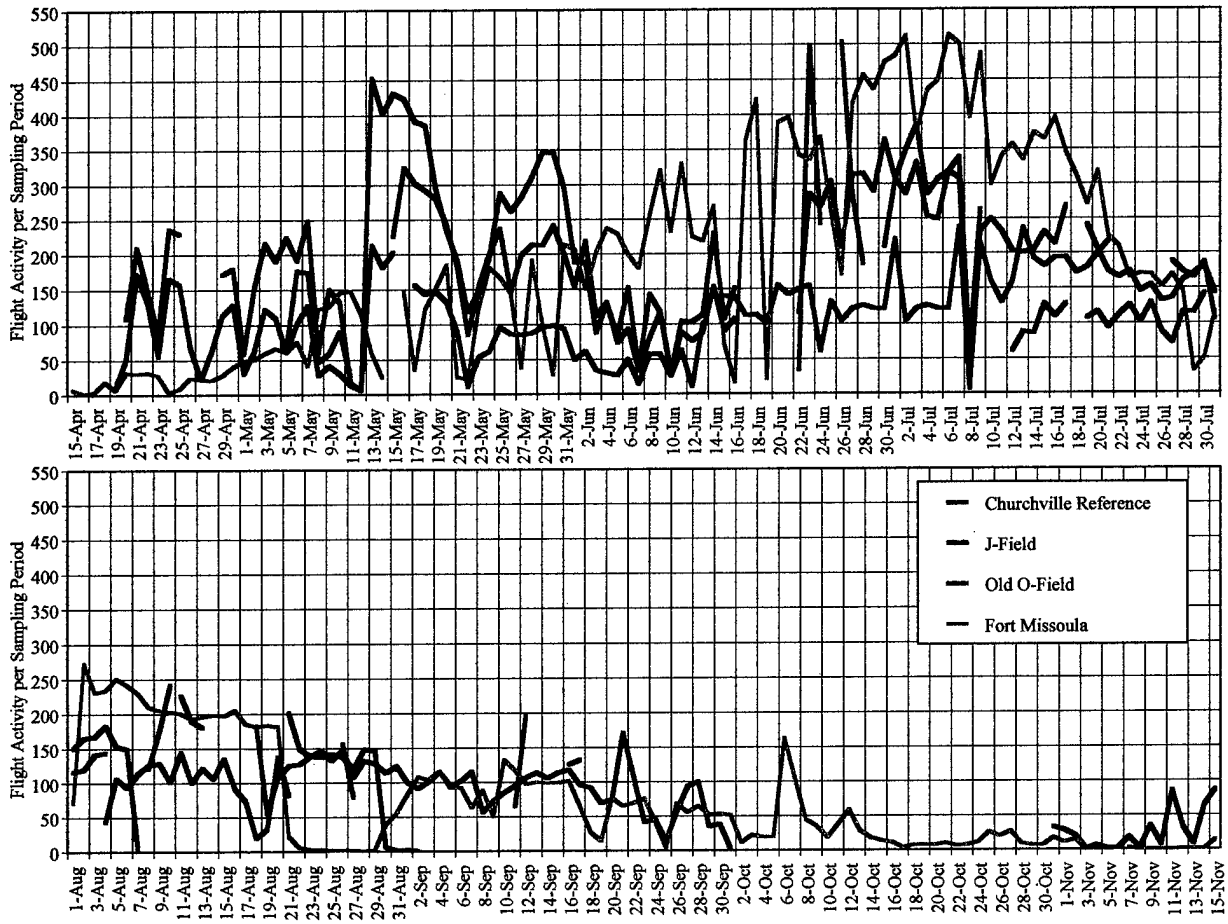


Figure 3.1 1998 APG site by site comparisons of adjusted total daily flight activity (entering + exiting) corrected for number of 30 second sampling periods collected per day.

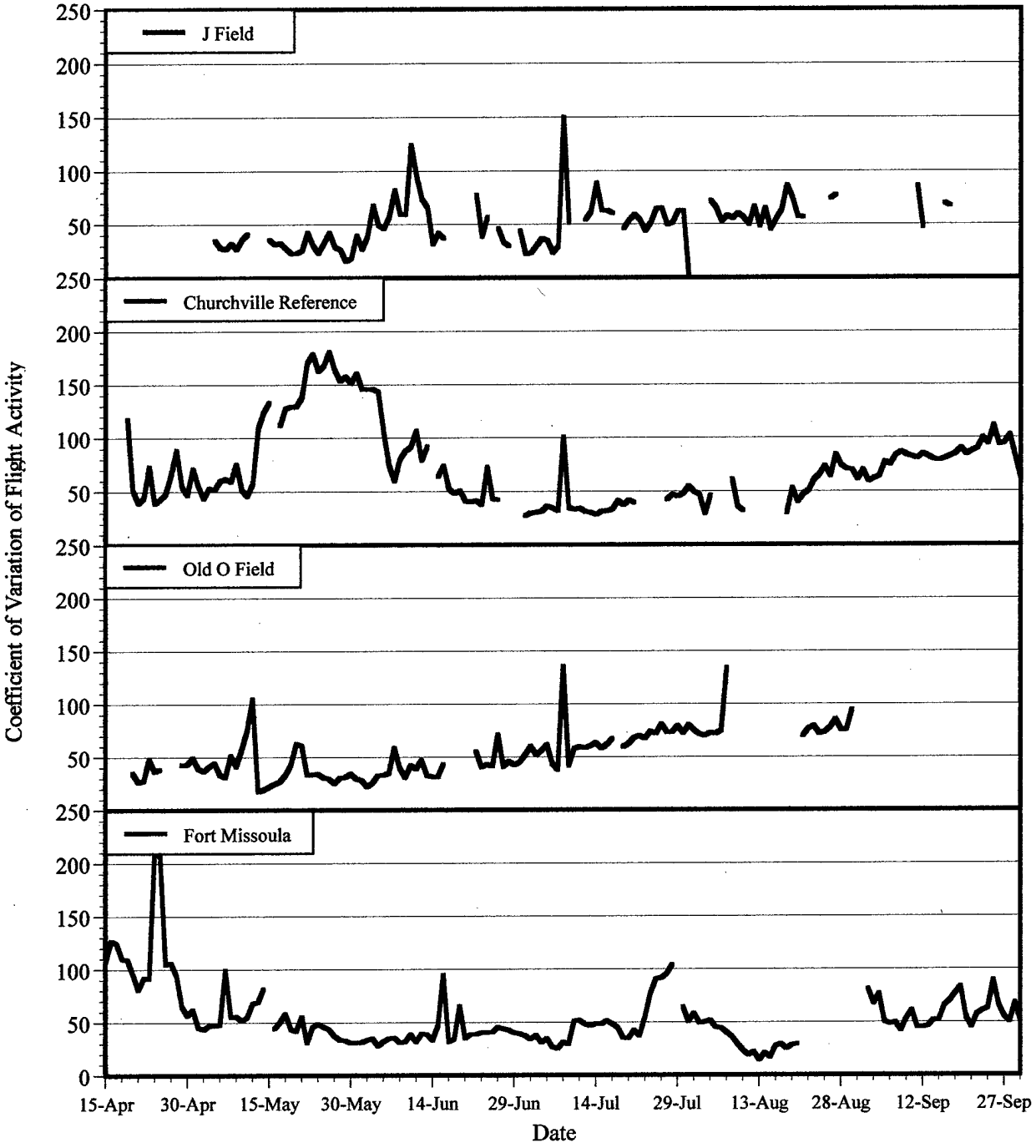


Figure 3.2 Site by site comparisons of coefficients of variation throughout the 1998 spring, summer, fall season. In 1998, the Churchville reference site experienced severe shortfalls in food resources and the bees had to be fed. These shortfalls were intensified by heavy rains that further suppressed foraging in May. The July 8 spike was induced by rainy weather experienced by all of the Maryland sites.

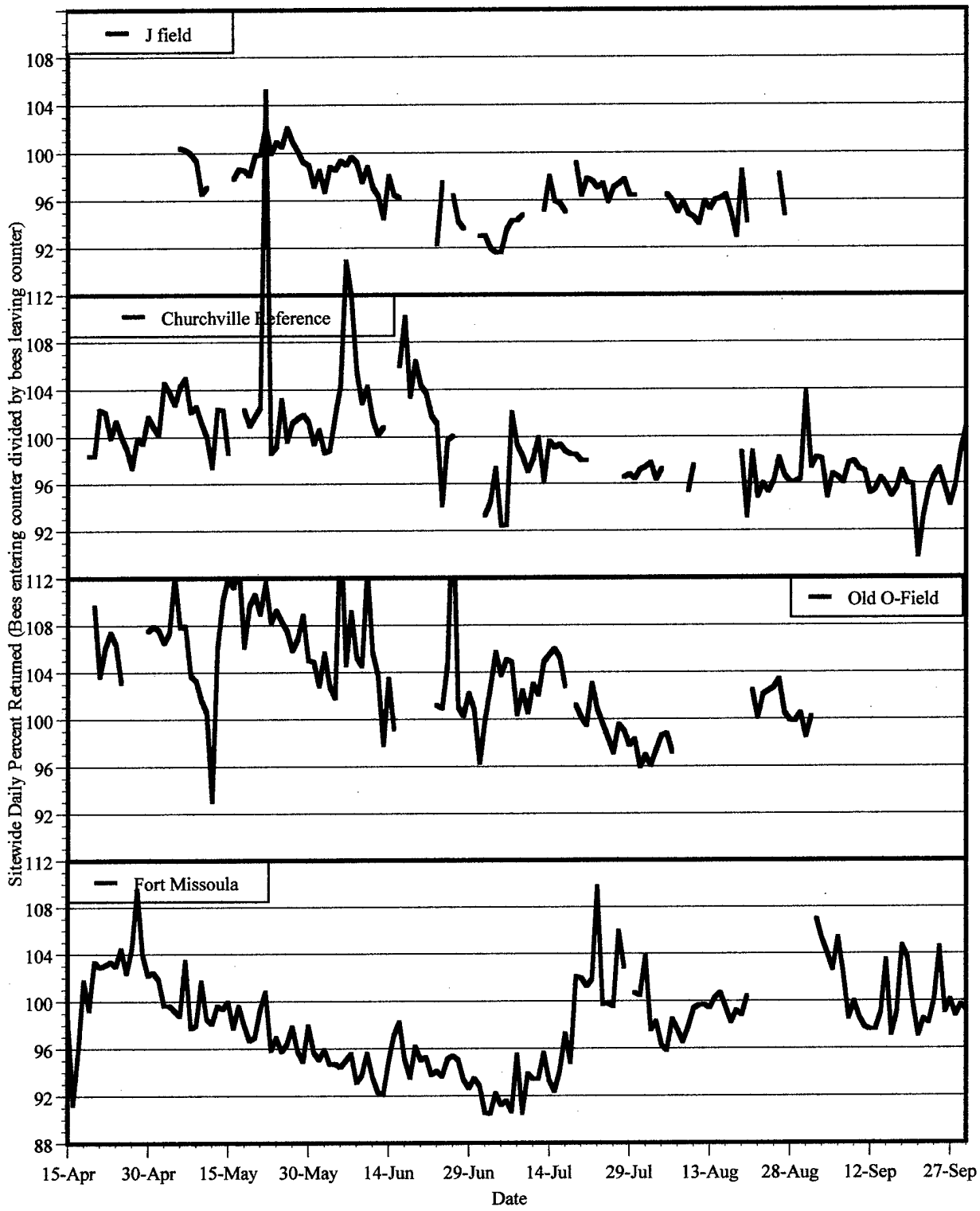


Figure 3.3 Cumulative Percent Return by site, spring, summer, fall, 1998

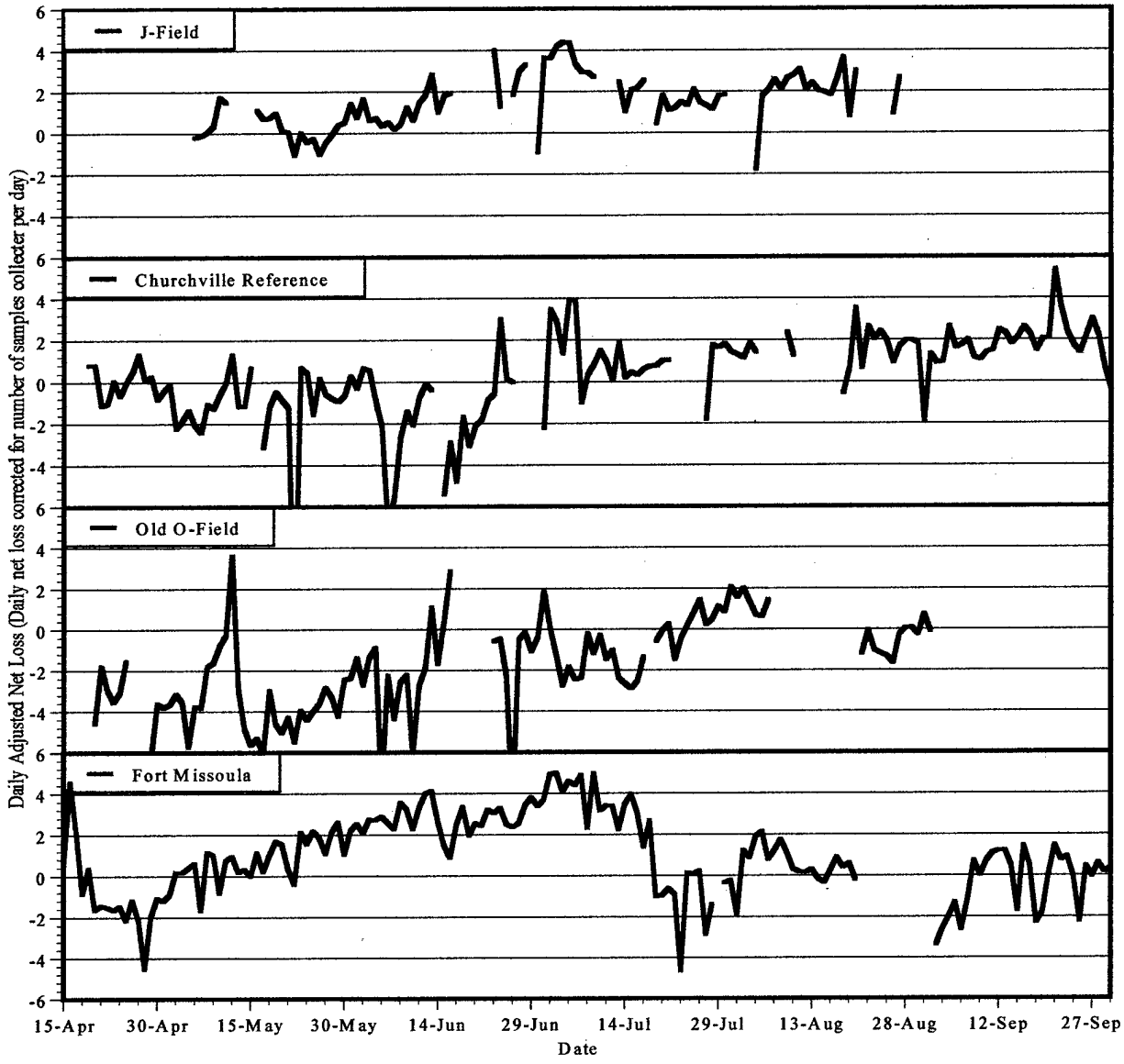


Figure 3.4 Site by site comparison of Adjusted Net Loss. Net loss is corrected for number of samples collected per day.

The percentage of bees returning to all of the hives at a site at the end of the day was calculated by the site-wide PRC analysis (Table 3.1). This metric provided an indication of the general health of the colonies at each site. Figure 3.3 illustrates the percent bees returning to the hive by the end of the day for all sites monitored in 1998.

Table 3.1

Percent Return to Colony, 100% Equals Return of All Bees to the Colony

PRC values reported represent "normal" observed colony return rates. Transient maximums induced by identified events such as severe rainstorms, swarming, power outages, or dose/response (toxicity) experiments at Fort Missoula have been removed from this data set. Sites not monitored in a given year are indicated by a dash in the appropriate cell.

Site		PRC		
		1996	1997	1998
Canal Creek	CC	90%-108%	94%-98%	-
Old O Field	OF	90%-102%	94%-104%	96%-112%
Churchville	CV	92%-100%	94%-98%	94%-105%
J Field	JF	-	92%-98%	92%-103%
Fort Missoula	FM	-	94%-108%	90%-104%

Generally, for J Field and the Churchville reference site, the sitewide PRC results stayed within the range of 92% to 105% during the foraging season. The site-wide PRC at Old O Field varied from 90% to 112% during the foraging season. The somewhat higher PRC at this site, compared to the other two Maryland sites, was due in part to warping of the floor of a counter on one of the hives. This warping allowed some of the outgoing bees to bypass the counter when leaving the hive. Incoming bees returned to the hive through the counter, consistently producing a PRC greater than 100% for this hive. The spike at Churchville in May reflects the food resource shortage and rain events experienced at this site in 1998.

The sitewide PRC at Fort Missoula varied between 90% and 108%. The extreme peaks in late-July and late-August corresponded with the periods following each of the methyl parathion dose/response experiments. After each trial, treated hives were removed from the condos and not replaced with new hives until some days later. Apparently, some of the field bees were not in their hives when the hive boxes were taken out of the condos. These field bees returned to empty condos, resulting in pulses of incoming bees with few or no outgoing bees.

Table 3.2 provides a comparison of Tier 1 values (coefficients of variation) for each site for 1996, 1997, and 1998. Bees were not deployed at J Field (JF) and Fort Missoula (FM)

(Montana) in 1996 or at Canal Creek (CC) in 1998, which explains the cells marked by a dash in Tables 3.1-3.3.

Table 3.2

Comparison of Tier 1 (Site-Wide) Metrics, Coefficients of Variation

Percent values reported represent "normal" observed colony variability. Transient maximums induced by identified events such as severe rainstorms, swarming, power outages, or dose/response (toxicity) experiments at Fort Missoula have been removed from this data set. Sites not monitored in a given year are indicated by a dash in the appropriate cell.

Site	C.V.		
	1996	1997	1998
Canal Creek CC	15%-60%	20%-50%	-
Old O Field OF	50%-130%	25%-75%	20%-80%
Churchville CV	50%-90%	15%-50%	30%-100%
J Field JF	-	15%-50%	20%-80%
Fort Missoula FM	-	30%-140%	20%-100%

In 1996, coefficients of variation for flight activity among all of the colonies at each Maryland site were highest at the Old O Field landfill, followed by the Churchville reference site. That same year, following a removal action in 1996, the C.V.'s for Canal Creek on the Edgewood Post were considerably lower than at the other two sites. In 1997, C.V.s at all three MD sites were similar, with lows of 15-25% and highs of 50-75%; a considerable improvement over the 1996 lows of 50% and highs of 90-130%. In 1998, C.V. values for the Maryland sites were again similar, although somewhat higher than in 1997. The low values ranged from 20-30%; the highs 80-100%. In 1998, the highest C.V.s for flight activity were observed at the Churchville reference site. C.V.s of 30-140% at the Fort Missoula research site reflected the numerous experiments conducted at the site as well as replacements of colonies after the dose/response trials.

PRC results from the 1997 compared to the 1996 monitoring season showed higher return rate and a smaller range of variation for all of the Maryland sites. For example, in 1996 West Branch Canal Creek colonies displayed PRCs ranging from 90-108%. In 1997, both West Branch Canal Creek and J Field colonies had PRCs of 94-98%. Similarly, the Churchville reference site bees changed from 92-100% in 1996 to 94-98% in 1997 (with the exception of the day of the storm). Over all three years, we observed some drift among Old O Field hives, with bees from weak colonies joining stronger colonies. This drift contributed to PRC values in excess of 100%. In addition, as mentioned before, in 1998, some of the bees exiting one of the Old O Field hives managed to avoid going through the counter. The most important finding of the PRC analysis is

that in general, in 1997 and 1998, 92% or more of the bees leaving each hive returned each day. This also can be seen in Figure 3.4, which presents site by site comparisons of Adjusted Net Loss. Based on five year's of data from Maryland and Montana, these return rates are typical of colony's judged to be healthy with no evidence of visible disease symptoms and no evidence of exposure to chemicals known to be toxic. Based on our prior modeling of honey bee populations and calculations of the average life of a forager bee, which is relatively short (i.e., a few days), some bees are expected to die of old age, get lost, or be eaten by predators. The 92% return rate agrees with the losses expected from natural sources of forager bee removal from the population. Finally, our methyl parathion trials clearly show short term depressions in return rates far in excess of 8%. Therefore, there was no evidence of any acute toxicity at any of these sites.

Table 3.3

Adjusted Net Loss, 0 Equals No Loss of Bees Returning to the Colony

ANL values reported represent "normal" observed colony return rates. Transient maximums induced by identified events such as severe rainstorms, swarming, power outages, or dose/response (toxicity) experiments at Fort Missoula have been removed from this data set. Sites not monitored in a given year are indicated by a dash in the appropriate cell.

Site	ANL		
	1996	1997	1998
Canal Creek CC	-4 to +4	0 to +5	-
Old O Field OF	-2 to +6	-2 to +3	-6 to +2
Churchville CV	0 to +4	0 to +3	-2 to +3
J Field JF	-	+1 to +5	-1 to +4
Fort Missoula FM	-	-4 to +3	-2 to +5

Finally, as seen in Table 3.3, variation in adjusted net loss by the end of each day improved dramatically in 1997 compared to 1996, but declined somewhat in 1998, mainly at Old O Field. This finding was in part an artifact of the trapping of outgoing bees in one of the counters. For 1999, the counter assemblies have been fitted with clear Lucite™ coverings, so that any entrapment of bees can be easily seen and corrected.

3.4 Results of Tier 2 Evaluations of Flight Activity Data

Daily flight activity data from individual colonies were compared with data from other colonies at each site. Sites that were part of a continuing study were compared to results from the

previous year using Tier 2 analysis methods. Figures 3.5 - 3.8 present total daily flight activity for the sites under study.

Variations in flight activity can be quantified as C.V.s among hives (Tier 1) or graphically visualized for individual hives (Tier 2) by using normalized flight activity color maps for each site as displayed in Figures 3.9-3.12. This analysis provides a visual benchmark on which to compare colonies based on the assumption that all colonies should contribute an equal amount to site-wide flight activity if the colony's are well matched in terms of population size and if weather and food resource conditions are optimal.

Colonies at the Churchville reference site displayed some obvious variance in total flight activity during 1998 as shown in Figure 3.5. Flight activity for colony #5 increased significantly from mid-May to mid-June. In late August, colony #1 experienced a decrease in flight activity compared to other colonies.

In Figure 3.7, two colonies (#3 and #4) at J Field displayed a higher than average contribution to the site-wide total flight activity, while colony 1 exhibited somewhat decreased levels of flight activity. While strong, vigorously flying colonies were common at this site both in 1996 and 1997, in 1998 the colonies demonstrated decreased flight activity levels.

At Old O Field in 1998, as shown in Figure 3.6, colonies #4, #6 and #7 exhibited high contributions to the site-wide total flight activity for the entire season. Colonies #1 and #2 both swarmed on May 18 and July 1, respectively. Colony #6 demonstrated bearding behavior from early to mid-July.

Figure 3.12 displays the normalized flight activity map for the Fort Missoula site. On July 20 the colonies were exposed to methyl parathion placed in the hive as Crisco patties. This experiment was also repeated on October 7. In both cases the treated colonies exhibited significant amounts of bees collected in the dead bee traps. While the number of dead bees collected in the traps showed an immediate increase, as shown in Figure 3.14, the flight activity levels gradually decreased for the treated colonies (Figure 3.15-3.16).

In general, colonies at all sites displayed lower levels of activity during the 1998 flight season than the 1997 flight season, although these levels were still higher than the 1996 flight season. Increased variability in total flight activity among the hives can be seen at most sites during the 1998 flight season, compared to 1997. Colonies at the Churchville reference site showed a much lower level of flight activity, and a slightly higher degree of variability than the previous year. At Old O Field, the flight activity was slightly lower and the degree of variability among hives was the same as the previous year until the end of June when the variation increased.

Finally, from May 26 to May 29, the colonies at J Field displayed flight activity patterns that were very similar to a brief smoke event. For the first three days these occurred in the morning at 9:35, 9:45, and 10:15 respectively. On May 29, the event occurred at 13:00 hours. This could be due to heavy machinery working in the vicinity of the condos and exposing the bees to exhaust fumes.

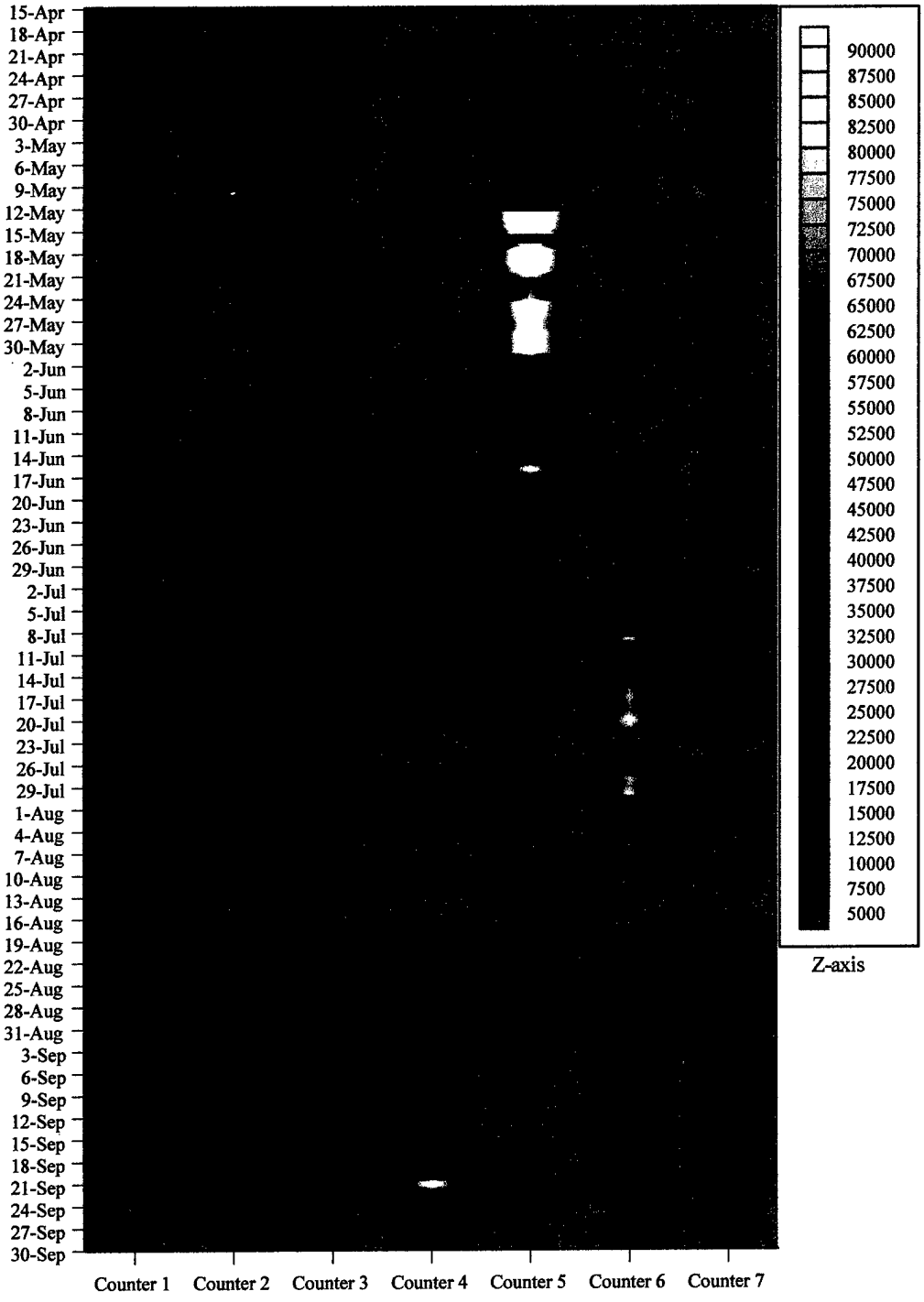


Figure 3.5 Total flight activity for colonies at the Churchville reference site, 1998. Hotter colors denote higher activity.

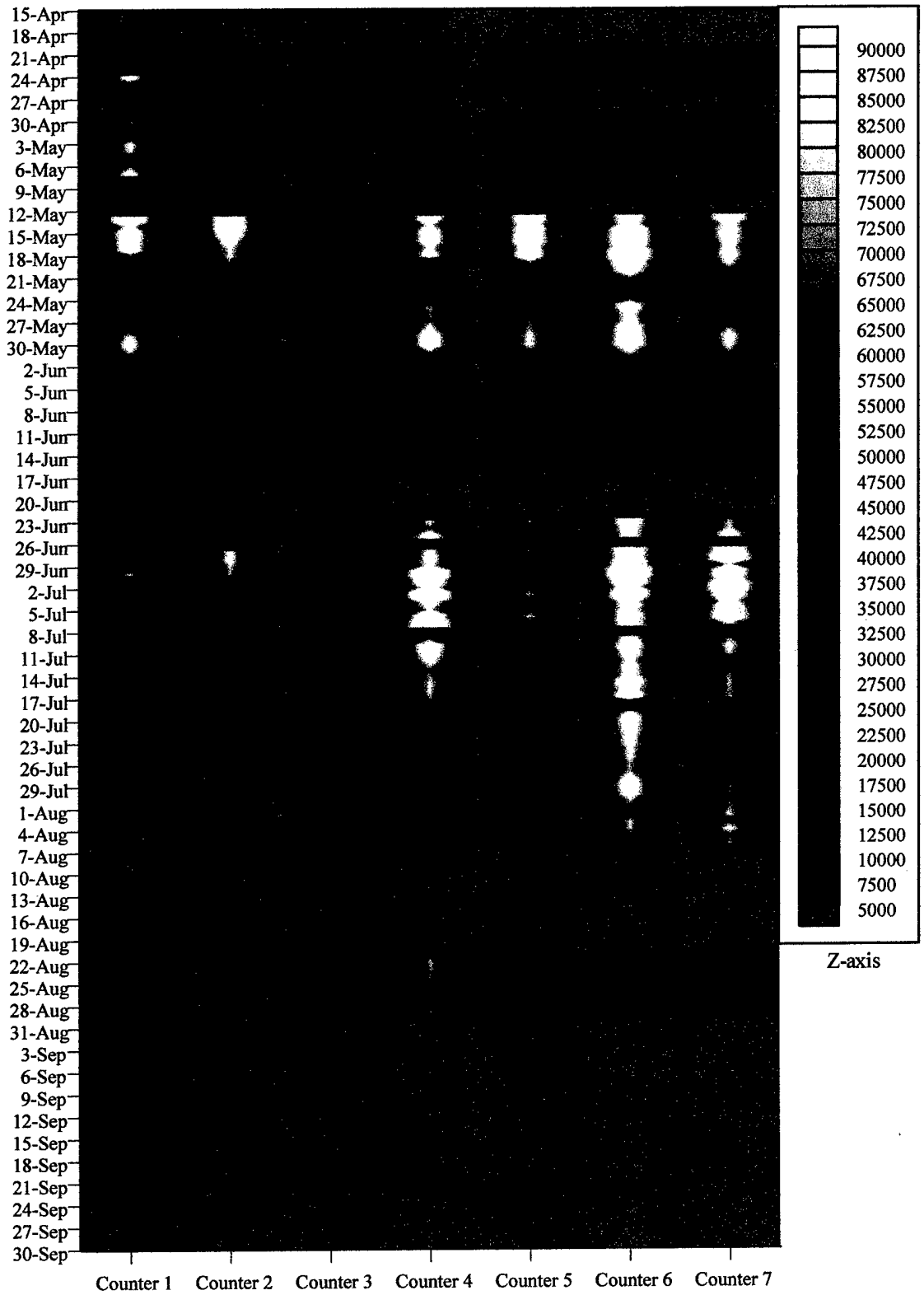


Figure 3.6 Total flight activity for colonies at the Old O Field site, 1998. Hotter colors denote higher activity.

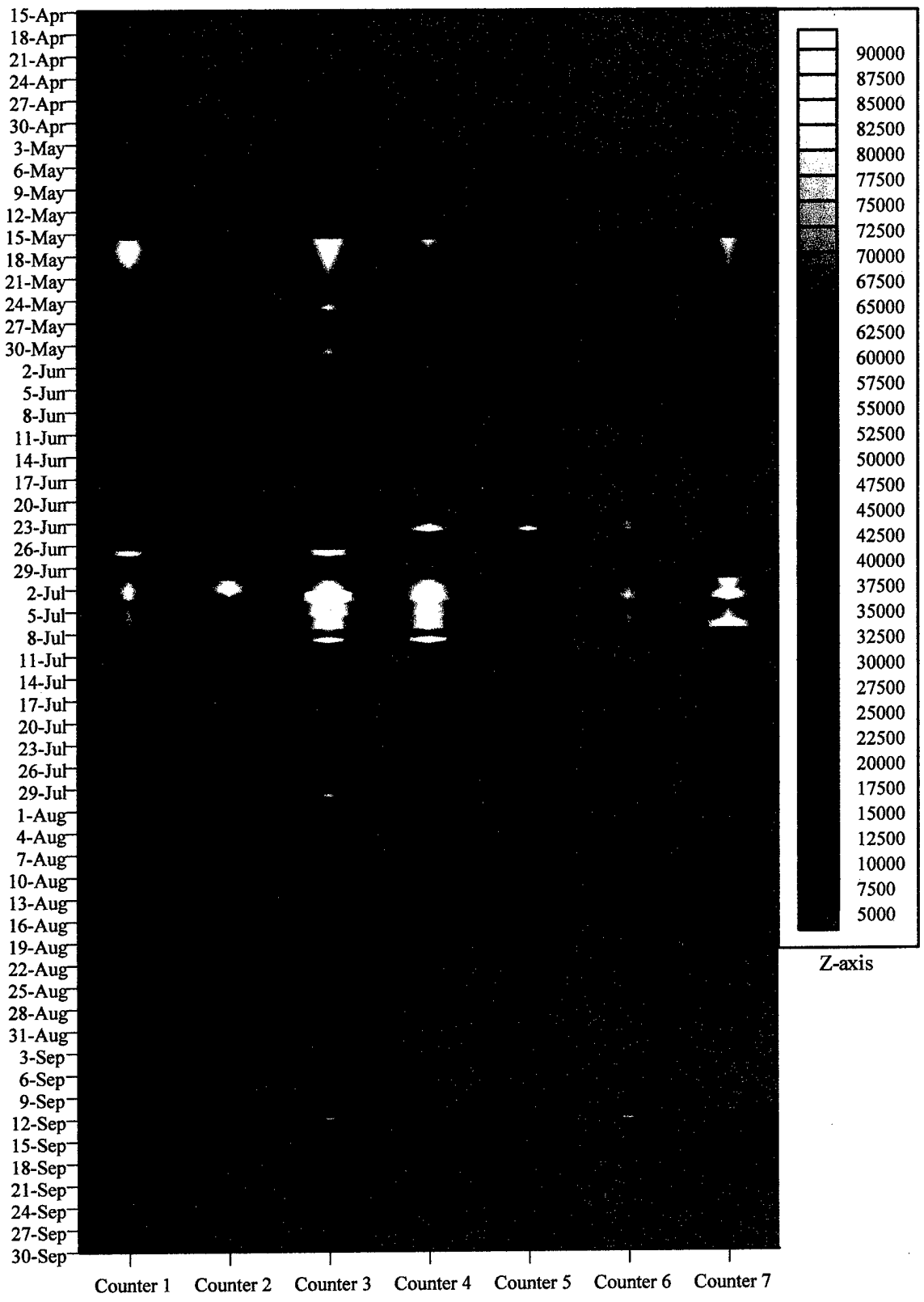


Figure 3.7 Total flight activity for colonies at the J Field Site, 1998. Hotter colors denote higher activity.

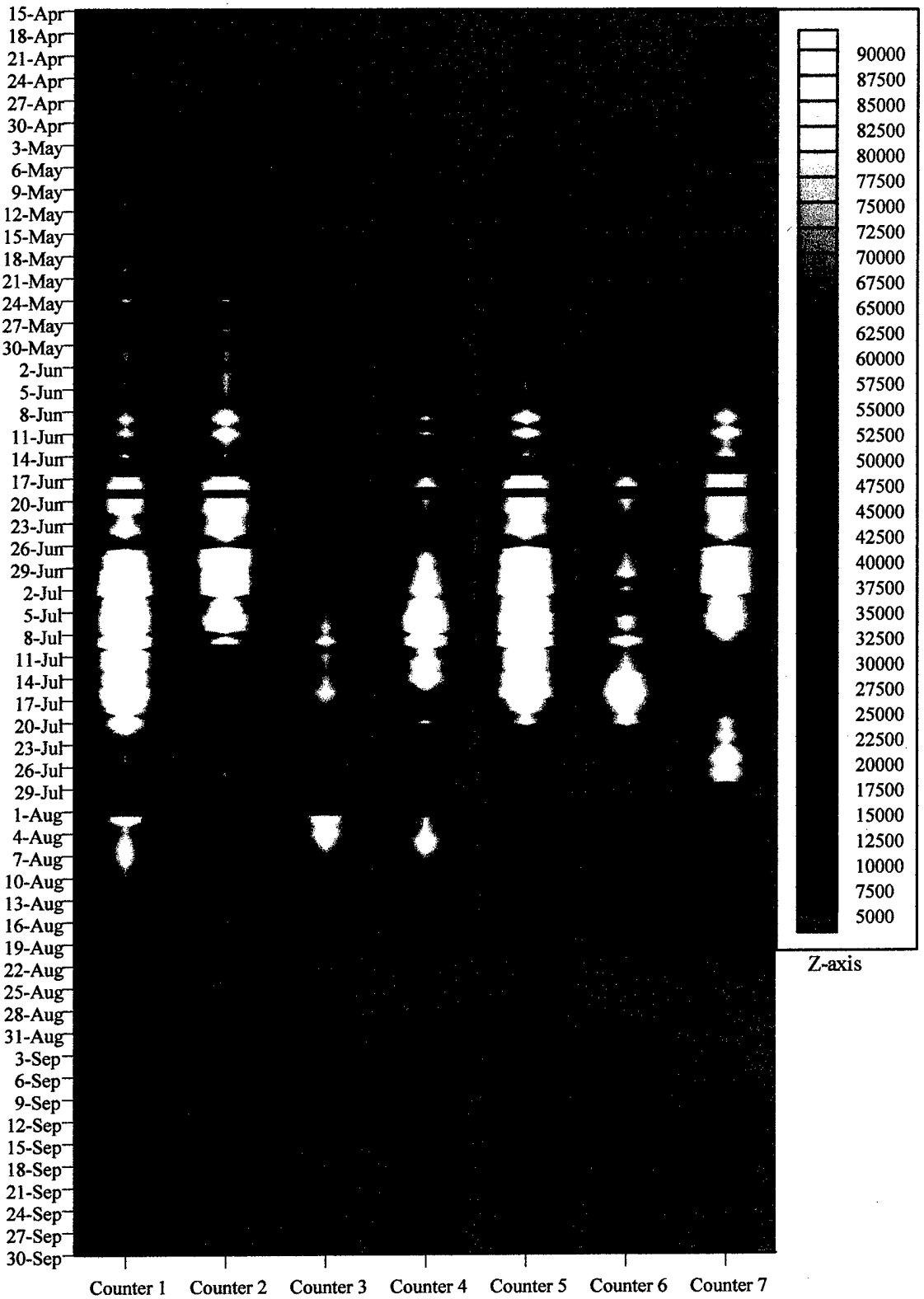


Figure 3.8 Total flight activity for colonies at the Fort Missoula Site, 1998. Hotter colors denote higher activity

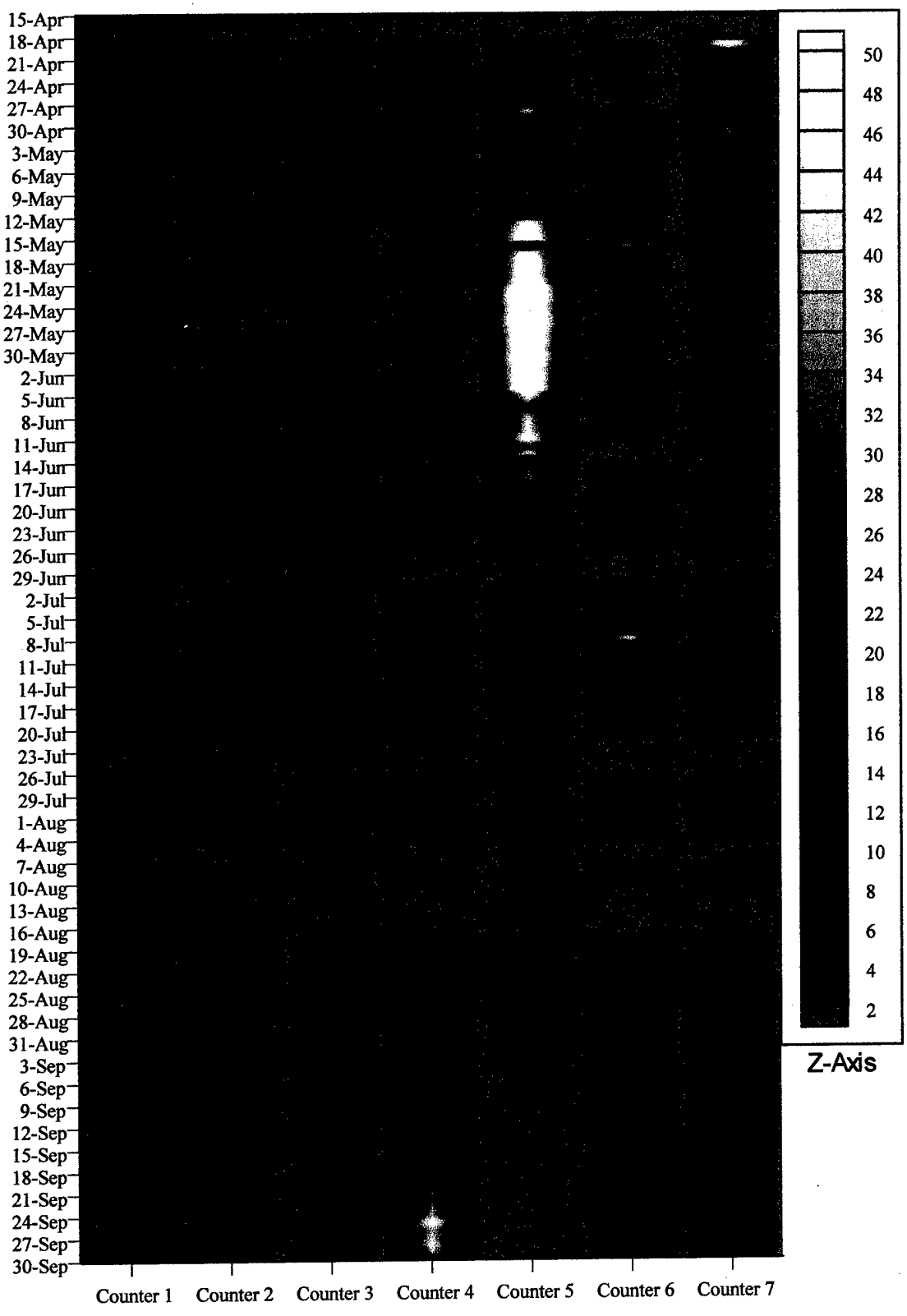


Figure 3.9 Normalized Percent Returned at end of day for Churchville Reference Colonies, 1998. Blue to purple colors are below 15%, indicating a weaker colony, while red to yellow are above 15% returned, indicating a stronger colony.

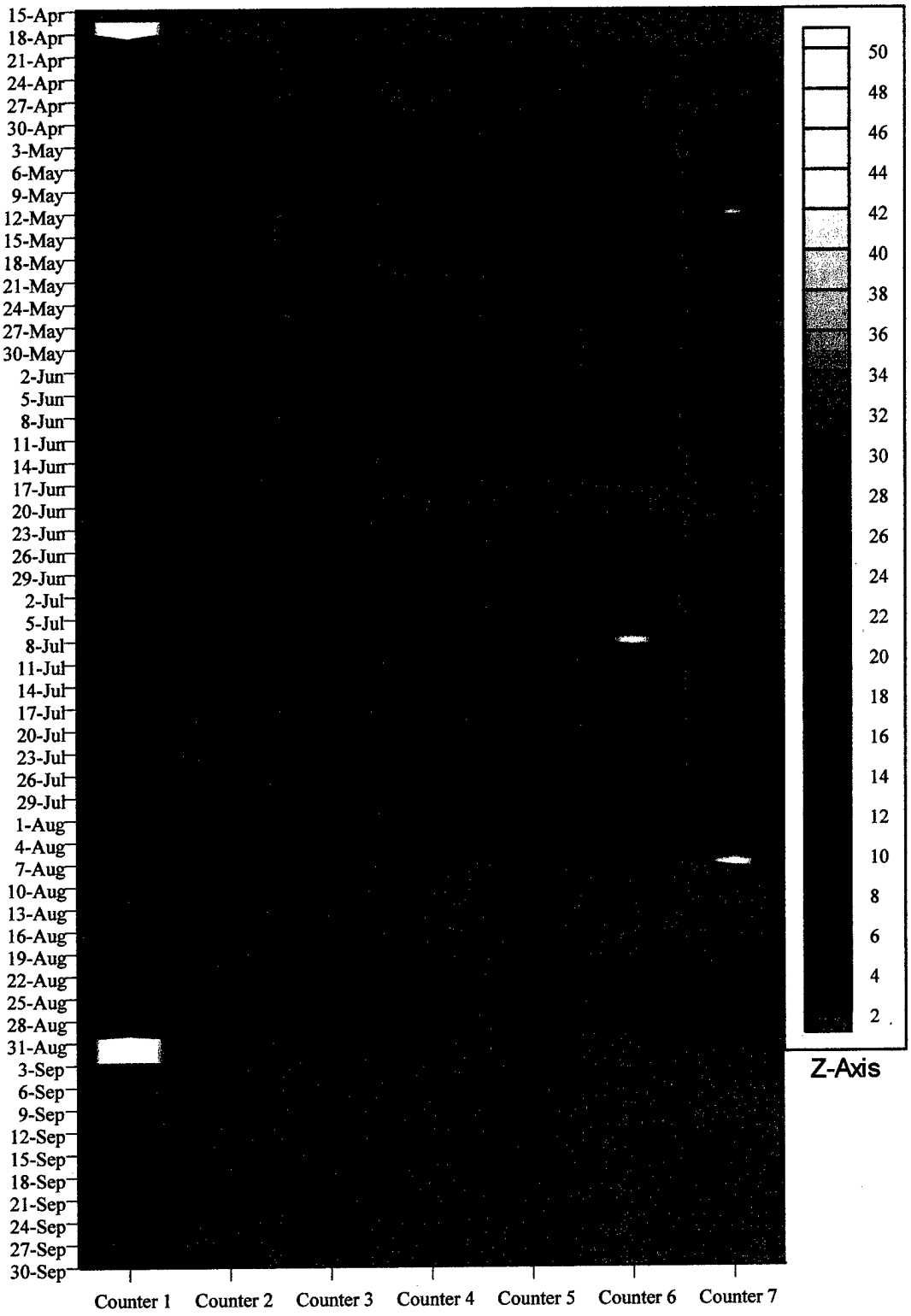


Figure 3.10 Normalized Percent Returned at end of day for Old O Field Colonies, 1998. Blue to purple colors are below 15%, indicating a weaker colony, while red to yellow are above 15% returned, indicating a stronger colony.

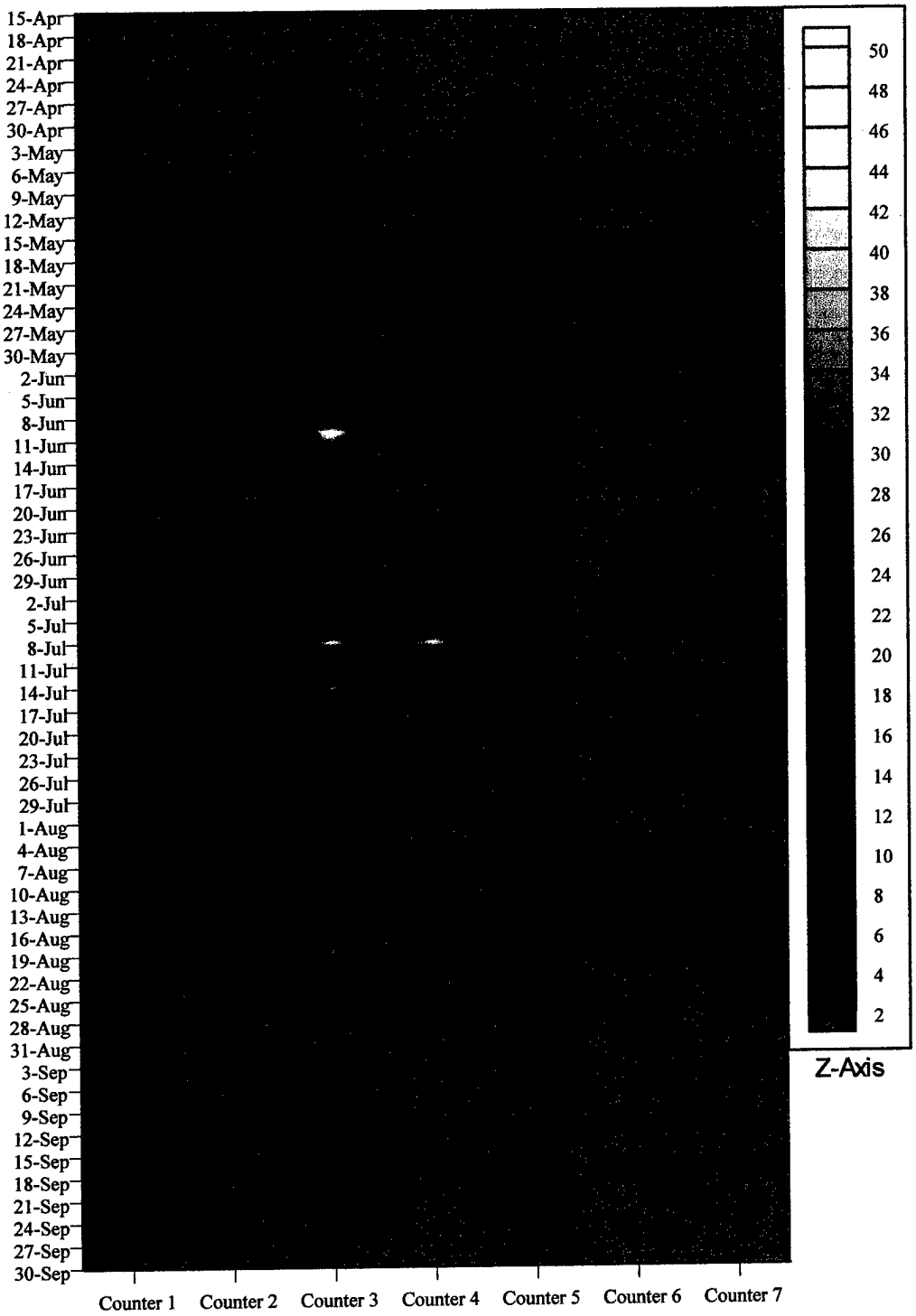


Figure 3.11 Normalized Percent Returned at end of day for J Field Colonies, 1998. Blue to purple colors are below 15%, indicating a weaker colony, while red to yellow are above 15% returned, indicating a stronger colony.

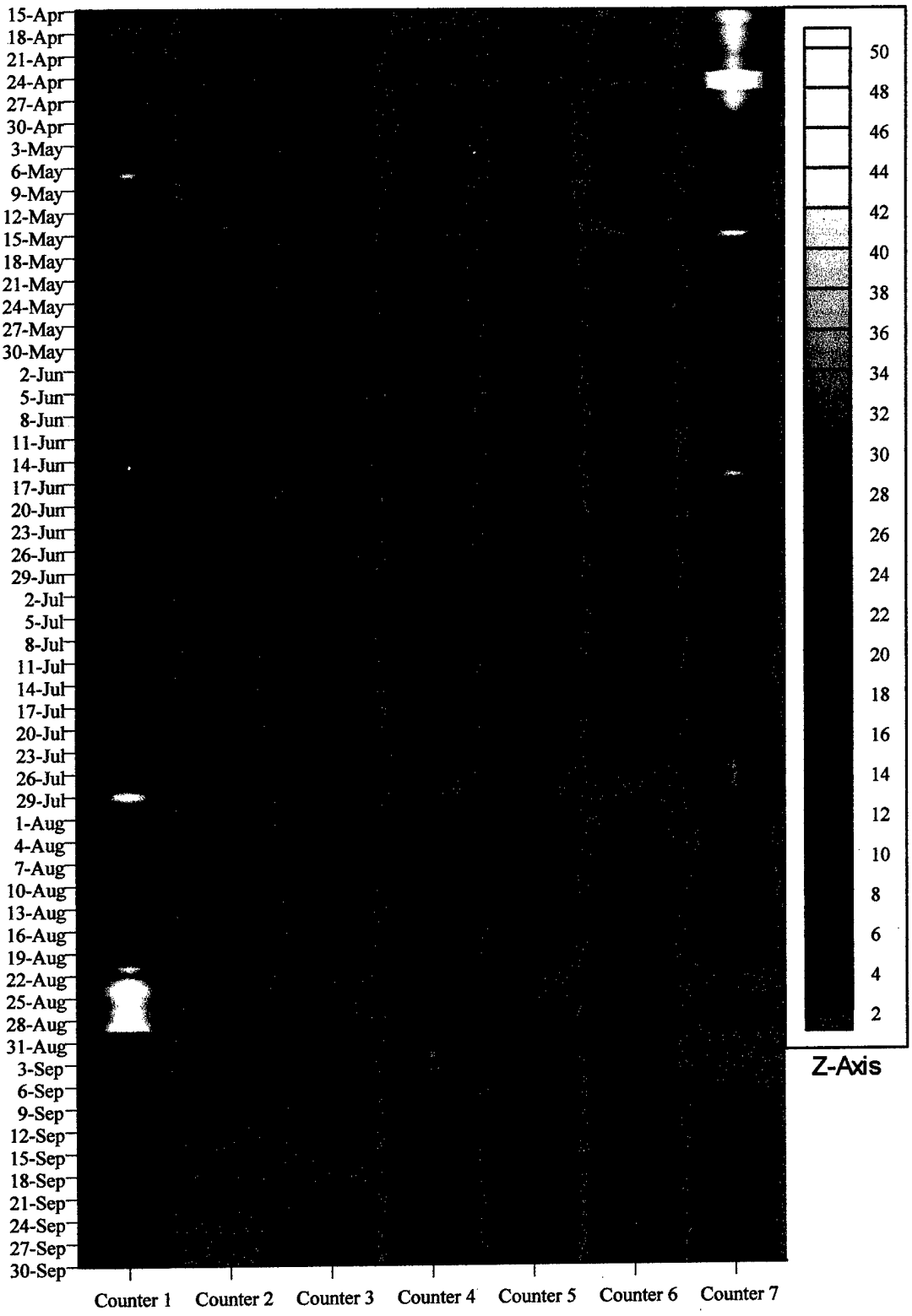


Figure 3.12 Normalized Percent Returned at end of day for Fort Missoula Colonies, 1998. Blue to purple colors are below 15%, indicating a weaker colony, while red to yellow are above 15% returned, indicating a stronger colony.

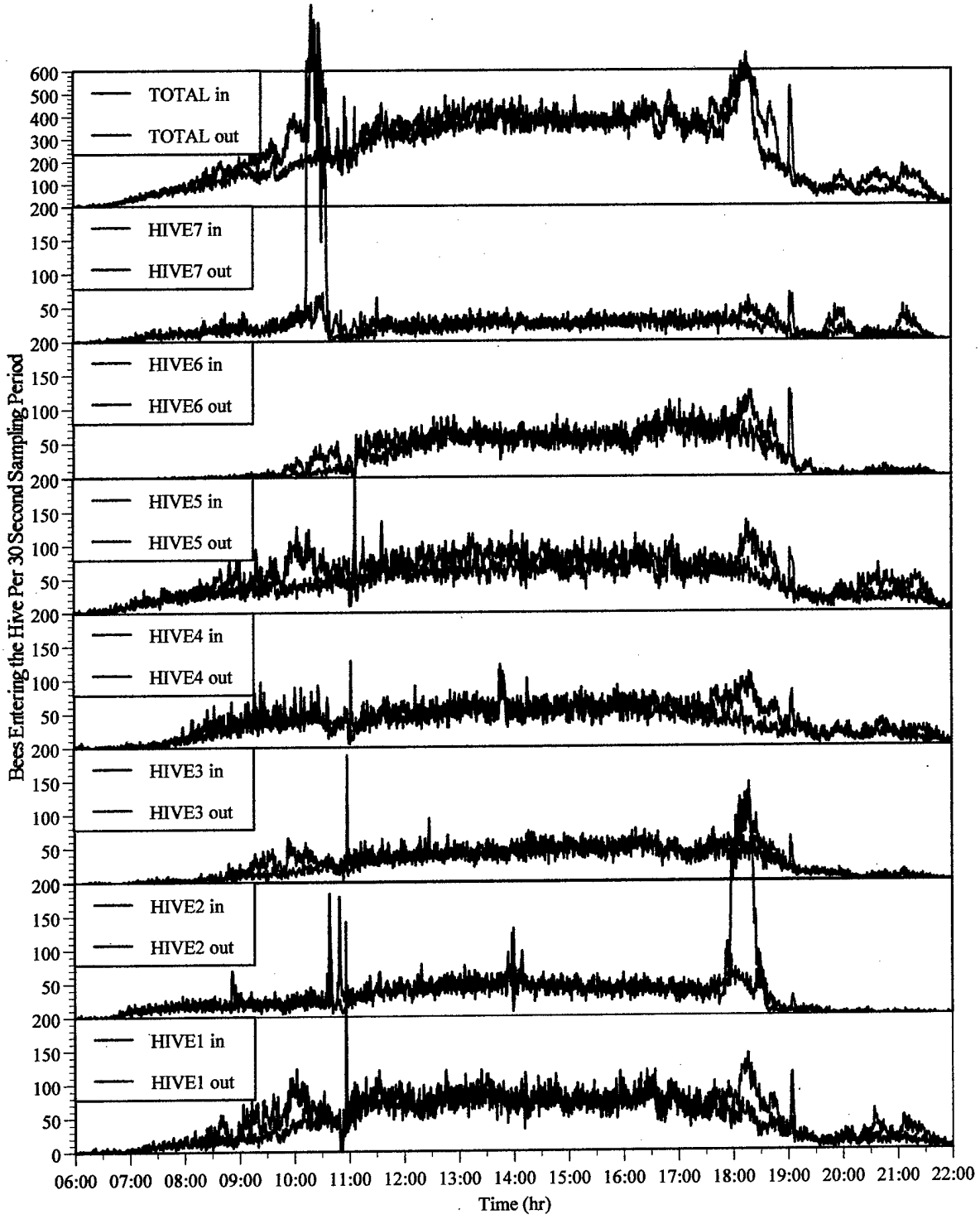


Figure 3.13 Swarms issued from hives 2 (2nd from bottom of plot) and 7 (2nd from top of plot). Spikes during the late morning were due to the conduct of hive inspections.

3.5 Results of Tier 3 Evaluations of Flight Activity Data

A main focus of this year's data analysis efforts was refining the application of methods developed in 1997. This was done in conjunction with field simulations and experiments such as weather events, hive maintenance activities, and chemical events/exposure performed at the Fort Missoula research and development site, with some parallel experimentation occurring at the Churchville reference site.

An increase in the number of swarms observed was due to starting data collection in May before the primary swarm season (usually May-June). Figure 3.13 demonstrates a single day at the Fort Missoula site where two different colonies swarmed. The spikes after the first swarm were produced by opening the hives for a visual examination of their condition. The second swarm was followed by an increase in the number of bees entering the non-swarming colonies. This seems to indicate that some bees leaving the swarming colony entered non-swarming colonies.

Another interesting behavior that was initially thought to be swarming or robbing was produced by bearding (a buildup of bees on the outside of the hive) at the entrance to a counter. Strong colonies often beard during the heat of the day or early evening, especially on hot days. The rate at which the bees leave the counter when bearding occurred was similar to swarming, but occurred later in the day.

3.6 Supporting/Calibration Studies

Many of the chemicals observed at APG are not acutely toxic. In order to calibrate the system, we initiated in 1998 and are continuing in 1999, a series of experiments designed to assess this real-time biomonitoring system in the context of exposure to chemicals known to be toxic to honey bees. Methyl parathion (MP) was chosen as a test substance because it reportedly continues to cause harm to honey bees used for pollination and because it may serve as a surrogate or simulant of chemical warfare agents which are also organophosphate chemicals.

A full report on these tests, including contact toxicity trials (1999), injection of controlled amounts of vapors into the hive (1999), exposure to MP vapors from pollen spiked with MP and placed inside the hive (1998), and consumption of MP in MP spiked sugar and Crisco patties (1998) will appear in the 1999 annual report. During 1998, the lesson learned was that the electronic surveillance system was sensitive to the act of placing the chemical into the colony. This "observer disturbance" to the colony masked any short-term (minutes - few hours) behavioral responses as indicated by honey bee flight activity or thermoregulation.

Placing the chemical into the hive at levels reported to be toxic to bees by numerous other investigators, simulated an internal poisoning event such as might occur as returning foragers brought pollen contaminated with toxic chemical(s) back to the hive. Although dead bee traps showed an initial severe bee mortality that then continued to a somewhat lessor degree for several days (Figures 3.14), flight activity displayed a general decrease in flight activity by the day following exposure to the chemical and tended to decline gradually for several more days (Figures 3.15-3.16). This decrease in flight activity continued to occur after the dead bee traps had stopped collecting excess dead bees. We did not observe a concurrent reduction in the

number of bees returning to the hive compared to those leaving the hive. Obviously, the toxic event killed bees in the hive before they could leave it.

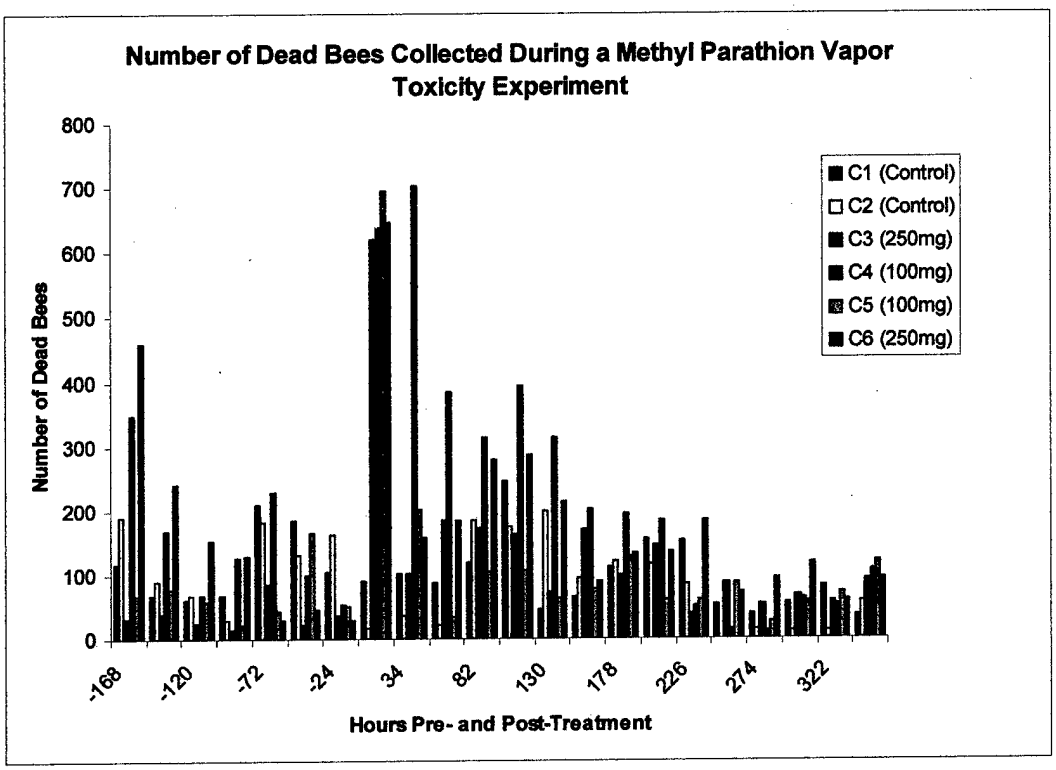


Figure 3.14 Typical dead bee trap results. The initial loss was due to relocation of hives prior to the actual dosing with MP, which occurred at 0 hours. The loss of bees from both the 100 mg and 250 mg doses was dramatic and sudden. After 200+ hrs, bee mortality had subsided.

Given the observed influence of the observer placing a chemical packet into the hive, we designed and fabricated in the fall and winter of 1998-1999 an electronic system for injecting chemical vapors into a hive, using a PC computer to control the system. A bubbler containing the substance to be tested is affixed to the hive. A metal tube inserted into the center of the hive allows for injection of the test material. Filtered, compressed air provides the propellant. One of these bubblers is attached to each hive to be tested. A bypass filter allows a flow of filtered air to pass into the hive. The observer then retreats to a building housing the controller system and monitors the flight activity of each hive as graphically displayed on a monitor. When bee flight activity returns to "normal" (as compared to the flight profiles for prior days), a click of the PC mouse will open a solenoid to divert the air flow through the bubbler. In this way, any instantaneous changes in flight activity or thermoregulation can be observed. In other words, the system is allowed to equilibrate before initiating the trial.

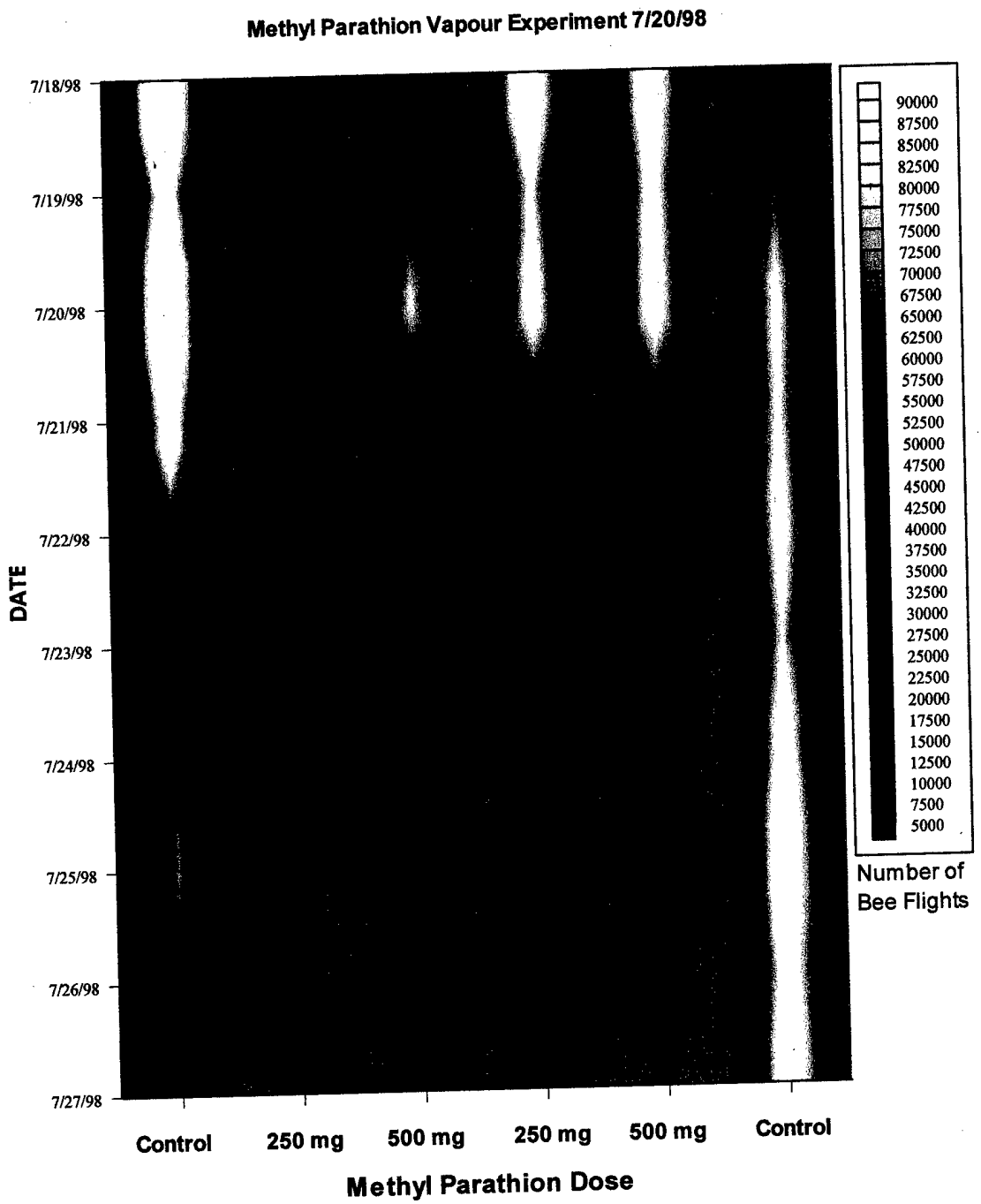
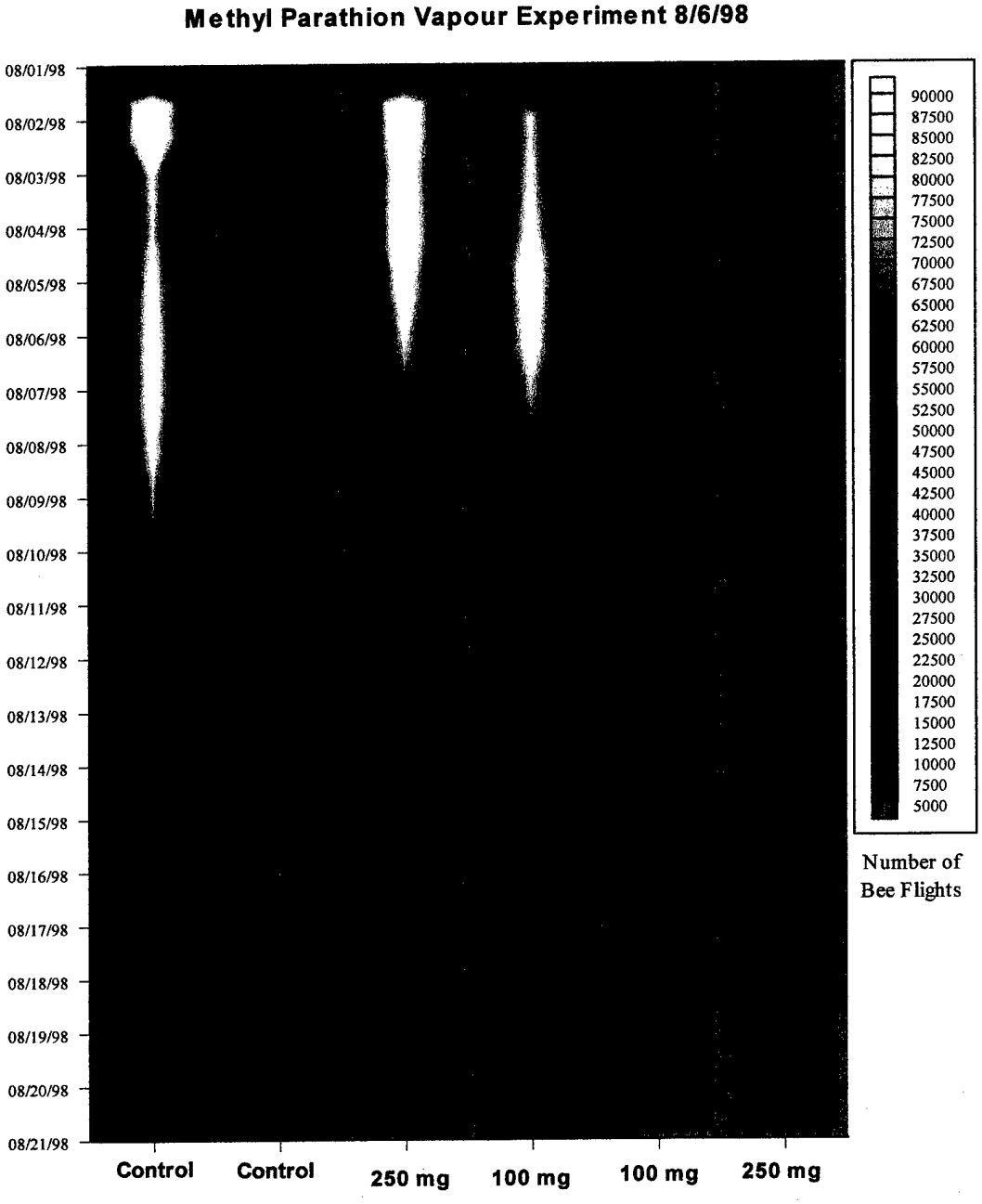


Figure 3.15 Flight activity before and following dosing of colonies with methyl parathion. Bees were exposed to vapors only. One of the control colonies actually increased in strength and activity level during the experiment. The four dosed colonies reflect a dramatic reduction in overall number of forager flights per day.



Total Daily Flight Activity for Colonies at Fort Missoula. Hotter colors denote higher activity.

Figure 3.16 Flight activity before and following dosing of colonies with methyl parathion. Bees were exposed to vapors only. Dose trials were conducted using smaller amounts of MP. Again, all dosed colonies displayed a major reduction in flight activity.

Also, in order to assess contact toxicity, in 1999 an extended porch will be mounted ahead of the counter porch. From the perspective of the incoming and outgoing bee, the porch will appear to be more or less the same as the counter assembly which will now sit within the added shell. This extended porch will have a negative pressure well where filter papers or foliage treated with the chemical to be tested can be placed. A vacuum system will be adjusted so that all air at the entrance is drawn away from the hive, preventing vapors from entering the hive. Incoming and outgoing forager bees will continue to be counted, but must pass over the chemical treated substrate as they leave and enter the hive. In addition, this same electronic control system can be used to activate pumps to sample the air inside the hive to measure and verify dose delivery rates.

3.7 Acute Toxicity

With daily return rates of forager bees at all of the Maryland sites averaging 92% or better (Table 3.1), no acute toxicity was measured at any of the APG sites as indicated by the numbers of forager bees leaving and returning to the hive. However, the MP dose trials in Montana indicate that an acute kill could occur within the hive and not be reflected by this metric. However, this was not evidenced by any obvious change in total flight activity throughout the summer (Figures 3.5-3.8). The change in total flight activity following exposure to a known toxic chemical (i.e., MP) was easily seen (Figure 3.15-3.16). Similarly, the amounts of bees recovered from the dead bee traps at each of these sites was minimal, with the exception of two somewhat larger (about 2 cups of dead bees over two weeks) seen in two of the J Field hives during midsummer.

By comparison, one colony of each pair at four of the Boundary Sites failed before the end of the summer. The cause of the colony loss seemed to be a general decline of the bee population rather than any acute toxicity event. In 1998, the second hive of each pair was placed on site early-summer, about a month after the first hive was put in place in the spring. These later season colonies were made up of splits from strong colonies kept at G Street. As such, these colonies did not have as large a bee population as the first colonies deployed at Boundary sites.

The loss of four of these hives was probably a consequence of starting with a smaller bee population and deployment after the main nectar flows had ended. Thus, we suspect that the loss of four of these hives was more a reflection of the bee management than it was of any exposure to toxic levels of contaminants. However, because of these losses, 20 strong hives (ten pairs) were placed back on the Boundary Transect lines in June of 1999 to provide a repeat of the test.

None of the condo or D Field colonies failed during the study which extended from April through the end of September. However, condo colonies were fed with sugar syrup when it became evident that late season nectar flows were inadequate to maintain the colonies and food stores were nearly depleted.

3.8 Summary of APG Results

The Tiered Analysis approach was applied to the monitoring trials at APG sites in 1996, 1997, and 1998. The results are discussed here in the context of other measures of colony performance and of the chemical exposure data. There was a general decrease in variability in the flight activity among hives at each of the Maryland sites in 1997 compared to 1996, with some increase in flight variability during 1998.

The Tier 1 indicators C.V., PRC, and ANL (Tables 3.1-3.3), were all used to provide metrics for comparing sites to one another. The colonies at Old O Field showed a slight degradation in C.V. values in 1998 (20-80%) compared to 1997 (25-75%). The C.V. values for 1998 at Old O Field were lower than those at the Churchville reference site (30-100%) due to late summer colony declines at Churchville, probably as a consequence of limited food resources. J Field also exhibited a slight degradation of C.V. values in 1998 (20-80%) as compared to 1997 (15-50%).

Occasional returns to a colony in excess of 100% seen at all of the sites can result from bees drifting from one colony to another or may be an indication of bees leaking (bypassing) from the counters. This happens as bees exit through small gaps in the counter without being counted on their way out, with the same bees returning through the counter after landing on the counter porch.

For 1999, all of the counters have been retrofitted with a clear Lexan shield to address the leaking problem. Also, a metal flange has been added to bridge the gap from the door to the plate under the hive body. With a clear shield, any leaking or trapping of bees can be immediately seen and corrected. That is, leaking bees usually pass through or end up in the bottom of the counter assembly. Any bees spotted inside the area wrapped with the clear plastic shield will be an indication of a leaking counter and steps can be taken to correct the installation.

The PRC metric showed little change from 1997 to 1998 for both the Churchville reference site and J Field, while Fort Missoula displayed a decrease in PRC. The Fort Missoula decrease in PRC may have been a response to the MP trials being conducted in the apiary. One of the counters at Old O Field (on the strongest colony) had a noticeably leaking counter which caused the PRC for the entire site to be somewhat skewed.

The trends listed in Tables 3.1-3.3 indicate similar, but slightly degraded behavior for 1998 compared to 1997. This still represents a dramatic improvement in comparison to the sites that were monitored in1996. The degradation in performance does not appear to be significant (averaged over the season, although significant for individual colonies on individual days) and is probably attributable to variations in cumulative stresses, both anthropogenic or natural. For example, some degradation of colony performance occurred at Churchville because of a food shortage.

The Methyl Parathion experiments demonstrated the ability of the counters to monitor the course of exposure to a toxic chemical over short and long period of time. The decrease in flight activity, although not immediate, was quite obvious, and continued to increase with time. This correlated well with the number of bees found in the dead bee traps. It also demonstrated that the impact of an acute toxicity event may continue long after the initial bee drop (kill). Also, a

reduced foraging force would impact the ability of the bees to collect nectar, pollen, and water as well as alter pollination efficacy. As mentioned, these trials are part of an ongoing series of trials aimed at better identify a spectrum of substances and dose response relationships.

Monitoring of colony performance at Maryland sites, especially before, during, and after an action such as capping a landfill, proved to be useful for documenting changes in both colony performance (discussed in this Section) and exposures to bioavailable chemicals (Section 4). Based on earlier studies of West Branch Canal Creek and of Old O Field, removal and capping activities both reduced exposures of bees to organic solvents and resulted in improved colony condition. The MP trials, which will be continued in 1999, clearly indicate that the real-time biomonitoring system's behavioral metrics can clearly identify a toxic exposure to bees, if it occurs. Determinations of dose-response thresholds for chemical commonly found at APG will be continued in 1999.

SECTION 4 MONITORING OF EXPOSURES TO BIOAVAILABLE CONTAMINANTS

4.1 Chemical Sampling and Analysis

Chemical sampling and analysis for this project are guided by three overall objectives:

- Measure chemical agents in the ambient air as well as those that are bioavailable to honey bees from multiple sources.
- Make site-to-site comparisons with respect to these chemical exposures— at sites on the Aberdeen Proving Ground (mainly Edgewood) and in the communities surrounding APG.
- Provide chemical exposure data needed to characterize relationships between exposures to specific chemical agents and effects (lethal to sublethal, acute to chronic, dying bees to behavioral changes) as measured in honey bee colony populations.

For the 1998 field season, colonies of honey bees were monitored for exposures to volatile (VOC) and semi-volatile (SVOC) organic compound residues by pulling hive atmospheres (i.e., the air inside the hive box) through chemical traps. Also, at each colony location, a concurrent sample of the ambient air was trapped. For analysis, these hive and ambient air samples were thermally desorbed into a gas chromatograph/mass spectrometer (TD/GC/MS), following EPA Methods T0-1 and T0-2 (EPA/600/4-89/017). This chapter presents the results of the 1998 monitoring of VOCs and SVOCs and compares them to the results from 1996 and 1997.

Samples of pollen, live bees (forager) and dead bees (from hive-mounted traps) were collected for analysis for heavy metals, other inorganic elements of concern (Be, As and Se) and radiochemicals. These specimens have been oven-dried, ground, and forwarded to other labs for quantitation. Upon receipt of these results from the cooperating laboratories, a report will be forwarded immediately to USACEHR and to APG as an addendum to this document.

Several new aspects of chemical sampling are presented in this year's report. First, the organics sampling train that precedes the air pumps has been improved by the addition of a three-tube "JAG" box. JAG boxes employ a drying tube and a guard tube ahead of a 4-phase chemical trap to capture and remove water and high molecular weight compounds. This has dramatically reduced the number of samples that are lost or rendered unusable due to moisture in a trap or from a buildup of terpenes and carbohydrates in the TD/GC/MS analysis system. Second, quantification of TD/GC/MS results has been improved by refining the internal standard's calibration protocol as well as increasing the frequency of calibration checks. Third, improvements in flowmeter technology provided a more accurate measure of the volume of each air sample than could previously be accomplished, based on the pump manufacturer's counter system. Finally, additional studies conducted in Missoula have verified that several of the chlorinated solvents commonly found in APG groundwater can be brought back to the hive by water-collecting bees and subsequently detected in hive atmospheres.

The results of chemical studies for the 1998 field season are separated into six parts:

- **Year 3** of biomonitoring at the **Old O Field** landfill site
- **Year 2** of biomonitoring at the **J Field** phytoremediation site
- **Year 1** of a **Boundary Survey** study -- levels of contaminants at sites near the APG boundary and along three 21-mile transects radiating out from APG into the residential and farm communities of Cecil, Harford, and Baltimore counties
- **Year 1** of an assessment survey of **D Field** sites
- **Year 3** of the **Churchville** Off-Base reference site
- **Year 4** of corroborating studies conducted at **The University of Montana** research apiary sites in Missoula.

A previously unreported chemical, methenamine, is included in the list of compounds reported at APG for 1998. One of the uses of this compound is in the manufacture of RDX explosives. Therefore, it may serve as evidence of the presence of explosives at APG field sites. Other possible sources of methenamine are discussed in this report under the Boundary Study section. In addition, traces of the high explosive RDX were found in soil, bee, and goldenrod samples collected at a D field site and analyzed by Sandia National Laboratories as part of our USACEHR and DARPA (Defense Advanced Research Projects Agency) funded technology development.

Overall, chemical concentrations seen in 1998 field studies did not reveal any short-term, pulsed, or severe exposures of bee colonies to volatile and semi-volatile organic chemicals at APG sites. Most of the detected chemical exposure values in hive atmospheres or ambient air were in the part per trillion range (ppt). Many sites demonstrated frequent or continuous exposures to low levels of these organics. The highest exposures to these chemicals often occurred at sites substantially distant from APG, sometimes in concentrations in excess of those seen at sites under restoration on the Edgewood peninsula.

4.2 Experimental Methods and Materials

4.2.1 Sampling Design/Frequency - 1997 APG Field Applications: Samples were collected from a total of 77 Maryland-based honey bee colonies. Three sets of instrumented condo units (seven colonies each) were returned to the same sites as in the 1997 APG field applications: 1) the capped Old O Field landfill site, 2) the J Field phytoremediation site, and 3) a reference site at a hobbyist beekeeper's farm near Churchville, MD. Nine pairs of freestanding colonies were distributed at new sites on D Field. Three sets were placed near Briery Point; two sets were set out along the road proceeding south from that point; and four sets were positioned immediately adjacent to the Moving Target track area at Sand Point. Other freestanding colonies were deployed to augment chemical sampling at the instrumented condo sites -- at Old O Field (two hives) and J Field (five hives). Finally, three 21-mile lines of hives were established to gather comparative data at sites progressively further from APG sites. These radiated out to the north, the northwest and southwest from the upper Edgewood peninsula area and included additional sites at Grace's Quarters, Carroll Island, Westwood, and on the Aberdeen peninsula area.

4.2.2 Hive- and Ambient-Air Sampling: Sampling of outside ambient air and of hive atmospheres (i.e., air inside the hive) took place periodically between April 13, 1998 and

October 29, 1998. A list of the actual sample dates appears in Table 2.2, Section 2. The number of samples taken on any given date was constrained by the number of constant flow pumps in our equipment inventory. Dates carrying an "@" prefix indicate baseline sampling of colonies at our APG G-Street beeyard prior to deployment for the 1998 field season.

In addition to sampling the air inside the survey or condo beehives, for each sample date an ambient air sample was collected in the same vicinity and over the same duration. The sampling pump and tube were clipped at a height of about 2m to the top of the metal honey bee biomonitoring signs at each site. Ambient air samples from the freestanding colonies were gathered in a similar manner unless otherwise noted. The dates on which ambient air was sampled in the vicinity of the colonies are also incorporated with the colony sample schedule of Table 2.2.

As can be seen from the sampling dates in Table 2.2, a few samples are missing — usually no more than one of the several samples taken from a given location and day. Samples were mainly lost during sampling due to bees plugging the copper sampling tubes, moisture from rain or high humidity conditions wetting the Carbotrap desorption bed, or pump failure because of premature battery failure. Occasional samples were lost because of tube breakage during shipping. Many of these problems were eliminated or reduced by the JAG box introduced early in the 1998 sampling period. ***Once the JAG boxes were employed, only three 1998 sample tubes were lost to moisture problems throughout the rest of the season.*** The JAG box system is fully described in Section 4.2.2.1.

With the exception of bees plugging sampling tubes, which is beyond our control, the other sources of sample tube loss have been virtually eliminated and should not be a problem for the 1999 field sampling season. The JAG box design has been ungraded by the addition of an ***aluminum rod-stabilized and -guided rack*** that should further reduce sample tube breakage, both in the field and during transport to and from field sites. Sample tube breakage during shipping between Montana and Maryland was reduced in 1998 (compared to previous years) by the use of foam-insulated, plastic "coolers" as a recyclable, tough, cushioned, shipping container. At the end of the 1998 field season, ***stainless steel canisters with hinged "sealable" polycarbonate covers*** were found to replace the wide-mouth sample jars that we had been using to store and ship the Carbotrap tubes. These steel canisters fit securely within the shipping coolers and provide an extra measure of protection. In addition, the canisters have straight sides with no restriction at the mouth (under the cover). This allows wrapping the tubes with plastic bubble wrap before placing them in the canister and further reduces the chance of "fumbling" and dropping tubes when putting them into or taking them out of the container.

The battery failure problem also has been solved for 1999. The pump manufacturer supplies small rechargeable batteries that sometimes stop before the end of an 8-10 hr sampling period. This has been a particular problem at APG sites with restricted access like J Field, D Field, and Old O Field. Downrange activities on the Edgewood peninsula sometimes delayed a re-entry to the site within the expected sampling period, resulting in stopped pumps by the time that the field technician finally was able to get onto the site. Instead of relying on the rechargeable batteries supplied by the pump manufacturer, we designed and now use transformers that allow the use of inexpensive, long-life, dry cell or larger rechargeable batteries. ***The dry cell batteries***

have proven capable of running pumps continuously for three or more days with no showdown of the pumping rates.

4.2.2.1 The JAG Box Sampling Train: Extended periods of rainy weather in the spring of 1998 led to moisture penetrating our sample train. Wet sorption tubes cannot be successfully desorbed and chromatographed. They led to a "grassy lawn" chromatogram and severe chemical carry-over among the tubes being desorbed. What had been an occasional problem in prior years became a frequent problem, which was resolved by the JAG boxes—a new sampling train was devised early in the 1998 field season.

It was prompted by two recurring problems: 1) loss of samples due to moisture in the sorbent bed, and 2) coating of transfer lines in the GC from high molecular weight terpenes and carbohydrates in beehive atmospheres. The alteration to the sample train consisted of a series of three tubes housed in a plastic enclosure -- what we term a JAG box (Figure 4.1).

An air sample pump connected to the outlet end of the JAG box actively pulls air through the improved sampling system. The JAG box itself consists of three tubes connected in-line with brass Swagelok fittings and 1/8 inch copper tubing housed in a 710 ml Rubbermaid "Servin' Saver"™. The Rubbermaid "Servin' Saver"™ provides a waterproof housing for the sample tubes. Three holes are drilled in the short ends of the "Servin' Saver" for copper tubing and Swagelok™ fittings to run through and be anchored. The JAG box inlet uses the copper tubing nozzles that we have always employed. The copper nozzle inlet tube connects to an SKC model 226-44-02 drying tube packed with 9000 mg of anhydrous sodium sulfate. Water molecules are absorbed by the sodium sulfate as air is pulled through the tube. Next, we added a Supelco Carbotrap 150 tube to act as a guard-tube to remove the larger terpene and carbohydrate molecules that impair GC/MS desorption processes. It is packed with 20/40 mesh Carbotrap C, a graphitized carbon black with 10 m^2/gram surface area for trapping and efficiently releasing molecules in the C9 to C30 range. The third and final tube in the sample train is a multi-phase Supelco Carbotrap 300 or 400 tube on which the analytes of interest to APG are sorbed.

A side benefit of using the JAG boxes is that we were able to observe a potentially important new contaminant, methenamine. Introduction of the Carbotrap 150 guard tube removed high levels of carbohydrates that had previously masked its presence as an underlying coelutent. JAG boxes also improved the overall performance of the GC/MS instrument. We saw sharper peaks and fewer broad baseline anomalies. Finally, by removing the larger terpene and carbohydrate chemicals in the sampling train prior to desorption, the wear and tear on the GC/MS was significantly decreased. Previous to the employment of the JAG Box, the GC/MS required a complete cleaning and rebuild after every 100 to 150 samples were run. The JAG Box allowed over 700 samples to be run with nothing more than routine maintenance of the instrument.

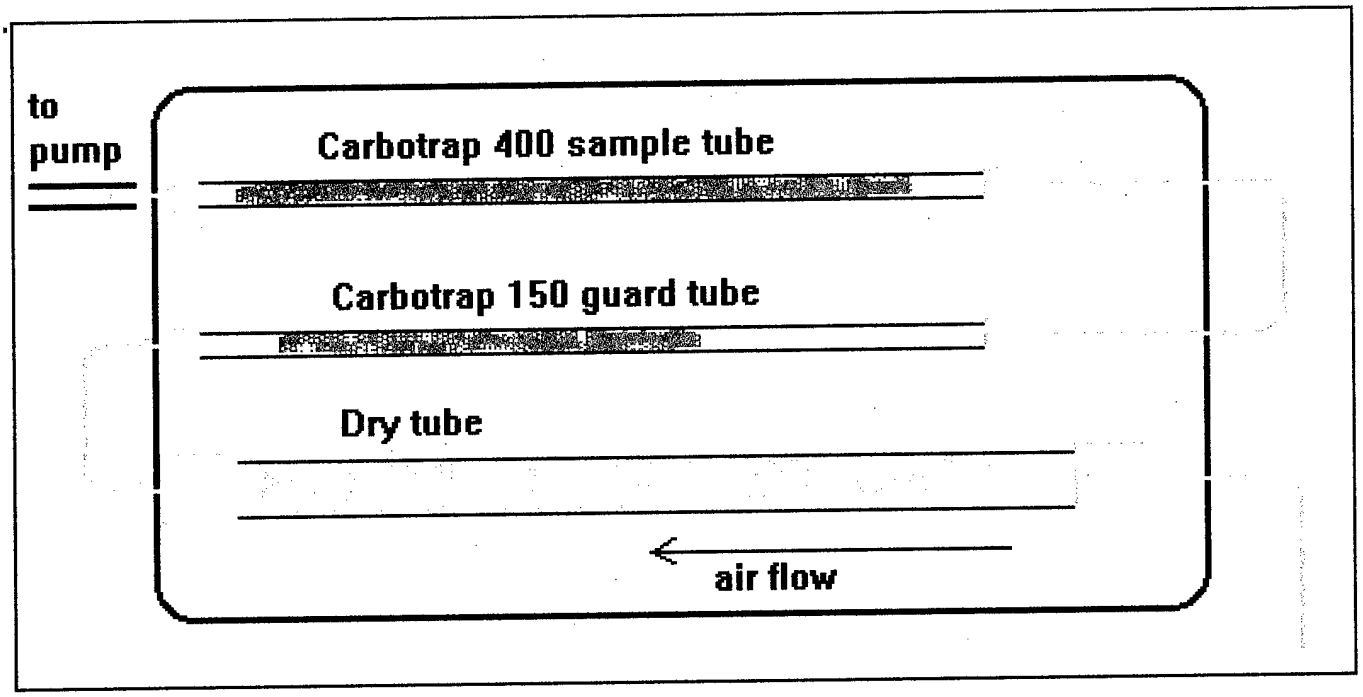


Figure 4.1. Schematic diagram of the new JAG box sampling train. Inlet is at lower right via copper tubes from the hive. The sample air is drawn through an anhydrous sodium sulfate drying tube, a Carbotrap 150 guard tube and a Carbotrap 400 sampling tube. The air sampling pump is attached via Tygon tubing to the JAG box outlet at upper left.

Pump flows for 1998 field samples were adjusted to exactly 100 ml/min. This allowed 48 liters of air to be sampled over an eight-hour pumping period. This pumping period has proven optimal in our sampling trials. It is sufficient to see the low levels of contaminants present in the hives, but does not overwhelm the GC/MS with the high levels of other organics present in bee colonies. We achieved better limits of detection in this manner and were successful in seeing a variety of contaminants.

Trip blanks were collected from each site on every sampling date. In each case the trip blank consisted of a complete set of tubes in an assembled JAG box. As with the sample boxes, both the inlet and outlet ports were sealed with paraffin wax sheets. The blank JAG box was left at the site near the cassette pumps throughout the duration of the sampling period, and stored and transported with sample tubes until analysis time. Trip blanks were thermally desorbed into the GC/MS and analyzed as part of the same batch as the sample tubes they accompanied.

For the 1998 APG samples, the total volume of air sampled was obtained by multiplying the pump flow rate by the pumping time. In previous years, pumping volumes were derived from the "click" counters built into the digital pumps, as recommended by the manufacturer. The multi-bed sorbent tube array used in the JAG boxes imposed a drag resistance on the pumps. We found that the manufacturer's counters were not accurate when used with the JAG box system. Pumping volumes indicated from counter "clicks" overestimated pumped volumes by about 30%. Therefore, once the JAG boxes were put into service in 1998, the pumps were calibrated with digital flowmeters and bubble film meters prior to deployment.

Sample tubes were sealed in individual vials and stored in a refrigerated chamber to enhance retention of volatile contaminants. Samples from APG were air expressed, with trip blanks, to our University of Montana labs. Once in Missoula, they were stored in a dedicated 4 °C sample refrigerator until analyzed.

4.2.3 Thermal Desorption GC/MS Analysis: Air samples were analyzed by thermal desorption GC/mass spectrometry. Sample tubes were placed in an 8-station thermal desorption unit (Dynatherm MTDU 910). After a five-minute helium purge (Liquid Air, ultra high purity grade) at 46 °C, tubes were subjected to a 10-minute desorption cycle at 300 °C. A final 5-minute cooling flush was used to remove residual contaminants trapped in the sorbent bed and transfer line. All phases of the desorption utilized a helium flow rate of 35 ml/min.

A system blank or a trip blank was inserted after every two samples. The frequent blanks provided continual assurance that peaks appearing in a sample's chromatogram were real, not laboratory artifacts or carry-over from earlier sample tubes.

Thermally desorbed contaminants from the sample were captured by a 6" Vocarb 3000 trap from Supelco (10 cm Carbopack B graphitized carbon, 6 cm Carboxen 1000 molecular sieve and 1 cm 1001 molecular sieve) installed in a Tekmar LSC2000 Liquid Sample Concentrator. From there, the sample was introduced into the gas chromatograph by heating the Vocarb 3000 trap to 260 °C and flushing it with 40 ml/min of ultra high purity helium. The entire helium flow from the trap entered the GC for 15 seconds and was split 1:50 thereafter.

Chromatographic separations were accomplished on a Hewlett Packard GCD instrument containing a 60 m x .32 mm ID Restek RTX-502.2 capillary column (phenylmethyl polysiloxane, 1.8 mm coating). Helium flow was 1 ml/min and the total time for an analysis was 48 minutes (5 min initial temperature 40 °C, ramp 5 °C/min to 220 °C, 7 minute hold time at 220 °C). Detection of the mass spectrum generally covered a range of 35 to 260 m/z.

Prior to any analyses, we performed a daily manual calibration tune of the mass spectrum detector to match previous performance characteristics. 1 uL of a methanol solution containing 25 ng of bromofluorobenzene (BFB) was injected and the resulting total ion chromatogram and mass spectrum examined. The mass detector was adjusted until the absolute abundance of the BFB peak on the total ion chromatogram was 11,000 $\pm$ 10% and the relative abundances on the mass spectrum were within guidance limits as outlined in earlier reports.

If the instrument did not pass on all eight tests (TIC and seven relative abundances), the mass detector was adjusted and a new BFB injection made. Given these procedures, relative sample concentrations, expressed as characteristic ion abundance/liter of air pumped, were reproducible with a maximum error of 10%.

4.2.4 Calibrations with Analytical Standards: The quantity of a contaminant represented by an absolute ion abundance was determined by volatilizing and sorbing known quantities of certified analytical standards into Carbotrap tubes.
The sorption was conducted in an apparatus designed to mimic, as much as possible, the same conditions found in field sampling (Figure 4.2). Ultrapure helium (Liquid Air) was metered through a 15- mm variable area flowmeter (Cole Parmer, E-03217-50) into a silanized quartz T

housed in an insulated block of ceramics. The flow rate was set to 120 ml/min to match the draw rate of the cassette pumps. Solutions containing known amounts of volatile organic analytes from ***EPA Method 524.1 (Supelco, VOC Calibration Standards Kit 4-8804)*** were injected through a GC septum in a second branch of the quartz T.

A resistance heater was used to volatilize the injected aliquot. The quartz T was maintained at a temperature of 100 °C, adjusted with a Calrad VC-5 variable power supply and monitored via a dual input digital thermometer (Omega HH12), to assure that the entire sample was volatilized. A Carbotrap tube to sorb the sample was attached through Teflon fittings to the final branch of the quartz T that placed it outside the ceramic block. A second temperature probe, outside the oven outlet where the sorption tube connected, was also monitored by the HH12 and assured that heat from the analyte volatilization process did not unduly warm the Carbotrap tube above temperatures experienced in field sampling.

A series of solutions was made such that 50, 100, 200, 400, 600, 800 and 1000 ng of each analyte could be easily dispensed from a gas-tight syringe into the heated quartz T of the calibration apparatus. An internal standard consisting of 250 ng 1,2-dichlorobenzene-d_4 (Supelco, 4-8948) was present in each final solution of the calibration procedure. Also added, as surrogates, were 250 ng each of fluorobenzene and 4-bromofluorobenzene (Supelco, 4-8083). Ion abundance *vs.* ng analyte data were plotted to evaluate linear response of the MS unit over the quantitation range chosen.

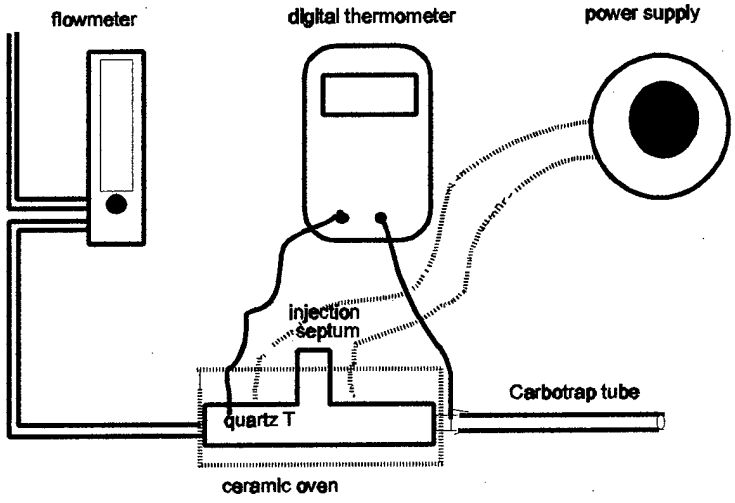


Figure 4.2. Calibration device for introducing the internal standard and system monitoring compounds into the TD/GC/MS sampling tubes. Samples are "pushed" onto the trap by a helium carrier gas flow from the flowmeter, but beyond that, sorbed in an analogous manner to field samples.

Throughout 1998, we followed a regular schedule of weekly calibration checks with certified standard solutions and a daily check with a derived standard mix. Concentrations contained in this report have been generated from the numeric factors of the closest weekly calibration check sample. Corrections for slight variations in thermal desorption and transfer were made using internal standard peak areas.

4.3 Chemical Results and Discussion

Ambient versus Hive Air Samples: ***A final comment about the nature of our reported hive atmosphere concentrations (air inside the beehive) is always warranted since these are fundamentally different from standard environmental samples.*** The ambient air samples taken at each site represent the more traditional, single point-in-space, single media sampling approach to environmental sampling. As with many air samples, these samples are time-averaged over several hours (usually 8-12 hrs).

The air samplers were not run for 24hrs from midnight to midnight, as if often done when monitoring air quality because high moisture conditions, as discussed, can lead to wet sample tubes and degradation or loss of the sample. At APG on the narrow Edgewood peninsula that extends into Chesapeake Bay, evening thunderstorms are common, the relative humidity tends to remain high, and the dew-point often is reached by morning. Inside a bee hive, the relative humidity tends to remain high, aggravating the moisture problems, especially at night. Because of these moisture problems, we have not sampled hive- or ambient air at night since our preliminary trials in 1995. The 1998 JAG boxes reduce but cannot eliminate this problem under severe wetting conditions such as are likely to be encountered at night at APG.

Hive atmospheres represent temporally- and spatially-weighted averages of accumulated bioavailable chemicals from multiple environmental sources. From a toxicological perspective, the cumulative exposure(s) to bioavailable chemicals constitutes the exposure hazard to a receptor (i.e., sentinel species, population, community, or ecosystem) at a specific site or location. Often, this is the exposure characterization metric that is the most useful but also the most difficult to obtain for use in an ecological risk assessment. In this case, the actual exposure to a sentinel population, which is considered to be a keystone species, has been directly measured and does not need to be estimated from concentrations in other environmental samples (i.e., water, soil, air, vegetation).

However, the concentrations reported for hive atmospheres can not be used to estimate or predict the concentrations that would actually be encountered in any single source, media, or point (time or space) within the surrounding environment. Because each of the hive air samples are collected over an 8 to 12 hr pumping period, they carry no indication of how the contaminant was sorbed over time. The colony could have been exposed to a constant low level or to a series of chemical spike episodes. Without additional "detection work," one can not be certain about how the contaminant made its way into the hive -- whether it wafted in from the ambient air, was carried in by a small or large fraction of the forager bee population, originated from an air-borne, dust-borne, water, or vegetation source (pollen, nectar, resin), was from a nearby or distant source(s), or resulted from a combination of some or all of these source and transport scenarios.

Although we have considerable knowledge about how the efficiency with which bees take up and transport various inorganic contaminants from a variety of sources, we are still building a knowledge base for organic chemicals, as well as about the role that electrostatic charges play in the uptake and transport of particulates (dusts and microbes) by bees. It is possible that some contaminants are retained to a greater degree than others. For example, certain lipophilic compounds may dissolve into the bees' exoskeletons while more polar compounds roll off after direct contact.

Finally, until recently, we did not have a direct method of determining where individual forager bees were going. We could look for bees at water sources, watch their incoming flights to determine direction, watch their dances to estimate direction and distance, or determine plants being visited by identifying the types of pollen spores being brought back to the hive. At this point, we have now taken delivery of the first of a variety of tools ranging from perfluorocarbon taggants to microchips that can be carried by individual bees and read as they return to the hive for use in marking and tracking of forager bees.

Sample Location Groups: For purposes of reporting and discussing the results of the chemical sampling, sample locations will be broken into the following groups: ***Old O Field, J Field, D Field, the Boundary Survey study and the Churchville reference site.*** Since we conducted sampling at some sites during the 1996 and/or 1997 field seasons, we include some comparative summaries as well in this report. Only 1998 results are available from D Field and the Boundary Survey sites, so no comparisons can be drawn for these three study areas.

The body of this report contains only a summary of the chemical results. A complete report by chemical, sample type, date, and site will be made available to APG project officers on a set of CD-ROMs. The CD's will provide the chemical data on a hive-by-hive basis for each in the form of Excel spreadsheets with accompanying maps and test. Even compressed, a complete three-year chemical data set combined with the real-time behavioral monitoring data set from APG requires more than a single CD-ROM for storage and retrieval. The entire data set is far to large to present in a useful form as a written report with raw data tables.

For each location we present a Table that indicates the highest **chemical exposure concentration** measured for each contaminant found within a hive at a given location or in the ambient air. These are tabulated in columns labeled with **"Max."** Again, it should be emphasized that the levels reported are time-weighted averages -- concentrations accumulated over the entire pumping period. A better indicator of the **long-term, low level chemical exposures** is tabulated in columns labeled **"Mean."** These values represent composite means over all of the hive or all of the ambient air samples gathered at a site. Unless otherwise noted, concentrations of organic contaminants are given in parts per trillion (by volume).

4.3.1 Volatile and Semi-Volatile Organic Contaminants—Overview: Hive atmospheres during the 1998 field season contained the same general types of volatile and semi-volatile organic contaminants as were seen in 1996 and 1997. Chlorinated hydrocarbon solvents found included perchloroethylene (PCE), trichloroethylene (TCE) and tetrachloromethane (TCM). Hexachloroethane (PCA), used in smoke obscurrants and sometimes disposed of in APG landfills, was only found at Old O Field in 1996. It was again completely absent from hive atmospheres in 1998. Dichlorobenzene (DCB), a solid pesticide often used to repel moths, was

seen, but only at low levels in most locations. There was an anomalous spike recorded for DCB on one occasion in the ambient air at Old O Field, but in the absence of other concurring data, this seems to be an uninterpretable artifact. The BTEX group of petroleum and gasoline residuals was almost ubiquitously present. We measured benzene (Benz), toluene (Tolu) and ethylbenzene (Etbnz) from the BTEX group. One day in late September, Old O Field had unusually high ethylbenzene levels in hive and ambient air samples; especially those collected from the site nearest the water treatment plant. We also found naphthalene (Naph), a component of diesel and creosote, present at most sites. We also noted the presence of acetophenone (AcPh) at some APG sites, which we suspect may have resulted from historic production of tear gas and subsequent degradation. Finally, we include levels of methenamine observed during 1998. While it is the principal reagent from which RDX is manufactured, it also sees other uses such as in adhesives, the vulcanization of rubber. Its presence in high levels at agricultural non-APG sites suggests that it has sources in agrochemicals. These have yet to be determined.

PCE and TCE in hive atmospheres were often at levels significantly in excess of ambient air. We suspect that either the bees were accumulating these chemicals from the air or these contaminants were contacted in some other form or source such as an organic film on standing water or moist soil particles. This was especially true with respect to the maximum levels seen; rarely were the ambient air maxima above the hive air maxima. TCM, BTEX and Naph occurred in ambient air at levels near those in the hive atmospheres. Since BTEX and naphthalene are known components of gasoline and diesel exhaust, they likely originate from vehicles or equipment in the vicinity of our sampling units. Increased levels of BTEX and naphthalene at Old O Field were seen following reopening of the road past the Old O Field landfill. The highest levels of these chemicals often were seen at those Boundary sites nearest to Baltimore or major highways. Both of these observations tend to support vehicles as a contributing source.

4.3.1.1 Old O Field: The 1998 field season marks the third year of data collection at Old O Field. Data from 1996 were collected while active site restoration activities were underway with the installation of an earth cover and sprinkler system. Data from 1997 represented the first year of post-capping measurements. We now have a second year of post-capping measurements (Table 4.1).

Maximum levels observed in 1998 for most contaminants followed the 1997 trends quite strongly. TCM, PCE and benzene were essentially unchanged from the previous year. Toluene, the contaminant seen at the highest concentration before completion of the cover, decreased following the capping, and showed another reduction in 1998, with the concentrations dropping from 1074 ppt in 1997 to 787 ppt in 1998. An abnormally high day for ethylbenzene was encountered on September 29th. Values rose to an unprecedented maximum of 5424 ppt in Condo 4 and 1063 ppt in the ambient air near the water treatment facility. Seven of the eleven samples surpassed 300 ppt and three topped the 1000 ppt mark. As can be seen in the seasonal averages, this single, high-day event skewed the average values for ethylbenzene. These are the highest ethylbenzene levels seen to date in the entire APG study since 1995. During the entire season, only three other readings at Old O Field exceeded 100 ppt. It is of interest to note that toluene and benzene, although higher on this date, were not the predominant BTEX members present. Usually, toluene has the highest ppt level, benzene is second and typically ethylbenzene is third.

Naphthalene was seen in the hives at a higher maximum value in 1998 (78 ppt) than in 1997 (10 ppt), but this was still only half of what was observed during 1996 (142 ppt) before the cap was

Table 4.1
Comparison of 1998, 1997 and 1996 Hive Atmosphere and Ambient Air (air) Levels of Old O Field Volatiles and Semivolatiles
Values are ppt by volume

Compound	1998 Max	1997 Max	1996 Max	1998 Mean (n=37)	1997 Mean (n=34)	1996 Mean (n=25)
TCM (air)	136 (131)	138 (82)	113 (37)	19 (50)	20 (25)	27 (24)
TCE (air)	11 (6)	172 (10)	188 (15)	1 (2)	13 (6)	19 (8)
PCE (air)	117 (38)	115 (81)	2814 (50)	14 (15)	21 (28)	207 (38)
DCB (air)	14 (308)	28 (21)	30 (6)	1 (32)	5 (7)	10 (3)
Benzene (air)	382 (192)	240 (165)	710 (197)	91 (104)	66 (78)	180 (113)
Toluene (air)	787 (858)	1074 (625)	3197 (323)	194 (236)	274 (227)	605 (206)
Ethylbenzene (air)	5424 (1063)	512 (146)	219 (220)	274 (158)	58 (48)	38 (4)
Naphthalene (air)	78 (18)	10 (17)	142 (4)	9 (4)	5 (6)	19 (1)
Acetophenone (air)	160 (37)	---	---	14 (5)	---	---
Methenamine (air)	353 (138)	---	---	57 (32)	---	---

completed. There was also a single, unusual DCB event (308 ppt) observed in the June 2nd sampling of Old O Field ambient air. Since DCB has not previously been detected in any of the hives at a level above 14 ppt, at this point we are classifying this as an unexplained anomaly.

Mean values for contaminants at Old O Field paralleled the maximum concentrations, though the averaging operation 'smoothed' the magnitude of the ethylbenzene and DCB episodes. There is evidence that both benzene and ethylbenzene exposures increased in 1998 compared to 1997.

There was also a continued presence of TCM in ambient air (50 ppt), with a mean that was somewhat more than in either of the two preceding years (25 ppt in 1997 and 24 ppt in 1996). TCM levels have always seemed to suggest a soil vapor release. The 1998 data continue to support this notion.

4.3.1.2 J Field: The incomplete restoration, the phytoremediation trees, and the ongoing removal activity at J Field, centered around the trenches immediately to the north of the instrumented condo cluster, probably contribute to the overall higher levels of several contaminants seen in both hive and ambient air samples compared to other APG sites. Samples collected during the 1998 field season were high in TCM, TCE, PCE, benzene, ethylbenzene and acetophenone compared to other sites.

J Field is definitely the APG site with the strongest presence of TCE. In 1998, both hive and air samples from J Field exhibited the highest single measurement values and the highest seasonal mean (Table 4.2). The 1998 hive maximum for TCE at J Field showed about a 75% drop compared to 1997, but an ambient air value captured on August 29th by a pump hanging in the yellow poplar grove was the highest seen in the four years of sampling at APG. The 1998 hive mean for TCE was 42% lower (10 ppt vs. 24 ppt). The ambient air TCE mean was unchanged from the previous field season (20 ppt in 1998 vs 18 ppt in 1997).

Another contaminant seen in 1998 J Field samples at elevated levels was TCM. On August 8th, a sample from one of the hives at the southeast margin of the poplar grove exhibited a reading of 403 ppt TCM, nearly twice as high as previously seen in any APG sample from any year. The 1998 TCM mean was nearly five times greater than in 1997 (47 ppt vs. 10 ppt). The 1998 ambient air TCM mean was also elevated over 1997 levels (34 ppt vs. 20 ppt). Because the hive and ambient air means are close in magnitude, a vapor release, possibly from soil, seems most likely for the general exposure path.

J Field also showed a pronounced presence of PCE in the air relative to other 1998 field sites. An ambient air sample collected on June 27th displayed a reading of 226 ppt PCE, the highest ambient level noted in the entire APG project. Perhaps the active trench work close to the sampling pump at the condo cluster was responsible for an enhanced release of volatiles that would have otherwise had more resistance to release. The PCE hive maximum at J Field for 1998 was more than four times that of the maximum in 1997 (64 ppt vs 15 ppt), more evidence that it was also available for the bees to contact in some manner. While the seasonal PCE mean for hive samples was lower than in 1997 (15 ppt vs. 23 ppt), the ambient air mean was about half again as high as ambient levels in 1997 (34 ppt vs. 23 ppt). The 34 ppt average was the largest PCE mean among 1998 APG field sites, although lower than for some of the Off-Base sites in the 9-mile radius subset.

In J Field samples, acetophenone levels, which may reflect the presence of tear gas breakdown products, were among the highest for all of the 1998 field samples. The 162 ppt measured on

Table 4.2
Comparison of 1998 and 1997 J Field Hive Atmosphere and Ambient Air (air) Volatiles and Semivolatiles
Values are ppt by volume

Compound	1998 Max	1997 Max	1998 Mean (n = 56)	1997 Mean (n = 24)
TCM (air)	403 (101)	58 (78)	47 (34)	10 (20)
TCE (air)	52 (80)	224 (36)	10 (20)	24 (18)
PCE (air)	64 (226)	118 (38)	15 (34)	23 (23)
DCB (air)	9 (9)	45 (23)	0.4 (0.9)	3 (6)
Benzene (air)	218 (272)	170 (160)	86 (131)	55 (55)
Toluene (air)	618 (738)	1786 (285)	204 (243)	206 (163)
Ethylbenzene (air)	1486 (234)	305 (45)	95 (68)	36 (32)
Naphthalene (air)	43 (22)	135 (21)	5 (5)	18 (8)
Acetophenone (air)	369 (162)	---	55 (32)	---
Methenamine (air)	221 (235)	---	51 (41)	---

June 27th in the ambient air near the survey hives to the north of the trench activity was the largest single value for the field season. Overall, the mean hive air values were somewhat higher than the means for air. Also, the mean concentrations of acetophenone for the collective hive air and for the ambient air samples (55 ppt and 32 ppt, respectively) were the highest of all of the sites sampled in 1998.

For the most part, contaminant levels for the remainder of contaminants found at J Field were similar to those seen at many other locations. Among the BTEX suite, a 272 ppt ambient air level for benzene, captured by a pump suspended from a limb of a yellow poplar in the phytoremediation grove, was close to the maximum air level observed outside a hive. Other BTEX levels were well below maxima seen elsewhere. Also somewhat elevated was the

ambient air level of methenamine, among other things, a precursor of RDX. Its value of 235 ppt was second only to the 265 ppt recorded in a sample from Carroll Island.

4.3.1.3 D Field: The D Field sites are divided into three subgroups based on their locations: DF1 - at the northern end of the road ending at Point Briery; DF2/3 - locations to the east side of the access road leading to Point Briery; and DF4/5 - north of the moving target track and tank range near Sandy Point. DF4 hives were located on the bench north of the tank range and slightly east of the tower structure. The DF5 hives were on a shoulder near Sandy Point, about 50 m north of moving target track.

With the exception of TCE, D Field samples contained the seasonal highs, of one type or another, for every contaminant we quantified (Table 4.3) at the 1998 on-post APG sites. It should be mentioned, however, that some Off-Base sites had values in excess of those collected at D Field locations.

The six survey hives situated in the vicinity of Briery Point exhibited the seasonal highs for TCM in ambient air. This was true for both the single sample high (186 ppt on August 9th) as well as the seasonal mean (123 ppt). Because ambient levels of TCM were at or above hive levels, a soil vapor source is suggested. The highest PCE levels in hives were also recorded in the D1 area. The hive designated as DF1.5 exhibited the highest single sample value of 369 ppt during the September 30th sampling round. The seasonal average for hive PCE was 40 ppt, definitely influenced by the single large value from DF1.5. Unlike TCM Values, the hive levels were substantially higher than ambient air at D1, so this contaminant was either concentrated from the air by the bees, entered the hive with the pollen, nectar, or resin, or, most likely, was probably encountered as a film on standing water or moist soil where puddles had subsided. Hive DF1.4 gave rise to the highest hive DCB level observed (67 ppt in DF1.2 on August 12th) plus the highest hive toluene level (1320 ppt in DF1.2 on August 12th). These probably came into the hive together as components of an organic film mixture. The highest hive acetophenone level (582 ppt) was seen in the sample from hive DF1.4 also on August 12th. Because hive DF1.4 was physically located about 150 m east of DF1.2, bees from this colony were probably collecting water from a different source, since water gatherers usually go for the near by water. Finally, the DF1 site showed the largest mean level of ethylbenzene in ambient air -- a value of 208 ppt. This was more than twice the magnitude of mean ethylbenzene levels in ambient air at any other site.

Only two seasonal maxima were encountered at the DF2/3 sites and both were associated with BTEX components. The DF2/3 hive mean for benzene (121 ppt) was identical to that found at DF4/5 and the highest benzene mean recorded among APG sites. This was substantially less,

Table 4.3
1998 D Field Hive Atmosphere and Ambient Air (air) Volatiles and Semivolatiles
Values are ppt by volume

Compound	DF1 Max	DF1 Mean n=13	DF2/3 Max	DF2/3 Mean n=5	DF4/5 Max	DF4/5 Mean n=16
TCM (air)	183 (186)	40 (123)	57 (41)	26 (14)	342 (111)	48 (39)
TCE (air)	15 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)
PCE (air)	369 (24)	40 (12)	37 (15)	17 (10)	46 (29)	12 (13)
DCB (air)	67 (0)	6 (0)	30 (0)	6 (0)	47 (0)	3 (0)
Benzene (air)	224 (223)	68 (116)	178 (169)	121 (138)	722 (198)	121 (108)
Toluene (air)	1320 (171)	349 (155)	660 (1222)	321 (514)	481 (227)	184 (121)
Ethylbenzene (air)	1301 (611)	179 (208)	444 (52)	124 (20)	265 (138)	71 (39)
Naphthalene (air)	40 (2)	5 (1)	13 (27)	4 (11)	96 (40)	7 (7)
Acetophenone (air)	582 (21)	48 (7)	31 (0)	12 (0)	194 (41)	20 (6)
Methenamine (air)	314 (85)	63 (64)	86 (41)	33 (14)	432 (79)	71 (25)

however, than the highest Off-Base hive benzene mean of 162 ppt seen for the northwest transect of the Boundary Study. The second DF2/3 maximum was established by the ambient air level of toluene recorded at DF3 on August 12. This value was undoubtedly a reflection of the pump placement atop a 55-gallon drum. The inlet end of the copper tube was placed near the edge of the barrel lid to characterize any emissions that might emanate from the barrel contents. This is probably not a reflection on the actual background level at that site. High as it is, however, it is less than half that seen in the air at Lohr's Orchard, Off-Site, on June 9th.

The final D Field area monitored by hives, the DF4 and DF5 sites, housed eight survey colonies. The original DF4 pair were located immediately to the east of a line of trees that separated from an abandoned tower structure. Two additional pairs of hives were added after the initial sampling round indicated substantial presence of some contaminants, especially acetophenone.

By the field season's end, four seasonal maxima were associated with DF4/5 measurements. The mean hive level of benzene at 121 ppt was the same as that for DF2/3, but lower than the Off-Site mean previously noted for the northwest transect. The maximum single hive level for benzene (722 ppt) over the entire 1998 field season, though, was noted in one of the original DF4 hives, Hive DF4.1, on August 9th. This was two times greater than the maximum Off-Base benzene maximum of 396 ppt.

The 40 ppt of napthalene in the ambient air at the DF5 site on a hot day, August 12th , was the highest air concentration recorded at APG. This episode might be explained by the proximity of the moving target track. Railroad ties, treated with creosote as a wood preservative, often off-gas naphthalene when heated by direct sun. Based on field and laboratory blanks, this value did not appear to be form a sampling or analytical error.

Finally, the largest APG hive level of methenamine (432 ppt) was detected in a sample collected from Hive DF4.5 on September 30th. The sample from Hive DF4.4 was also high (320 ppt) on the same date, indicating that the substance was contacted by more than a single hive in that vicinity. D Field is not an unexpected location at which to see methenamine since it is adjacent to an active tank firing range. Active shooting, in fact, required special arrangements for sampling access during the September sampling trials.

Both of these hive levels are well below the Off-Base hive maximum for methenamine. A value of 1917 ppt was recorded in a Lohr's Orchard hive on October 27th, a level of 1558 ppt at the Cylburn Arboretum on August 14th, and a high of 2513 ppt in a Silver Lake Drive hive on October 29th. Obviously, nonexplosive sources of methenamine are present at these Off-Site locations.

4.3.1.4 The 1998 Boundary Study: The 1998 Boundary Study was designed to evaluate how much Off-Base drift of chemical contaminants might be occurring. The study design established a network of on-base survey hives placed at six locations and three directional transects generally oriented to the north, the northwest and the southwest. In addition, survey hives were situated along each transect such that a radial pattern was also established at 3 miles, 9 miles and 21 miles from the base. For purposes of this discussion, data are presented as the northern set of base sites, the southern set of base sites, a direction-based summary of Off-Base sites and a radial-based summary of Off-Base sites.

4.3.1.4.1 The Northern APG Site Subset: The north subset of APG sites for the 1998 Boundary Study included three survey hives near the Youth Center, a pair of survey hives at Cluster 13 and a pair of survey hives near the Fragmentation Pit facility on the main Aberdeen upper post area. Among these sites, four 1998 seasonal maxima were encountered for APG sampling locations. Beyond that, these sites could be characterized as having below average BTEX levels, a slight presence of TCM and some moderate levels of methenamine (Table 4.4).

The Fragmentation Pit site held the highest mean methenamine level for ambient air, 159 ppt. Given the character of its setting, a high value here could represent an indication of RDX residues in the vicinity.

Custer 13 held the highest maximum (400 ppt) and mean (183 ppt) for methenamine in any of the hives on APG Edgewood sites. It was about half the high hive mean for methenamine off the base, a reading of 304 ppt on average for the southwest transect which had exceptionally high individual readings at both the Silver Lake Drive and Cylburn Arboretum locations. Cluster 13 was also the location of the largest seasonal hive maximum and mean for DCB at 26 and 7 ppt, respectively. This is comparable to last year's highest mean, a 10 ppt level at Cluster 3. Cluster 13 also displayed elevated napthalene in hive atmospheres compared to other northern base sites, 101 ppt maximum compared to 7 ppt at the Fragmentation Pit and 4 at Youth Center. However, the hives at Carroll Island contained three times as much napthalene (309 ppt).

The Youth Center only gave rise to one APG-wide maximum for the 1998 season -- the mean naphthalene in ambient air concentration -- at 12 ppt. This might be a consequence of its proximity to major streams of traffic entering the Edgewood Area of the base and of the large, paved parking areas immediately to the southeast of the hive placement. Naphthalene is strongly associated with diesel fuel residuals. Most of the other APG sites on the upper post were farther removed from traffic flows and parking lots.

4.3.1.4.2 The Southern APG Site Subset: The Southern subset of APG Boundary Study sites included two survey hives near the bend on Westwood Road, two survey hives east of the tower at Graces Quarters, and two survey hives at the south end of the middle promontory of Carroll Island. Carroll Island had a number of rather high levels of contaminants when compared to other APG sites (Table 4.5). The Westwood Road site was prominent in its ambient air levels of benzene. Graces Quarters displayed moderate levels of most contaminants.

The Carroll Island site held APG season highs with respect to hive mean for toluene (355 ppt), hive maximum for naphthalene (309 ppt), hive mean for naphthalene (46 ppt) and ambient air maximum for methenamine (265 ppt). The levels seen in the CI1 sample (hive 98120) of June 18th show relatively hive levels across the whole spectrum of contaminants. This hive was queenless in the July 7th hive log and a new one was introduced. A subsequent round of

Table 4.4

1998 Boundary Survey Study Hive Atmosphere and Ambient Air (air) Volatiles and Semivolatiles - Northern Base (APG) Sites

Values are ppt by volume

Compound	Y Ctr Max	Y Ctr Mean n=7	Cl 13 Max	Cl 13 Mean n=4	Frag Pit Max	Frag Pit Mean n=4
TCM (air)	57 (22)	23 (14)	24 (45)	11 (27)	55 (0)	17 (0)
TCE (air)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
PCE (air)	28 (17)	10 (6)	18 (17)	8 (15)	28 (0)	13 (0)
DCB (air)	13 (0)	2 (0)	26 (0)	7 (0)	0 (0)	0 (0)
Benzene (air)	144 (178)	109 (121)	77 (95)	51 (69)	113 (24)	58 (24)
Toluene (air)	778 (102)	268 (79)	633 (119)	241 (71)	624 (155)	222 (155)
Ethylbenzene (air)	172 (26)	39 (12)	62 (39)	30 (19)	100 (53)	34 (53)
Naphthalene (air)	4 (36)	1 (12)	101 (3)	28 (2)	8 (4)	4 (4)
Acetophenone (air)	4 (0)	1 (0)	27 (0)	8 (0)	7 (0)	3 (0)
Methenamine (air)	289 (71)	91 (30)	400 (0)	138 (0)	118 (159)	66 (159)

Table 4.5
1998 Boundary Survey Study Hive Atmosphere and Ambient Air (air) Volatiles and Semivolatiles - Southern Base (APG) Sites
Values are ppt by volume

Compound	Wwd Max	Wwd Mean n=6	G Qtr Max	G Qtr Mean n=6	Crl Is Max	Crl Is Mean n=7
TCM (air)	49 (65)	14 (35)	68 (75)	25 (65)	161 (40)	39 (21)
TCE (air)	0 (14)	0 (8)	0 (0)	0 (0)	16 (0)	2 (0)
PCE (air)	31 (25)	8 (16)	30 (13)	12 (8)	38 (20)	10 (11)
DCB (air)	14 (9)	2 (3)	0 (0)	0 (0)	24 (0)	3 (0)
Benzene (air)	194 (275)	115 (159)	101 (96)	52 (81)	248 (119)	78 (74)
Toluene (air)	635 (329)	236 (204)	895 (133)	245 (77)	775 (269)	355 (168)
Ethylbenzene (air)	255 (162)	93 (100)	79 (10)	34 (3)	154 (35)	54 (25)
Naphthalene (air)	14 (16)	5 (5)	8 (8)	3 (4)	309 (15)	46 (4)
Acetophenone (air)	21 (13)	5 (4)	11 (8)	2 (4)	180 (7)	29 (2)
Methenamine (air)	28 (11)	5 (5)	112 (5)	25 (2)	217 (265)	104 (115)

sampling on August 10th again showed very high levels of BTEX compounds in the hive. When the hive was reinspected on October 15th, no live bees were present and wax moths were feeding on hive stores.

The Westwood Road site proved to have the highest APG levels of benzene in ambient air from both a daily maximum (275 ppt) and a seasonal mean (159 ppt) standpoint. A Westwood ambient air sample from September 29th contained 275 ppt benzene. The seasonal mean for ambient air benzene at Westwood was 159 ppt. Both the daily and seasonal mean maxima at Westwood Road were considerably lower than ambient benzene maxima measured at Off-Base sample locations. The Off-Base ambient air benzene maxima were a daily reading of 513 ppt on

July 7th at Farview Manor and a seasonal mean for ambient benzene on the northwest transect of 213 ppt.

4.3.1.4.3 The North Off-Base Transect: The north Off-Base transect consisted of three pairs of survey hives—1 pair at an Estuary Science Center and 2 pairs in orchards. The 3-mile pair was located on the edge of a wooded area next to a paved parking lot at the Otter Creek Point Estuary Science Center. The 9-mile pair was located underneath some trees that bordered produce fields and orchards at Lohr's Orchard. The 21-mile pair was located beneath a hedge row of trees adjacent to fruit orchards near Conowingo, MD in Cecil county. The Otter Creek location was near Highway 40; the others were located farther from major highways.

Contaminant levels on the Off-Base transects qualitatively often looked fairly similar to those at APG sites (Table 4.6) and, in numerous instances, were even greater than the maximum values observed at hazardous APG sites. On June 9th, for example, the level of toluene seen in Hive 98111(1391 ppt) was greater than any seen at APG during the 1998 field season. The other hive at this location was listed as "dead" on the June 29th hive inspection log. We speculate that the high levels of contamination being brought into the hive may have stressed or killed this hive. A possible source of the toluene was a pair of auto repair shops. The owner of the orchard property said that he had complained to Maryland officials about oil slicks on his ponds from the repair shops.

Lohr's Orchard on this transect also showed some high contaminant levels. Among the Off-Site hives, a Lohr's Orchard sample from June 9th held the maximum ambient air levels of PCE (88 ppt), DCB (20 ppt), toluene (2667 ppt) and ethylbenzene (221 ppt). The toluene concentration was more than twice that seen anywhere among APG sites.

The north transect, by virtue of the high air sample at Lohr's Orchard held the highest seasonal averages among non-APG directional sites for DCB (4 ppt), toluene (580 ppt) and ethylbenzene (51 ppt).

Table 4.6
1998 Boundary Survey Study Hive Atmosphere and Ambient Air (air) Volatiles and Semivolatiles - Directional Summary of Off-Base Transects
Values are ppt by volume

Compound	North Max	North Mean n=16	NW Max	NW Mean n=16	SW Max	SW Mean n=18
TCM (air)	75 (69)	16 (31)	252 (156)	25 (72)	87 (102)	36 (55)
TCE (air)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
PCE (air)	31 (88)	9 (23)	75 (80)	29 (30)	54 (54)	13 (11)
DCB (air)	0 (20)	0 (4)	27 (0)	2 (0)	0 (0)	0 (0)
Benzene (air)	238 (362)	86 (157)	396 (513)	162 (213)	275 (210)	115 (107)
Toluene (air)	1391 (2667)	248 (580)	1357 (526)	563 (207)	1479 (181)	373 (96)
Ethylbenzene (air)	92 (221)	19 (51)	660 (139)	170 (41)	182 (53)	31 (23)
Naphthalene (air)	10 (14)	2 (4)	32 (40)	6 (9)	25 (20)	4 (7)
Acetophenone (air)	51 (19)	4 (5)	130 (41)	13 (7)	11 (4)	2 (1)
Methenamine (air)	1917 (35)	121 (19)	640 (18)	102 (5)	2513 (61)	304 (12)

4.3.1.4.4 The Northwest Off-Base Transect: The northwest transect of Off-Base sites include pairs of hives, each set in a farming/orchard location. The 3-mile survey hive pair was set between two fields at the Jones Farm nursery and produce stand on Old Philadelphia Road, the 9-mile pair was tucked under some power transmission lines at the Tower Hill Farm near Fallston, and the 21-mile pair at the corner of a house at the Farview Manor landscaping and

nursery in Shawsville, MD. Despite the more rural appearance of these three sites, Jones Farm and Tower Hill were both near the intersections of high traffic roads.

Samples from the northwest transect were generally higher in contaminant residues than the other two transects (Table 4.6). Each of the three sites contributed more than one Off-Base high for the season. The Jones Farm site gave rise to the highest Off-Base levels of PCE in hive air (75 ppt), benzene in hive air (396 ppt), naphthalene in ambient air (40 ppt) and acetophenone in ambient air (41 ppt). Jones Farm sits on the intersection of two roads, is just off Highways 95, 40, and 24, and is adjacent to several small businesses such as convenience stores and a motorcycle dealer. Tower Hill Farm held the Off-Base seasonal highs for DCB in hive air (27 ppt) and ethylbenzene in hive air (660 ppt). Farview Manor samples exhibited seasonal Off-Base highs for TCM in both hive and ambient air (252 ppt and 156 ppt, respectively) and benzene in ambient air (513 ppt). The benzene hit at Farview Manor exceeded any APG sample level. Although vehicular traffic appeared to be lighter at this site than at the other two northwestern sites, there was heavy equipment and outbuildings nearby as part of the landscaping business. This site was also on the edge of a small town and next to a gasoline station/convenience store complex.

The northwest transect showed seasonal means above the northern and southwestern transects with respect to PCE, benzene and acetophenone. The benzene and toluene means for this set of sites (213 ppt and 580, ppt, respectively) were greater than that for any APG Boundary Study site. We speculate that this may be related to incidents such as petroleum spills, agricultural chemicals, and fugitive emissions that are common in residential and farm communities and usually less closely regulated and monitored than similar scenarios on a military post. Spills lead to organic films that can be contacted by honey bees foraging for water in standing puddles or vegetation growing in contaminated soils. Ambient air levels are probably high in BTEX compounds because of sources such as displaced headspace vapors during refueling operations and unburned tailpipe hydrocarbons. The BTEX components are more resistant to photochemical processing and, thus, persist longer in the airshed. Again, two of these three sites are near heavily traveled roads.

4.3.1.4.5 The Southwest Off-Base Transect: The southwest set of Off-Base sites employed a 3-mile pair of survey hives under an apple tree at the Rumsey Mansion, a 9-mile pair at Silver Lake Drive near White Marsh, and a 21-mile pair placed at a lawn margin of the Cylburn Arboretum north of downtown Baltimore. These sites were by far the most urban. All three are located in residential neighborhoods. The Silver Lake and Arboretum sites are close to the Baltimore Beltway and feeder highways.

Contaminant levels on the southwest transect were generally moderate in nature (Table 4.6). Only the Silver Lake Drive site yielded individual samples with seasonal maximum levels and only three directional means were highest on the southwest transect. Like the other Off-Base transects, some of the BTEX compounds tended to be high along this transect compared to the southern base sites. For example, toluene maxima for the southern on-post hives ranged from 775-895 ppt in hives compared to a maximum of 1479 ppt in a sample from the Silver Lake Drive site on June 18th. This was the highest daily level among all 1998 field sites for toluene in hive air. Despite this high concentration, the colony was reported as healthy on the June 29 hive inspection report; although one of the Silver Lake hives was lost before the end of the field

season. The same hive at Silver Lake Drive provided a sample on October 29th that contained the 1998 high for methenamine (2513 ppt). In the absence of a gradient of methenamine concentrations radiating out from APG, this adds further support to the contention that methenamine has off-post sources other than RDX residues.

The southwest transect led the Off-Base seasonal means in three categories - TCM in hive air (36 ppt), DCB in hive air (2 ppt) and methenamine in hive air (304 ppt). The first two were considerably less than means seen at some APG sites. Given the very high methenamine levels seen on October 29th at both Silver Lake Drive (2513 ppt) and Cylburn Arboretum (1558 ppt), the southwest transect seasonal mean for methenamine exceeded levels found in any APG seasonal mean. A likely source for high hive levels of methenamine could be standing pools of water in old tire casings since methenamine is used as a vulcanizing agent in some rubber compounding.

4.3.1.4.6 Radial Trends in Off-Base Boundary Study Sites: To assess any general distance trends in Off-Base drift of contaminants, the Boundary Study sites on the three transects were also summarized on the basis of their radial distance from a central APG site, the Youth Center hives (Table 4.7). Several useful results can be drawn from these data.

When organized into radial subsets, Boundary Study sites at 9-miles seem to predominate as sites giving rise to higher levels of contaminants. This supports that the source of these agents is not related to APG sources. There is little likelihood that Off-Base drift could consistently overfly the 3-mile sites without some deposition occurring. Sites at 9- and 21-miles give ambient air cumulative means that exceed any other 1998 means for benzene (218 ppt) and toluene (627 ppt). The 9-mile ambient ethylbenzene mean (57 ppt) is highest among Off-Site means. Benzene toluene and ethylbenzene are strongly associated with petroleum resides and predominate at this radius because major highways happen to be routed closest to this subset.

Table 4.7
1998 Boundary Survey Study Hive Atmosphere and Ambient Air (air) Volatiles and Semivolatiles - Radial Summary of Off-Base Sites
Values are ppt by volume

Compound	3 Mi Max	3 Mi Mean n=17	9 Mi Max	9 Mi Mean n=15	21 Mi Max	21 Mi Mean
TCM (air)	87 (80)	19 (50)	75 (97)	22 (45)	252 (156)	37 (74)
TCE (air)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
PCE (air)	72 (22)	18 (11)	57 (88)	16 (28)	75 (80)	17 (33)
DCB (air)	0 (0)	0 (0)	27 (20)	2 (4)	0 (0)	0 (0)
Benzene (air)	396 (201)	102 (119)	354 (362)	151 (172)	275 (513)	128 (218)
Toluene (air)	1357 (243)	262 (103)	1479 (2667)	518 (627)	1391 (526)	430 (192)
Ethylbenzene (air)	508 (139)	50 (29)	660 (221)	135 (57)	147 (53)	43 (31)
Naphthalene (air)	28 (40)	5 (8)	32 (14)	4 (7)	18 (15)	3 (5)
Acetophenone (air)	130 (41)	8 (5)	48 (19)	5 (4)	51 (15)	6 (5)
Methenamine (air)	248 (35)	72 (11)	2513 (33)	380 (22)	1558 (0)	171 (0)

4.3.1.5 Edgewood Area Youth Center: Because chemical residues measured at the Youth Center in previous years had consistently higher hive contaminant levels than other Edgewood residential and office area sites, the YC results have been separated out into a 3-year summary (Table 4.8). The troublesome high levels of chlorinated organic solvents from previous years seem to have subsided, especially with respect to TCE and PCE. TCM has remained relatively steady over the 3-year period, suggesting a permanent source such as soil vapors as the origin of this contaminant.

Only levels for fossil fuel related contaminants remained high at this site in 1998. Again, we speculate that this may be from vehicular traffic since the hives were located close to one of the main entrance gates to the Edgewood area.

Contaminant levels observed in locations at APG known to be exposed to potentially hazardous chemicals substantially eclipse the 1998 Youth Center levels. Samples from Old O Field, J Field and D Field and Carroll Island easily surpass those seen here. At this time, we speculate that either construction activities, occurring nearby during the 1996 and the first part of 1997, or a portable organic analysis laboratory placed just across the street from the hives, may have given rise to the previously high chlorinated solvent samples.

4.3.1.6 Churchville Reference Site: As in previous years, a farm near Churchville, MD was used as an Off-Base reference site to provide a snapshot of regional levels of contaminants that do not originate at APG. The farm's owner, David Simmons, is a chemical engineer/hobbyist beekeeper. The farm is approximately 10 miles to the north of the Edgewood area, generally upwind of APG and local industry. It fell just slightly off the north transect for the Boundary Study, but was close enough to add an extra data point in that direction for trend analysis.

A three-year comparison (1996 to 1998) among contaminant levels of volatiles and semivolatiles at Churchville is summarized in Table 4.9. The data reveal that levels measured at this site, like others, are subject to isolated episodes of specific releases. In 1998, for example, there were two very large naphthalene "hits" in hive samples from October 27th. These were 4130 ppt in CV2 and 3710 ppt in CV5. We speculate that this may have resulted from scenarios such as two colonies collecting water from a standing pool that had a diesel film covering it or the parking of tractor and mower near the hives while moving wires and walkways out of the way. Naphthalenes comprise about 85% of persistent diesel residues. Taken together, these two samples skewed the overall mean at Churchville to a 1998 field season high of 479 ppt, well above any other location On- or Off-Base.

Churchville also had a relatively high presence of methenamine in the ambient air samples. Its daily ambient air maximum of 135 ppt and seasonal ambient air mean of 45 ppt were highest among Off-Base sites.

Table 4.8
Comparison of 1998, 1997 and 1996 Levels of
Youth Center Volatiles and Semivolatiles
in Hive Atmospheres and Ambient Air (air)
Values are ppt by volume

Compound	1998 Max	1997 Max	1996 Max	1998 Mean (n = 7)	1997 Mean (n = 11)	1996 Mean (n = 4)
TCM (air)	57 (22)	68 (21)	69 (53)	23 (14)	26 (8)	39 (49)
TCE (air)	0 (0)	50 (1)	355 (67)	0 (0)	13 (0.4)	96 (39)
PCE (air)	28 (17)	18 (22)	158 (148)	10 (6)	10 (10)	53 (84)
DCB (air)	13 (0)	6 (6)	23 (20)	2 (0)	2 (2)	9 (13)
Benzene (air)	144 (178)	77 (131)	468 (382)	109 (121)	32 (59)	206 (476)
Toluene (air)	778 (102)	233 (345)	861 (593)	268 (79)	140 (166)	364 (417)
Ethylbenzene (air)	172 (26)	47 (74)	58 (42)	39 (12)	20 (34)	27 (29)
Naphthalene (air)	4 (36)	5 (15)	26 (8)	1 (12)	2 (6)	8 (8)

Table 4.9
Comparison of 1998, 1997 and 1996 Levels of
Churchville Volatiles and Semivolatiles
Values are ppt by volume

Compound	1998 Max	1997 Max	1996 Max	1998 Mean (n=18)	1997 Mean (n=19)	1996 Mean (n=19)
TCM (air)	93 (86)	66 (15)	79 (52)	32 (29)	16 (13)	18 (34)
TCE (air)	0 (0)	21 (18)	2564 (17)	0 (0)	5 (9)	183 (6)
PCE (air)	38 (11)	183 (48)	70 (36)	14 (4)	16 (24)	18 (26)
DCB (air)	9 (0)	16 (65)	23 (12)	1 (0)	4 (32)	5 (5)
Benzene (air)	167 (147)	86 (110)	275 (106)	92 (93)	37 (72)	67 (186)
Toluene (air)	421 (93)	409 (896)	1422 (328)	218 (44)	101 (472)	283 (204)
Ethylbenzene (air)	171 (10)	72 (274)	74 (22)	49 (6)	19 (140)	18 (17)
Naphthalene (air)	4130 (2)	50 (13)	253 (12)	479 (1)	6 (7)	19 (1)
Acetophenone (air)	52 (0)	---	---	9 (0)	---	---
Methenamine (air)	225 (135)	---	---	64 (45)	---	---

4.3.1.7 Summary Comparison of APG vs. Off-Site Contaminant Levels: Several data presentations have been generated to permit comparisons of contaminant levels seen on and off APG sites. The 1998 daily maxima and 1998 season meals have been collected into Tables 4.10 and 4.11. These are presented without additional discussion since each maximum entry in the two tables was addressed within previous sections for specific sites. It is worth repeating, however, that the highest levels of nearly all contaminants are associated with known or suspected sources of these hazardous materials (e.g., landfills, removal activities, contaminated groundwater, vehicular traffic, agricultural chemicals, explosives, tear gas). These chemicals were detected by sampling of the bees, hive atmospheres, and the ambient air. The bees served

as a sampling device for detection these chemicals and did not reveal any unlikely chemical exposure scenarios. In other words, the results were consistent with the historical and current activities the would have contributed to the chemical exposure fingerprint for each site.

Finally, an overall picture of the seasonal and spatial distribution of contaminants from the 1998 Boundary Study can be seen in the three chemical tree maps that have been prepared. On the maps, a chemical tree has been drawn in the vicinity of each site that pictorially represents the contaminant levels in each hive and the ambient air as shown in Figure 4.3.

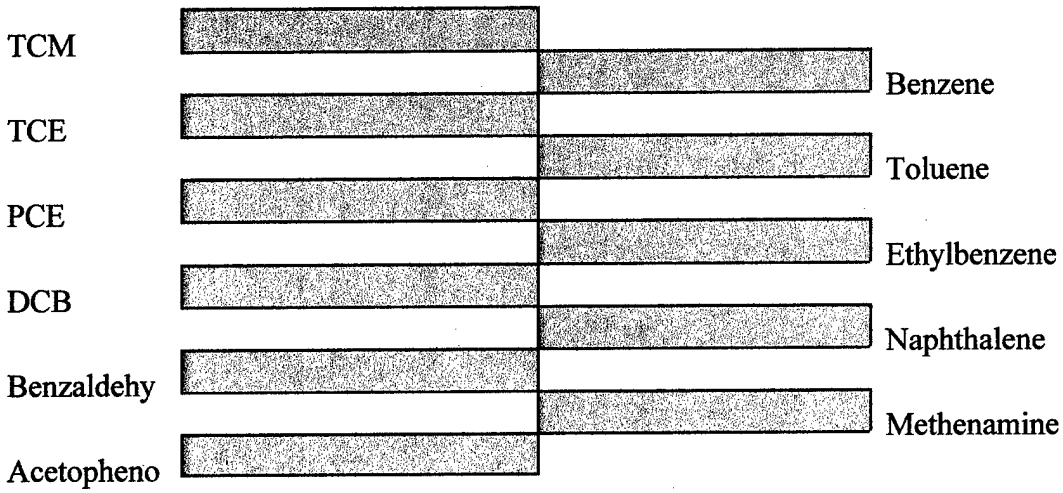


Figure 4.3. Contaminant assignments for chemical tree branch locations. Length of branches is scaled such that the maximum concentration seen in Table 4.10 is assigned a full branch length.

There is one map for each of the sample rounds -- early summer (June samples), late summer (August samples) and fall (October samples). Branch locations identify which contaminant is being addressed (see the maps in Figures 4.4-4.6 below) and branch length gives a comparative concentration relative to other sites. Branch lengths were scaled such that the seasonal daily maximum of Table 4.10 is assigned a full length. Note that the "fullest" chemical trees are often associated with Off-Base sites, particularly those members of the 9-mile radius subset. Contaminants are grouped with chlorinated organics appearing on the top left, petroleum-based contaminants on the upper left, and compounds of interest to the military on the lower branches of both sides.

Finally, the data presented in Tables 4.4-4.6 also is plotted in Figures 4.7-4.9. Although somewhat redundant to the data presented in the Tables, these plots underscore the effects of local sources on the levels of VOCs and SVOCs at sites near to and in the communities surrounding APG Edgewood.

Table 4.10
Comparison of Hive Atmosphere and Ambient Air (air) Daily Maxima for APG Sites Versus Off-Post (Boundary) Sites
Values are ppt by volume

Compound	1998 APG Max	1998 APG Site	1997 APG Max	1997 APG Site	Off-Post Max	Off-Post Site
TCM (air)	403 (186)	J Field (DF1)	209 (83)	Canal Ck (NG)	252 (156)	Farview (Farview)
TCE (air)	52 (80)	J Field (J Field)	224 (44)	J Field (BR3)	2574 (18)	CV '96 (CV '97)
PCE (air)	369 (226)	DF 1 (J Field)	158 (81)	Cluster 3 (O Field)	183 (88)	CV '97 (Lohrs Or)
DCB (air)	67 (308)	DF 1 (O Field)	91 (23)	Cluster 3 (J Field)	27 (65)	Tower Hill (CV '97)
Benzene (air)	722 (275)	DF 4/5 (Westwd)	282 (165)	Bush R 10 (O Field)	396 (513)	Jones Farm (Farview)
Toluene (air)	1320 (1222)	DF 1 (DF 2/3)	3156 (625)	Bush R 10 (O Field)	1479 (2667)	Silver Lk (Lohrs Or)
Ethylbenzene (air)	5424 (1063)	O Field (O Field)	512 (431)	O Field (Bush R 9)	660 (274)	Tower Hill (CV '97)
Naphthalene (air)	309 (40)	Carroll Is (DF 4/5)	135 (79)	J Field (Bush R 5)	4130 (40)	CV '98 (Jones Farm)
Acetophenone (air)	582 (162)	DF 1 (J Field)	---	---	130 (41)	CV '98 (Jones Farm)
Methenamine (air)	432 (265)	Cluster 13 (Carroll Is)	---	---	2531 (135)	Silver Lk CV '98

Table 4.11
Comparison of Hive Atmosphere and Ambient Air (air) Seasonal Means for APG Sites Versus Off-Post (Boundary) Sites
Values are ppt by volume

Compound	1998 APG Max Mean	1998 APG Site	1997 APG Max Mean	1997 APG Site	Max Off-Site Mean	Off-Base Site
TCM (air)	48 (123)	J Field (D Field)	27 (37)	Canal Ck (Up Edgwd)	36 (74)	SW (21 mi)
TCE (air)	52 (80)	J Field (J Field)	24 (18)	J Field (J Field)	183 (9)	CV '96 (CV '97)
PCE (air)	40 (34)	D Field (J Field)	23 (28)	J Field Old O Field	29 (33)	NW (21 mi)
DCB (air)	7 (32)	Cluster 13 O Field	10 (7)	Cluster 3 (Old O Field)	5 (32)	CV'96 (CV '97)
Benzene (air)	121 (159)	D Field (Westwd)	66 (78)	Old O Field (Old O Field)	162 (218)	NW (21 mi)
Toluene (air)	355 (514)	Carrol Is D Field	305 (284)	Bush R. (Cluster 3)	563 (627)	NW (9 mi)
Ethylbenzene (air)	274 (208)	O Field (D Field)	58 (48)	Old O Field (Old O Field)	170 (140)	NW (CV '97)
Naphthalene (air)	46 (12)	Carroll Is Yth Ctr	18 (15)	J Field (Cluster 3)	479 (8)	CV '98 (NW)
Acetophenone (air)	55 (32)	J Field (J Field)	---	---	13 (7)	NW (NW)
Methenamine (air)	138 (159)	Cluster 13 (Frag Pit)	---	---	304 (45)	SW (CV '98)

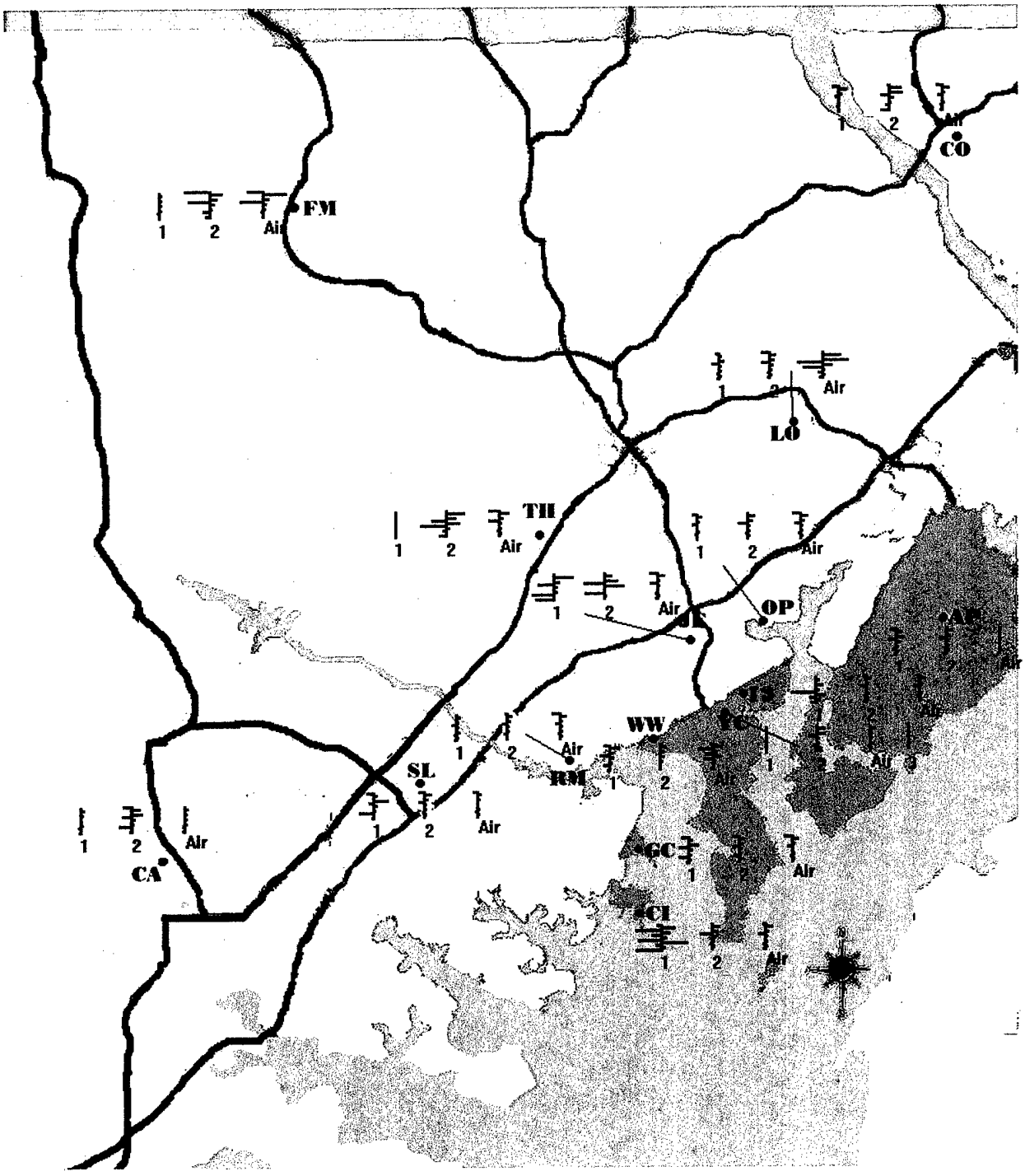


Figure 4.4 Boundary Study Sites with chemical trees for 06/18/98. 1 and 2 represent levels of organics in the air inside each beehive (of the pair) at a site; while air represents the levels in the ambient air.

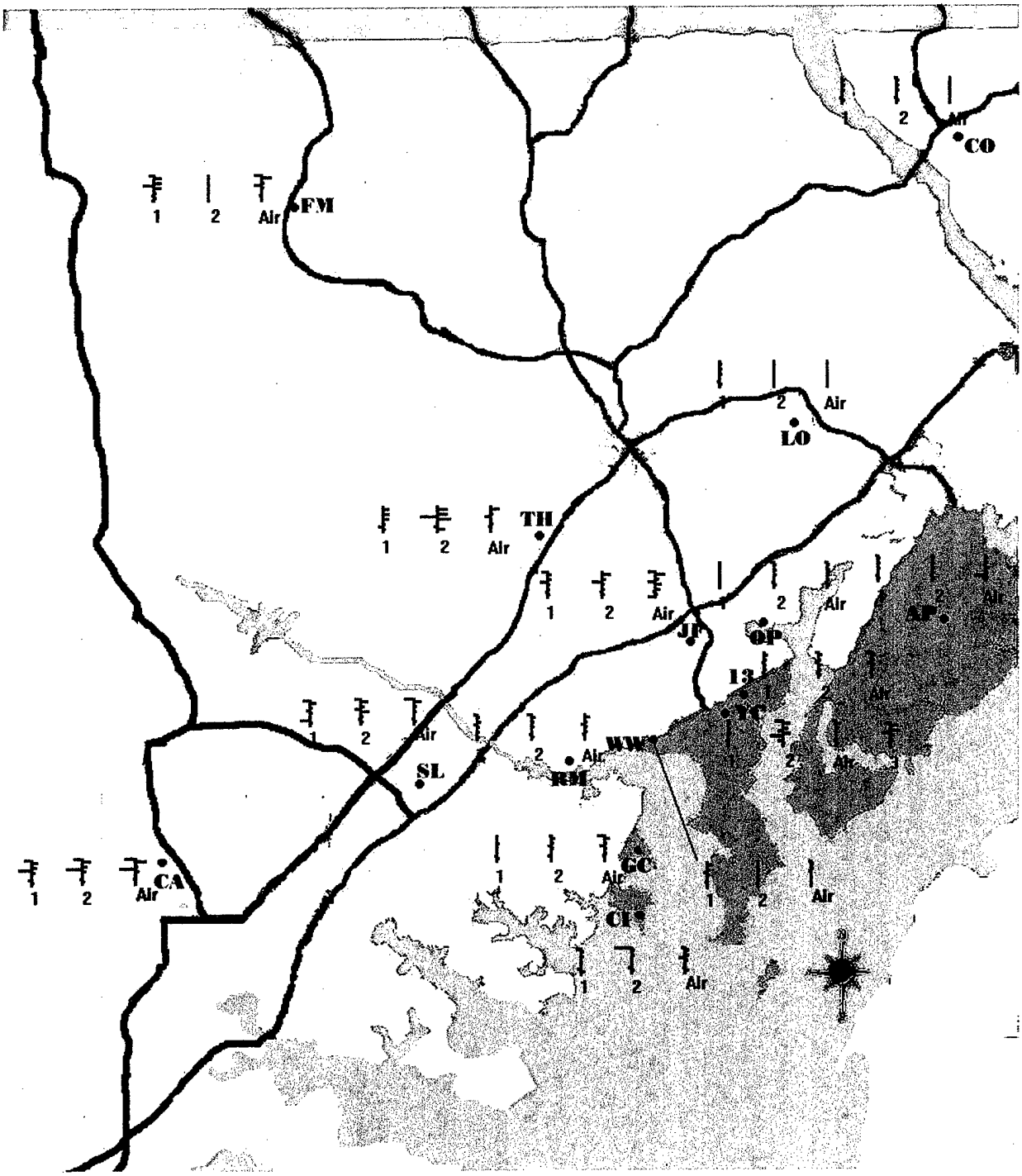


Figure 4.5 Boundary Study Sites with chemical trees for 08/14/98. 1 and 2 represent levels of organics in the air inside each beehive (of the pair) at a site; while air represents the levels in the ambient air.

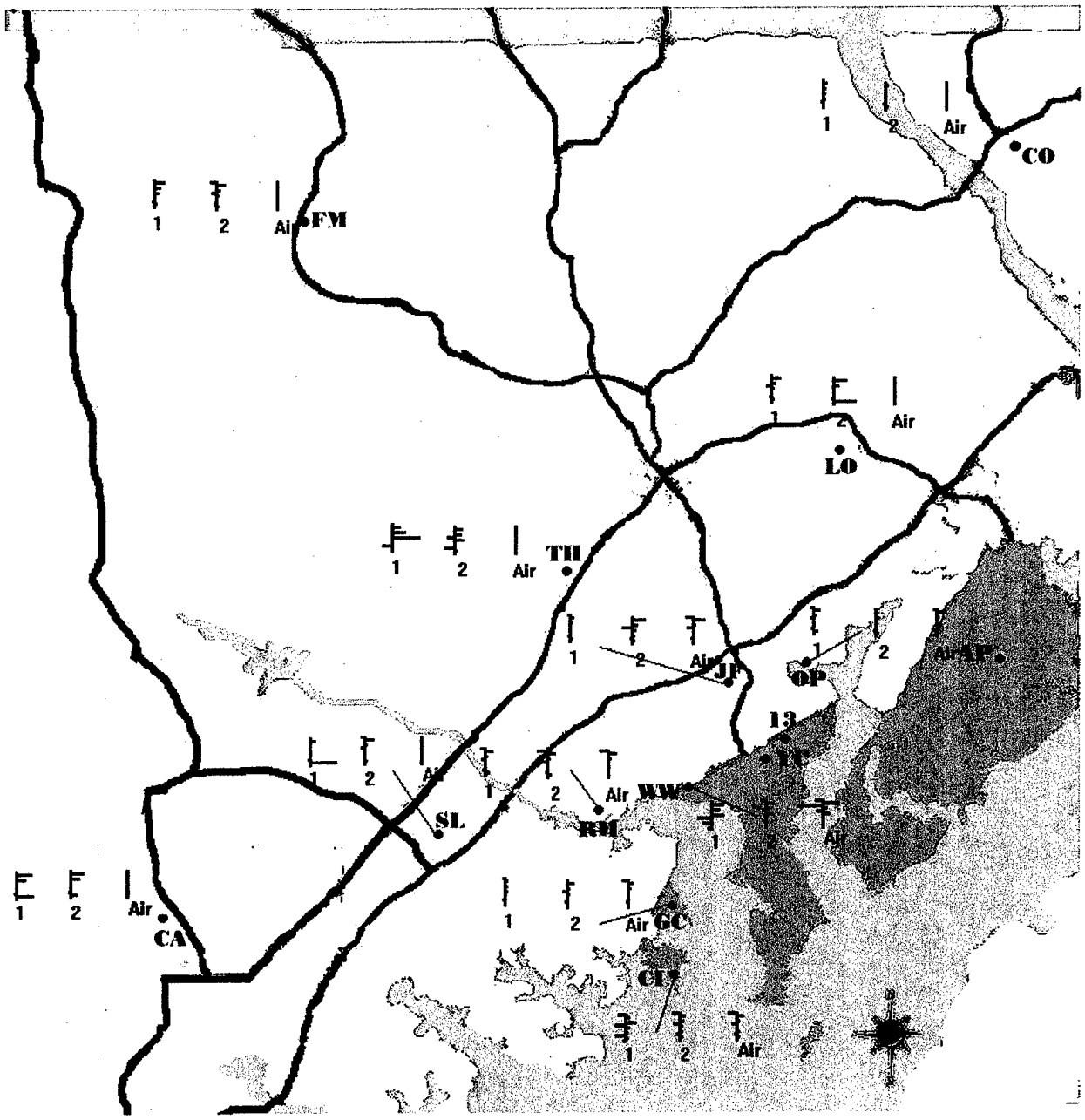


Figure 4.6 Boundary Study Sites with chemical trees for 10/29/98. 1 and 2 represent levels of organics in the air inside each beehive (of the pair) at a site; while air represents the levels in the ambient air.

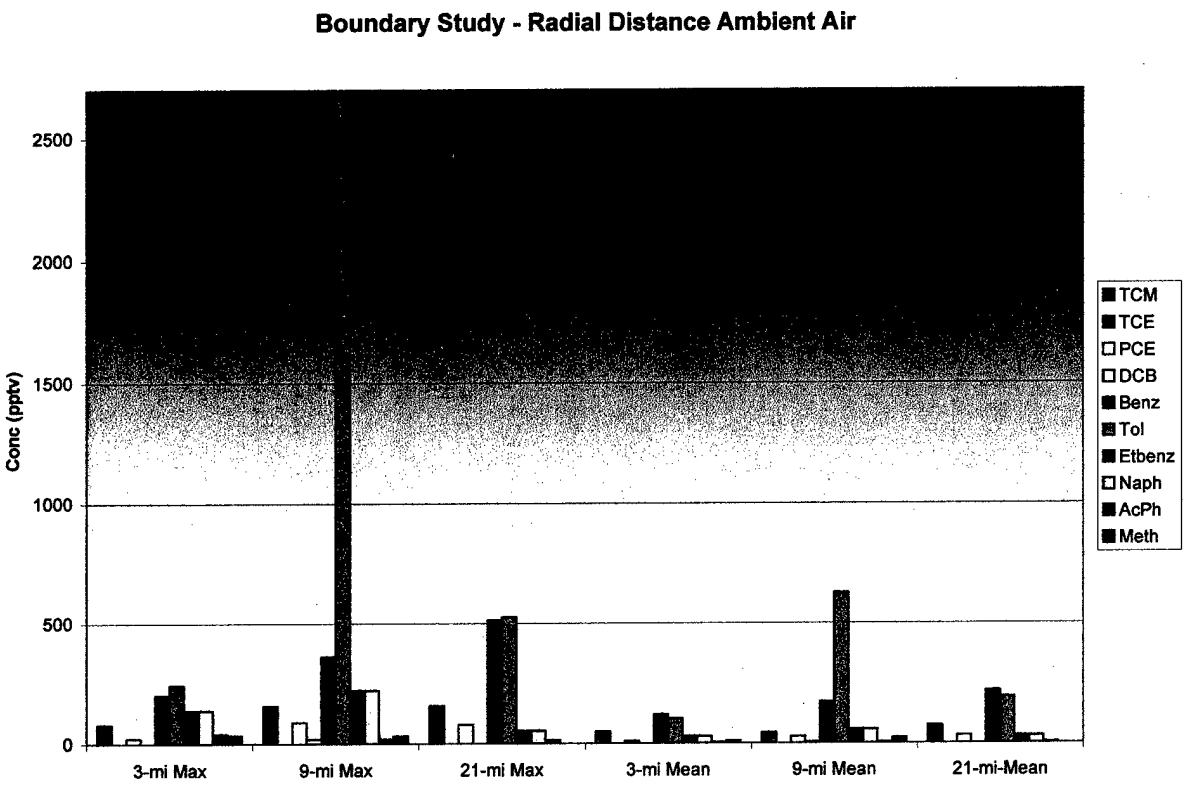


Figure 4.7 Maximum and mean concentrations (ppt) of selected organic chemicals in the ambient air at Boundary Sites located 3, 9, and 21 miles from APG.

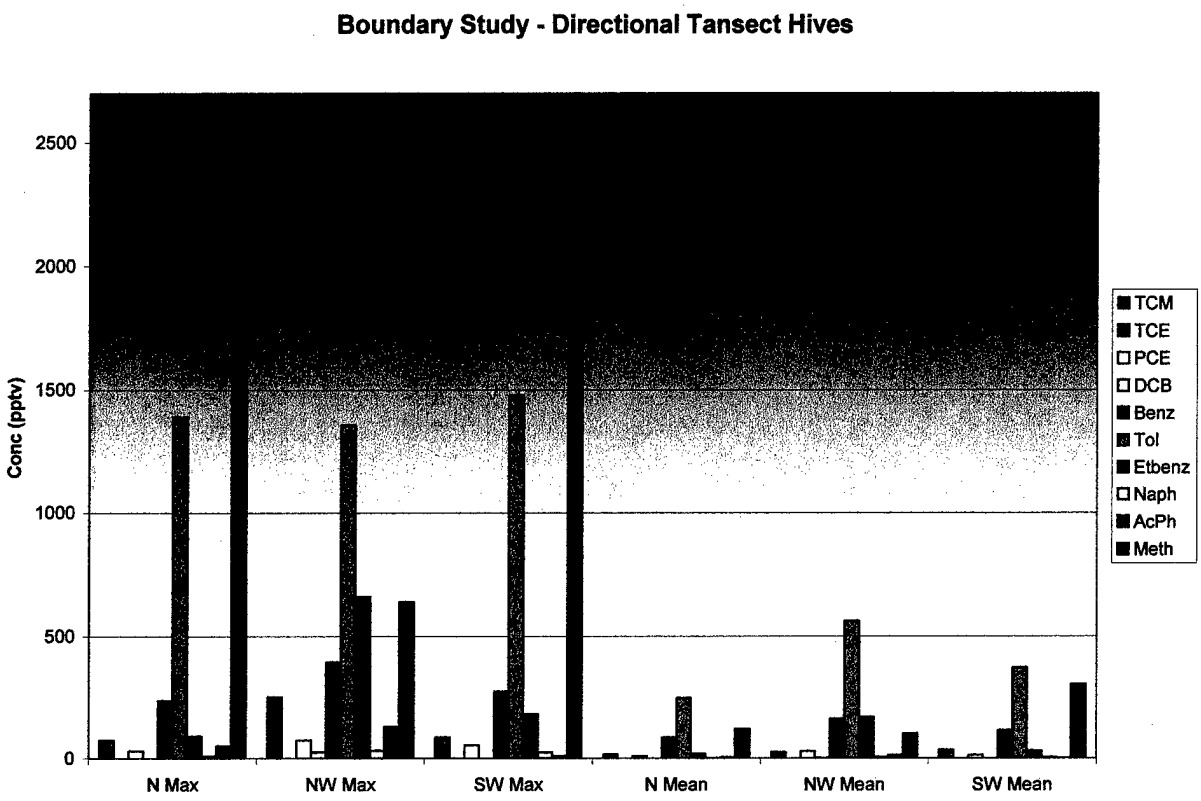


Figure 4.8 Maximum and mean concentrations (ppt) of selected organic chemicals in beehives at Boundary Sites located along transect running north (toward Shawsville), southwest (through Baltimore), and northwest (to Cecil County) from APG.

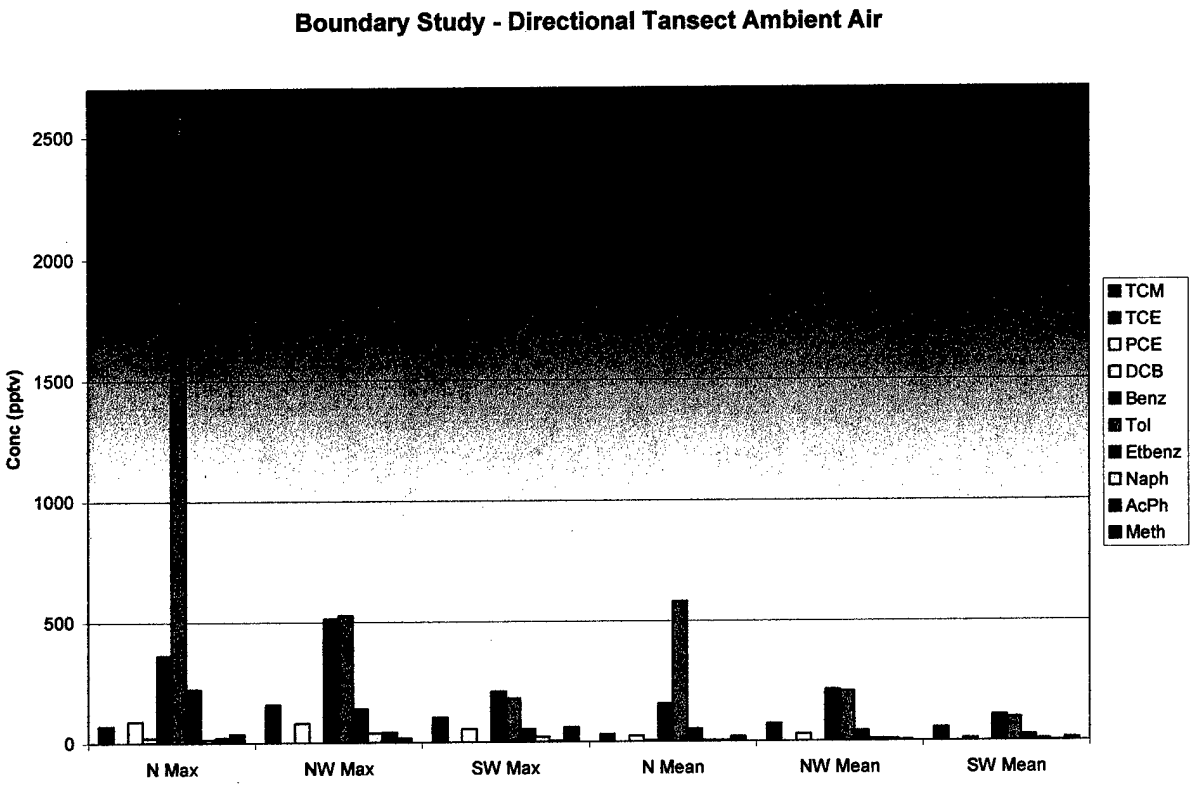


Figure 4.9 Maximum and mean concentrations (ppt) of selected organic chemicals in ambient air at Boundary Sites located along transects running north (toward Shawsville), southwest (through Baltimore), and northwest (to Cecil County) from APG.

Taking all of the results, Tables, charts, and maps into consideration, we conclude that in general the levels of contaminants seen at APG sites are no better or worse than those seen regionally Off-Base. This is easily seen on the two plots (maps) that compare On- and Off-Base sites from the 1998 Boundary Study. This finding is true despite the fact that Carroll Island, a known repository of hazardous contaminants, is included in the APG on-base site set. The accompanying sets of maps clearly reveal the predominance of BTEX components as ubiquitous contaminants at the off-post, Boundary Study sites.

SECTION 5.0 REPORTING

The results of the real-time colony effects biomonitoring and of the bioavailable chemical exposure characterizations have been summarized in this report and previous quarterly reports to USA CEHR. Due to the large amounts of data generated by real-time monitoring of colony responses and multi-chemical analysis procedures, all data collected has been archived on CD ROM disks and provided to USA CEHR.

SECTION 6.0 GLOSSARY

This list contains terms that have special meaning in beekeeping, computer electronics, chemistry, and risk assessment. It is provided to facilitate reading this report without having to refer to other references. The risk assessment terms follows definitions developed by EPA's Risk Assessment Forum, a committee of EPA scientists.

A/D Interface. An analog to digital signal conversion device placed between a computer and the electronic sensors.

Age polythethism. The changing of activities of members of the colony as they age.

Apiary. The place where a group of beehives is kept. Also called a beeyard.

Artificial Neural Networks (ANN). Software programs that learn real-valued functions from examples.

Assessment Endpoint. The environmental value that is to be protected.

Bioavailable. The chemical agent of interest can be collected and transported back to the hive in or on a bee or in or on the materials collected by the bee. For purposes of this report, bioavailable does not imply physiological uptake.

Brood. The immature members of the colony, including eggs, larvae, and pupae.

Brood nest. The region of a colony's nest where brood is reared, usually a central, roughly spherical area below the stored honey.

Burr comb. Pieces of comb that bridge the main combs in a hive.

Carbotrap. A form of thermal desorption sampling tube used to collect samples of volatile and semi-volatile organic chemicals.

Cell. The hexagonal tubes that make up the beeswax comb and in which the brood develops and food is stored.

Colony. The honey bee social unit, each consisting of a queen and workers. During the growing season the colony also will contain drones and brood. Normally, a hive contains a single colony.

Cluster. The behavior by which bees group together for warmth and then coalesce into a single group to conserve heat and to minimize surface area relative to volume. Clustered bees generate metabolic heat.

Coefficient of Variation (C.V.). A statistical function used to compare the relative amounts of variation in populations having different means. Also termed relative standard deviation.

Dead bee trap. A trap used to collect dead and dying honey bees.

Digital Interface. A digital signal device placed between a computer and the electronic sensors.

Direct effect. The consequence of a stressor acting on the ecological component of interest.

Drone. A male honey bee.

Forager. A worker bee that gathers and brings back to the hive water, resin, pollen, or nectar.

Flight counters. The hive mounted infra-red detector units used to track the numbers of incoming and outgoing bees.

Frame. The rectangular wooden structure that surrounds and supports each beeswax comb in a man-made hive.

Functional Organization. The organization of the colony that contributes to the survival and reproduction of the social unit.

Ecological Component. Any part of any ecosystem, including individuals, populations, communities, and the ecosystem itself.

Ecological Risk Assessment. An evaluation of the likelihood that adverse ecological risks may occur or are occurring as a result of exposure to one or more stressors.

Ecological Significance. The interpretation of risk estimates in the context of the types and extent of anticipated or observed effects. The interpretation step usually relies on professional judgement and considers the nature and magnitude of the effects, the spatial and temporal patterns of the effects, and the potential for recovery if the stressor is removed.

Exposure. Co-occurrence of or contact between a stressor and an ecological component.

Exposure characterization. The evaluation of the interaction of a stressor with one or more ecological components.

Exposure profile. A summary of the magnitude and spatial and temporal patterns of exposure used in the analysis phase of an ecological risk assessment.

Hive. The structure that houses a bee colony. Man-made hives are usually made of wood. Natural hives usually are cavities in trees.

Honey stomach. The expandable portion of the alimentary canal used to store and carry nectar and water.

Hydraulic pressure transducers. Electronic devices for measuring changes in pressure, used to weigh hives.

Hymenoptera. The order of insects that includes wasps, bees, and ants.

Hypopharyngeal glands. Glands in the head of the bee that produce proteinaceous secretions which are fed to the larvae and various enzymes that serve in the conversion of nectar to honey.

Inductively Coupled Plasma Mass Spectrometry (ICP/MS). An instrument used to analyze for trace elements and heavy metals (i.e., inorganic chemicals).

Indirect effect. The consequence of a stressor acting on supporting components of an ecosystem, which in turn influence the ecological component of interest.

Instar. Any stage between molts (casting off of outgrown skin) during the course of development of insects.

Larvae. The stage between the egg and the pupae in insects. In honey bees a crescent-shaped grub that floats on a pool of liquid food, intensively feeding, and rapidly growing.

Measurement endpoint. A measurable characteristic that is related to the valued characteristic chosen as the assessment endpoint. Measurement endpoints are often presented as statistical or arithmetic summaries of the observations that comprise the measurement.

Mini-hive. A small, mailbox-sized beehive used to house nucleus colonies for mating and for research.

Nasanov's gland. A gland on the apical end of a bee's abdomen that secretes a pheromone that attracts other bees.

Nectar flow. A period of intense nectar secretion by plants during which a honey bee colony collect large amounts of nectar and produce surplus stores of honey. Also called a honey flow.

No Observed Effect Level (NOEL). The highest level of a stressor that does not cause a statistically significant difference from the control.

Nucleus colony. A small colony of honey bees.

Nurse bee. An age-specific member of the colony that is specialized for the care of brood and other tasks in the brood nest.

Parallel port. A standard device usually used to connect a printer to a computer, usually the LPT port on an IBM-compatible computer.

Pollen basket. A specialized structure on each hind leg for the collection and transport of pollen back to the hive. A smooth area, bordered by a fringe of long curved hairs, on the outer surface of the leg.

Proboscis. The extensible, tubular mouthparts of a bee.

Propolis. The material used by bees to seal cracks in the hive, reinforce comb walls, and create a smooth coating over interior surfaces. Propolis is made up of plant resins collected by bees.

Pupae. The nonfeeding developmental stage between the larvae and adult form.

Queen. The reproductive female member of the colony. Generally, a colony only contains a single queen.

Queen cell. A special beeswax cell built to house a developing queen.

Queen excluder. A screen placed between the boxes of a hive to confines the queen to a particular region of the hive.

Queen loss. Disappearance of the queen from a colony without a concurrent loss of worker bees (see swarm). Queen loss may be a result of queen death or of the queen vacating the hive.

Queen right. A bee colony that contains a fully functioning queen.

Recruit. A forager bee that looks for new food sources after following the waggle dances of a returning forager in the hive.

Relative Humidity (RH) Probe. An electronic relative humidity sensor.

Relative Standard Deviation (R.S.D.) A statistical term that is equivalent to the coefficient of deviation.

Scout. A forager bee that looks for new food sources by independent searching.

Serial port. A standard port for connecting devices to a computer, usually the COM port of an IBM-compatible computer.

Social physiology. The highly organized functioning of a colony comprised of the integrated activities of the individuals and the group as a whole. An example is thermoregulation inside the colony.

Strain gauge. A bi-metal foil resistor that measures flexion of a beam. Strain gauges are used to measure hive weight changes.

Stressor. Any physical, chemical, or biological entity that can induce an adverse response.

Stressor-response profile. A summary of the data on the effects of a stressor and the relationship of the data to the assessment endpoint.

Supersedure. The replacement of the queen by her daughter.

Swarming. The method of colony reproduction in which the queen and a large portion of the worker bees leave the hive and find a new nest cavity.

Temperature probe. An electronic probe consisting of a thermal transistor.

Thermal Desorption/Gas Chromatography/Mass Spectrometry (TD/GC/MS). Instrumentation for the analysis of volatile and semi-volatile organic chemicals.

Waggle dance. The dance that causes foragers to leave the hive in search of specific food sources.

Weight of Evidence. A process that provides insights into the confidence of the conclusions reached in a risk assessment. Weight of evidence considerations often include the sufficiency and quality of the data, corroborative information, and the degree of correlation between the presence of one or more stressors and some adverse effect.

Worker. One of the non-reproductive females in the colony. The bulk of the population consists of workers.

SECTION 7.0 REFERENCES

Adamus, P.R. 1992. Choices in Monitoring Wetlands. *In*: Ecological Indicators. ed. McKenzie, D.H., Hyatt, D.E. and McDonald, V.J. London and New York. Elsevier Applied Science. Chapter 32: 571-592.

Bromenshenk, J.J.; Carlson, S.R.; Simpson, J.C.; Thomas, J.M.. 1985. Pollution Monitoring of Puget Sound with Honey Bees. *Science* 227:632-634.

Bromenshenk, J.J.; Smith, G.C.; King, B.E.; Seccomb, B.A.; Alnasser, G.; Henderson, C.B.; Loeser, M.R.; and Wrobel, C.I.. March, 1997. New and Improved Methods for Monitoring Air Quality and the Terrestrial Environment - Aberdeen Proving Ground Edgewood Area. Contract Annual Report to *USA CEHR, DAMD17-95-C-5072.*

Bromenshenk, J.J.; Smith, G.C.; King, B.E.; Seccomb, B.A.; Alnasser, G.; Henderson, C.B.; Loeser, M.R.; and Wrobel, C.I.. March, 1998. New and Improved Methods for Monitoring Air Quality and the Terrestrial Environment - Application at Aberdeen Proving Ground. Contract Annual Report to *USA CEHR, DAMD17-95-C-5072.*

Bromenshenk, J.J., Smith, G.C., and Watson, V.J. 1995. Assessing Ecological Risks in Terrestrial Systems with Honey Bees. In: *Biomonitors and Biomarkers as Indicators of Environmental Change*, F.M. Butterworth et. al., Eds. Plenum Press, New York.

Bromenshenk, J.J., Gudatis, J.L., Carlson, S.R., Thomas, J.M. and Simmons, M.A. 1991a. Population dynamics of honey bee nucleus colonies exposed to industrial pollutants. *Apidologie* 22:359-369.

Bromenshenk, J.J., Doskocil, J., Olbu, G.J., Degrandi-Hoffman, G. and Roth, S.A. 1991b. PC BEEPOP, an ecotoxicological simulation model for honey bee populations. *Environ. Toxicol. and Chem.* 10:547-558.

NRC (National Research Council); *National Academy Press*, 1991. ISBN 0-309-04046-9, 160 pp.

U.S. EPA. 1989. Warren-Hicks, W.; Parkhurst. B.R.; Baker, Jr., S.S.; *EPA 600/3-89/013.*

U.S. EPA. 1999. Guidelines for Ecological Risk Assessment. Risk Assessment Forum, NCEA, EPA/630/R-95/002F April 1998.

U.S. EPA. 1998. EFED Environmental Risk Assessment for the Methyl Parathion RED. Office of Prevention, Pesticides, and Toxic Substances. Washington, D.C. (Memorandum, October 27, 1998, through Environmental Risk Branch I/EFED 97507C).