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AIA	Absolute Ion Abundance	DIFF	Analysis
ANL	Adjusted Net Loss (of bees)	Ethbenz	Ethylbenzene
ANN	Artificial Neural Network	FM	Fort Missoula Site
APG	Aberdeen Proving Ground	GS	G Street Site
As	Arsenic	Hz	Hertz
Be	Beryllium	ipl	ions per liter
Ba	Barium	JF	J-Field Site
Benz	Benzene	JFN	J-Field North Site
BP	Beach Point Site	LC	Lauderick Creek Site (Cluster 13)
BR	Bush River Site	mg	milligram
BTEX	Benzene, Toluene, Ethybenzene, Xylene	Mg	Magnesium
BRSA	Bush River Study Area	Napth	Napthalene
CD-ROM	Compact Disk - Read Only	ng/mg³	nanogram per cubic meter
CCI	Memory	Ni	Nickel
CGI	Common Gateway Interface	NL	Net Loss of Bees
		OF	Old O Field Site
Cu	Copper	PCR	Percentage Return of Bees
CV CV	Conference Ste	ppt	parts per trillion
C.V.	Coefficient of Variation	QA	Quality Assurance
DAKPA	Projects Agency	QC	Quality Control
DCB	Dichlorobenzene	Pb	Lead
DF	D Field Site	PCE	Perchloroethylene
DER*	Derivative-Differential	Rb	Rubidium

$r^2$	<b>Regression Coefficient</b>
SSE	Summed Square of Error
SOPs	Standard Operating Procedures
SSYY MMDD	File Naming Format: Site, Year, Month, Day
Šr	Strontium
SVOCs	Semi-volatile Organic Chemical
TAL	Target Analyte List
TCE	Trichlorethylene
ТСМ	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

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### EXECUTIVE SUMMARY

The objective of this six-year study was 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.

### 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 exposures of ecosystems to bioavailable chemical agents.

In general, all of the measured hive metrics indicated similar, but slightly improved, colony performance for 1999 compared to 1998. The exception was at the Churchville, Maryland reference site, where, beginning in August of 1999, some of the hives were used for calibration trials to determine the ability and reliability of the electronic hive systems for detecting acute toxicity. These trials, and earlier (1998) trials conducted at the University of Montana show that real time measures of colony performance can identify and follow the course of the effects of exposure to a known toxic chemical such as methyl parathion. For these calibration trials, the number of bees found in passive traps installed below each hive to capture dead and dying bees provided a classical assay of toxicity. However, the electronic real time system showed that colony foraging activity levels continued to exhibit the effects of a toxic event long after bees stopped dying (as reflected by the traps) and that the recovery process (if any) could be closely followed by the real time monitoring system.

In 1999, with the exception of a colony that swarmed at J-Field, the only noticeable decline of bee colonies occurred among the survey hives at two of the off-post Boundary sites. The cause of these bee declines is unknown, but these events occurred at two of the four off-post sites that had experienced bee losses in the fall of the preceding year. Because of the bee losses at Boundary sites in 1998, bee colonies were re-deployed at most of the Boundary sites in 1999 to further investigate conditions at these locations and to obtain a complete autumn sample set for VOC and metals analysis.

# 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:

- Perform a verification survey of exposures to bioavailable chemicals at the Boundary areas,
- 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) as measured in honey bee colony populations.

In 1999, the analysis results for volatile organics demonstrated that BTEX contaminants were ubiquitous at all study sites. Concentrations of chlorinated organic compounds were generally lower at the off-base sites than at the APG-sites. The highest concentrations of chlorinated organic compounds are commonly observed at J-Field. Among the 20 organic contaminants followed in 1999, J-Field bee colonies yielded the seasonal maxima in 18 of 20 cases, and the ambient air samples recorded the season's maxima in 16 of 20 cases. The most serious exposure was to 1,1,1-trichlorethane (TCA), at nearly 3 ppb in hives and 0.5 ppb in air. Two dichloroethenes (1,1- and cis-1,2-) were common in bees at J-Field. Concentrations of TCM, TCE, and DCB were similar for on- and off-base study hives. Levels of PCE and perchloroethane were slightly higher off-base than on Aberdeen Proving Ground. 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.

There is no information available in the literature about the toxicity of these solvents to bees. However, we speculate that the variable flight activity responses at sites with elevated levels of these chemicals and the absence of evidence of toxic effects may reflect behavioral responses to these chemicals, which may act as irritants, induce alterations in the ability of bees to coordinate activities, or alter memory. These types of responses have been observed with respect to bees and other organic chemicals. The electronic hives documented changes in activity that were most pronounced at sites with higher exposures to a wider array of organic chemicals. Proving cause and effect for complex mixtures of these chemicals would entail a sizable, separate investigation.

The most serious threat to human health found in 1999 was the high levels of benzene recorded at most sites. Benzene is a known human carcinogen with a CRL of 31 ppt. Mean ambient air concentrations of benzene were 111 ppt, even at the Churchville reference site. However, any urban setting will often show benzene values above those found at Churchville, Cluster 3, or J-Field. A two-year study at the University of Montana produced a mean of 464 ppt. This ubiquitous exposure to humans in US cities is not well recognized by public health officials. In addition, some of the VOCs that were measured in ambient and hive air also were listed as chemicals of potential concern (COPC) in the published baseline risk assessments for Cluster 3 and J-Field. Based on 6-8 hr daily samples taken periodically throughout the study, some of the measured values exceeded EPA Region Three Human Health Risk Benchmark Characterization values. It should be noted that the values usually compared to HRBCs generally involve more measured (often continuous monitoring) values and some form of source modeling—caution should be exercised when comparing daily maximum to HRBC values.

The analyses of trace elements in forager bees and pollen showed no detectable levels of Be, Bi, Cs, and U, and Ti was only found at very low levels (below detection limit to 2.3 ppm). The trace elements As, Cd, Co, Cr, Ga, Se, Sr, and V were found at sub-ppm levels. Levels for Cu and Zn in forager bees were within the normal range, and there were no differences between the APG and the off-base sites. The levels of Mn and Sr in forager bees and pollen were highest at J-Field, second highest at Cluster 3, and lowest at the off base sites (P < 0.001) suggesting local sources of these elements in the J-Field area. Possible sources of Mn are human activities, and potentially higher Mn levels in the water of salt marshes in the vicinity of J-Field. Higher levels of Sr at J-Field are most likely due to military activities. Lead values were markedly elevated in some of the bee and pollen samples from J-Field, compared to all of the other sites. Finally, as the distance from APG increased, levels of Ba, Cd, Cu, and Mn in pollen were highest at the Cylburn Arboretum site, located in downtown Baltimore (P < 0.05)

Based on available information contained in the University of Montana's bee toxicity database and probit model programs, none of the toxic heavy metal concentrations observed in bees exceeded values estimated to be toxic to bees. A couple of pollen samples had lead levels high enough to warrant concern, but samples taken earlier and later in the season did not show sustained enhancement of this metal, so exposure was apparently of a relatively short duration. No information was available about the toxicity of strontium to bees or other terrestrial insects.

### **PROPOSED ACTIVITIES FOR 2000**

This USA CEHR contract is scheduled to terminate May 30, 2001. The emphasis for 1999/2000 has been:

- Detailed Evaluation of 1999 Investigations (this report)
- Technology transfer to commercial partners
- Final technical wrap-up covering the entire project period

### 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.

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### SECTION 1 OVERVIEW AND WORK PLAN FOR 1999

### 1.1 Previous Investigations

Previous investigations conducted at Aberdeen were covered in the annual reports for 1996 through 1998 (Bromenshenk *et. al.*, March, 1997, March, 1998, and February, 1999). 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 O 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 that began in the summer of 1998. Again, the J-Field bees provided a means of assessing exposures and colony condition during the restoration activities as well as the information needed for a post-removal action assessment. Overall, all of the measured colony performance metrics indicated similar, but slightly degraded, behaviors for 1998 compared to 1997. The only losses of bees occurred at three of the off-post Boundary sites and at Carroll island - the cause remains unknown. As in previous years, the same general types of volatile and semi-volatile organic chemicals were seen at APG sites. Taking all of the VOC and SVOC contaminants into consideration, in general the levels of exposure to organic chemicals at APG sites was not better nor worse than those seen regionally off-post.
- In 1999, we resumed the biomonitoring at J-Field, began monitoring Cluster 3 prior to the initiation of cleanup activities, and conducted a verification of the Boundary Survey. Calibration trials at the Churchville reference site confirmed results from our Montana reference site that indicated that the real time monitoring system could readily detect behavioral changes due to exposure to acutely toxic chemicals. The J-Field study provided ongoing monitoring of the removal activities at the site and added investigations of VOCs in the air and of possible transport of these chemicals into beehives that were placed around the J-Field phytoremediation grove. Also, in 1999, the number of detectable organic compounds was increased from nine to twenty compounds, in part because of improved sampling methods and instrumentation at the UM laboratory.

### 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 warranted continued monitoring. In addition, there is a need to verify the effectiveness of the trees being used for phytoremediation of subsurface VOCs and SVOCs and to replicate an assessment of 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 appeared to be unlikely based on preliminary studies, but prior to 1998 this potential route of transport into bee colonies had not been measured. Tree bud burst occurred earlier than expected in 1998, so bee colonies were placed on-site earlier in 1999 to be sure to be able to monitor this event.
- Many areas of APG had not been monitored for VOC and SVOC contaminants in the ambient air nor had 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 and was first addressed in 1998. These studies indicated that 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 suggested the existence of off-post sources of several of the same VOCs and SVOCs observed at locations at APG-Edgewood. The initial results showed elevated levels of a variety of volatile organic chemicals at some of the off-post Boundary sites, and colonies were lost at three of these sites. Verification of these findings appeared to be warranted. As such, colonies were again placed on Boundary sites, sampled, and monitored for acute toxicity in 1999.

### 1.3 Scope of Work

This subsection describes the work that was completed during the 1999 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

- <u>Subtask 1:</u> Conduct real time monitoring of colony behavioral responses to further document assessment metrics. Colony response investigations begun in 1997 were continued in 1998 and again in 1999 in order to monitor the removal actions conducted at J-Field.
- 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. Continue determinations of the potential for uptake and transfer of VOCs to bees from trees used for phytoremediation.

<u>Subtask 3:</u> Provide an expanded database (number of organics reported increased to twenty, and all data was indexed to a Geographical Information System) to facilitate a post-removal action assessment of exposures and the effectiveness of reducing potential risks to natural systems (i.e., bees and pollinators).

### Task 2. Cluster 3 Removal and Capping Activity Assessment

Conduct both real time monitoring of colony behavioral responses and chemical exposure surveys to document existing conditions in anticipation of a follow-on assessment in 2000 to monitor conditions during these cleanup activities and to quantify the degree to which capping the landfill reduced exposures or risks to bees, pollination systems, and any implications to human and ecological health.

# Task 3.Boundary Area Surveys: Chemical Exposure and Acute Toxicity Assessments<br/>of Colonies Deployed Along Transects Extending outward from APG

Deploy for a second year, chemical exposure survey hives at three locations along each of three transects extending area into Harford, Cecil, and Baltimore Counties from a fourth location, the Youth Center on the APG-Edgewood Post. Conduct early 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 and heavy metals that may accumulate through time in hive components, bees, pollen, and ambient air. Inspect hives for signs of 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, May, and July, and at Boundary sites in May and June. Monitoring began in late April, with chemical exposure pre-sampling started in early April. Routine sampling began in May. The 1999 field study was completed in October. VOC and SVOC analyses were completed by December, 1999. Sample analysis verification and interpretation was completed spring, 2000. Samples for inorganic analysis were prepared (i.e., dried and ground) in winter, 2000, and then submitted to USA CEHR for analysis. All of the colony behavioral response data was processed by late fall, 1999. 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 AND GENERAL METHODS 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 three areas of concentration: J-Field, Cluster 3, 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, and Cluster 3 at APG--Edgewood, and at an off-post site at Churchville
- Perform a follow-on survey of the off-post Boundary area transect sites and compare the results to those from APG sites— Youth Center, Cluster 13, J-Field, Cluster 3.

Objective 1 continued a multi-year monitoring of ongoing restoration activities at J-Field and also began monitoring of restoration activities started in 1999 at Cluster Three of the Bush River area. Objective 2 provided datasets that could be used to compare conditions in terms of exposures to bioavailable chemicals and acute toxic effects at sites distributed throughout the residential communities surrounding APG to those at APG sites.

Based on the estimate that 50 percent of the foraging honey bees in an eastern hardwood forest concentrate their efforts within 600 feet of the hive (Seeley, 1985), nucleus hives were spatially distributed in such a way to ensure that each of the main study locations was isolated from the others (i.e., the distance between primary sites exceeded bee flight range, so bee coverage did not overlap) and to optimize within each site the chance of the bees being exposed to bioavailable chemicals at each site. For example, at J-Field, colonies were grouped in sets of three at 5 locations circling the phytoremediation tree grove.

The probability of encountering contaminants (i.e., known or suspected chemicals present at each of the APG sites) was based on reviews of historical information and sampling data and analysis reports found in Remedial Investigation Reports for APG and RCRA Facility Agreements. Ideally, any site monitored with honey bees should have a readily available source of water and sufficient amounts of foraging vegetation in the surrounding area for maintenance of the colonies. However, honey bees can be used where conditions are marginal, if they are provided with supplemental food (syrup and pollen substitute) and water. Using this approach, bees were successfully used to monitor fluoride, trace elements, heavy metals, and radionuclides at sites on the Idaho National Environmental Engineering Laboratory that were mainly bare rock (basalt flows), with little vegetation, and no water (Bromenshenk et al., 1996).

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 (21 total) supplemented by additional chemical survey hives were established at J-Field, Cluster 3, and the Churchville reference site in 1999. 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 a 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 colonies cannot be used to generalize to all other rural areas, but they are useful for comparing chemical exposures and overall colony performance in this fairly rural region to the Maryland sites, of which even the most rural is still highly urbanized and industrialized when compared to western Montana. The Montana hives provide data that should approximate what a reasonably clean area might resemble. These comparisons are best used to evaluate the exposure data from Maryland; that is, the kinds and levels of chemicals to which bees are exposed. The data also can be used to develop life table functions such as the expected daily bee mortality in healthy bee colonies that are rigorously managed to suppress disease, are frequently inspected, and have been placed where food resources and water are plentiful (in other words, these resources are not limiting). The data cannot be used to compare patterns of activity such as daily flight profiles. For example, bees in Maryland generally fly from about 7 a.m. to 7 p.m. throughout most of the growing season, whereas MT bees may fly from 6 a.m. to 10:30 p.m. due to the extended hours of daylight in this northern state.

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 (Figure 2.3.0). 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, next to the USACEHR administration trailer (location B). An additional 15 full-sized survey hives were placed in groups of three at five locations (labeled A-E) surrounding the grove of trees being used for phytoremediation. A removal activity was carried out by a contractor throughout the spring and summer of 1998, and again in 1999. This restoration effort was focused on a location near the middle of the J-Field clearing that is more or less centered among the hives.

**2.3.2** Cluster 3, APG Edgewood: This is a heavily wooded site located in the western most portion of the Bush River Study Area (BRSA), north of the Bush River Road. Cluster 3 includes two sites: the Old Bush River Road Dump (OBRRD), which is Site 3 (DSERT #EABR03-A), and the Transformer Storage Area, which is Site 23 (DSERT #EABR02-B). A Remedial Investigation (RI) for Cluster 3 was completed in July of 1998, and a Record of Decision for the Site was signed in June, 1999.

Historical records and maps indicate that a former home site was located near the OBRRD. The site was active up to the mid-1940s. Wastes were pushed out toward Lauderick Creek, some wastes were not covered, and burning occurred at the site. Results from a 1997 survey of VOCs in ambient air and the air inside beehives at several Bush River sites indicated that several of the solvent-related VOCs commonly found at APG landfill sites occurred in relatively high

concentrations at Cluster 3, compared to other Bush River sites. Capping of the OBRRD began in 1999 and continued into 2000.

Just north of the Bush River Road and 16<sup>th</sup> Street intersection lies the Transformer Storage Area. Much of the surface of this area is covered with asphalt. Transformers were stored in this area from approximately 1981 to 1991. All transformers were removed in 1991.

During a RI, elevated levels of lead (643 ppm) were found in a surface soil sample just south of the OBRRD. The source and extent of the contamination is unknown, but the Ecological Risk Assessment indicated that soil invertebrates could be adversely affected by lead in this location.

In 1999, seven honeybee condos were situated for monitoring of Cluster 3. The hives were placed on the south side of the area of the OBRRD that was being capped, just east of the Transformer Yard. Monitoring of this location with electronic hives was continued in 2000.

**2.3.3 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. In 1999, this site was also used for calibration trials aimed at verifying that the honeybee real time monitoring system could reliably detect acute toxicity to bees. Use of this site was discontinued in 2000, when the electronic hive system was relocate to Worton Point, on the Eastern Shore of Chesapeake Bay.

**2.3.4 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 near the banks of a river on UM's College of Technology campus. This is a small campus with a just a few buildings, a small parking lot, and mostly open land consisting of alfalfa hay fields used by the High School Agricultural Program and large, weedy, old fields. The beeyard is in an open area at the end of short road, about 100 yards from the far corner of the paved parking lot. Large dense hedges and a large lawn separate the beeyard from the lot. No traffic drives past the beeyard. The cars parked typically number less than a few dozen, and these are parked on the far side of the lot from the beeyard. Traffic is minimal, consisting mainly of staff and students coming and going to class.

The Missoula area is much less industrialized and urbanized than Edgewood, Maryland. Some traces of BTEX compounds associated with gasoline and diesel use were found due to traffic in the parking lot, but the the levels of these compounds were minimal when compared to the bee locations in Maryland that often were near highways or expansive, crowded parking lots. 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. An extensive review of volatile organic chemicals found inside beehives was conducted by Smith et al. (2002).

This 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 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. Many of these studies could not have been conducted in Maryland due to the high background levels of the chemicals being assayed for toxicity.

**2.3.5** On- and Off-Post Survey Hive Study Areas (Figure 2.3.1 and 2.3.2): 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 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. Specific locations for the off-post Boundary locations employed both in 1998 and 1999 appear in Table 2.3.0. The following sites comprised the two-year Boundary study:

**2.3.6 Youth Center, APG Edgewood:** Investigations along approximately 700 acres of Canal Creek 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.

**2.3.7 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 21 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 nine pairs of hives, along with an extra location (Cluster 13) near the NE Transect are listed in Table 2.3.0. 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 2.3.0. Several of these locations were near heavily traveled highways.

### **Table 2.3.0**

## 1999 APG Boundary Study Sites

•

Transect Hub,	Youth Center
Location:	Youth Center, APG
Map coord:	HC29D06
Transect 1 - Site 1,	Cluster 13 (extra site)
Location:	APG Cluster 13
Map coord:	HC29F05
Transect 1 - Site 1,	3 mi (primary site)
Location:	Estuary/State Park
Map coord:	HC24H11
Transect 1 - Site 2.	9 mi
Location:	Orchard
Map coord:	HC19B08
Transect 1 - Site 3.	21 mi
Location:	Orchard
Map coord:	Harford County Index Map, 48mm from right edge, 39mm from top edge
1	(about the "w" in Rowlandsville Rd)
Transect 2 - Site 1.	3 mi
Location	Fruit Stand/Truck Garden
Man coord	HC24A12
Transect 2 - Site 2	9 mi
Location.	Farm
Man coord	НС22Н03
Transect 2 - Site 3	21 mi
Location	Landscaping Firm
Man coord	HC07E02
Map coord.	110071302
Transect 3 - Site 1.	3 mi
Location:	Private Residence
Map coord:	HC28C06
Transect 3 - Site 2,	9 mi
Location:	Private Residence
Map coord:	BC29G09
Transect 3 - Site 3,	21 mi
Location:	Clyburn Arboretum
Map coord:	BC34F01



Figure 2.3.0 Edgewood Peninsula Study Area Map Showing the Locations of Old O Field, D Field, and J-Field.



**Figure 2.3.1** Boundary Study Transects, 1999. More detailed maps of the Boundary Study Sites appear in the next Figure and in Sections 4 and 5 of this report.



**Figure 2.3.2** Closeup map of Boundary Sites established in 1998, including APG sites. The terminal site for each transect does not appear on this map, refer to Figure 23.1 for an expanded view of the area. Boundary sites surveyed in 1999 also appear on the maps in Sections 4 and 5 of this report. Four of the APG on-post sites used in 1998 (i.e., Westwood, Carroll Island, Graces Quarter, and the main Aberdeen Area) were not re-sampled in 1999, although all of the off-post sites were re-sampled in 1999.

#### 2.4 Sampling Frequency

Chemical sampling frequencies for VOCs, SVOCs, and trace elements and metals varied from biweekly to longer periods, depending on individual locations and specific study objectives. The most intensive and frequent sampling was conducted at J-Field. Most sites were sampled at least twice-in the early summer following the spring rains and nectar flow, and again in late summer or early fall. Ideally, for cost effectiveness and for improved chances of associating chemical exposures with bee population responses, chemical sampling frequency would consist of establishing a baseline, then sampling using an automated system that could be triggered by the electronic hives or remotely. That technology was being developed in 1999 and was delivered in 2000, but the lack of reliable communications still precluded its use at APG sites. At J-Field, in 2000, new telephone lines were installed prior to deploying the bees, but heavy equipment cut into the lines after about two weeks and the patched lines had a very high signal to noise level. At Eastern Shore, the phone had to be shared with the watchtower guard. It was only plugged in after he left for the day and as such was not available during the day light hours. At Cluster 3, lightening storms and computer hackers forced shutdowns of the system. Those problems have now been solved with a new system developed under our ongoing DARPA project. The new system is standalone, lightening resistant, and does not require a commuications link to work. However, this new technology did not exist in 1999.

Lacking the ability to sample on demand, we again used a sampling frequency intended to optimize our chances of seeing seasonal variations in chemical exposure and at the primary sites, more frequent sampling timed to coincide with the forager population turnover of the colony. Spring, summer, and fall sampling intervals provided an opportunity to examine wet and dry seasonal differences. Bee forager populations turn over approximately every two weeks, so the biweekly sampling was consistent with this turnover rate.

The limitation of this sampling approach is that we may miss a chemical exposure event of short duration, and when we see responses in bee performance, we often have to use after-the-fact sampling to see if we can detect elevated chemicals in or on the bees or inside the hive.

Electronic colonies were monitored continuously and periodically inspected for any symptoms of acute toxicity, disease or other stress-related responses. Survey hives along the Boundary Transects and at J-Field were visually inspected to determine colony condition. In all cases, inspection and sampling schedules were subject to weather—bee colonies can not be worked and sampled when it is raining. Sampling frequency and dates are detailed for organics in Section 4 and for inorganics in Section 5 of this report.

#### 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 exposures to ecosystems. These measurement and assessment methods can be divided into two categories:

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

Measurement, sampling, and analytical methods are fully described in the previous annual reports (Bromenshenk *et al.*, March, 1996, March, 1997, February, 1999). These methods are summarized below. Note also, that digital weather stations were run continuously at each of the primary sampling sites (i.e. J-Field, Cluster 3, Churchville).

Colony responses are measured by bi-directional bee counters and temperature probes in the hive. The counters collect data 200 times per second, and stores it every 30 seconds. The temperature probes were set to record data every 5 minutes, but could be set to any interval desired. Thus, the data can be collected in real time, but data transmission was near real time, due to bandwidth limitations. Where communications links were available at a site, summary data was posted to the Internet every 15 minutes. The entire data set was processed and transmitted to Montana during the night.

The systems designed and used at APG required stand-alone PC computers at each field site, and short time out periods occurred (less than a second) whenever data was processed and stored. The most recent system developed at UM under an ongoing DARPA project collects, processes, and stores data at the hive and on the fly, with no time outs. This system can be setup to send an immediate signal whenever an anomalous event occurs, rather than transmitting continuously or periodically. Only sending a signal when an event occurs, reduces bandwidth requirements and provides real time response capability - either automatically at the hive, or as a decision made by a human operator monitoring the system's output.

It should be noted that monitoring colony core temperature measures the ability of the colony as a whole to coordinate complex behaviors needed to maintain constant temperature in the brood nest and as such is a colony population level response. The flight counters can count individuals bees exiting and entering the hive. The resulting data provides measures of total flight activity and of any change in activity rates or of any failure of bees to come home. It also documents activities such as when a hive swarms (i.e., the original queen leaves with many of the workers to set up a new colony) or is so weak that it is being robbed (i.e., bees enter the hive in large numbers to rob it). Normally, bees in the morning first have to leave a hive, since they come home at night. Then, they return to the hive after foraging. That cycle continues throughout the day - bees go out, bees return. But if many more bees enter the hive than have left, the colony is being robbed. Similarly, contact with a toxic chemical in the field will kill bees, many of which die in the field before they can return to the hive. The counters are well-suited for flagging these events.

With respect to chemical contaminants, some are toxic, some are not, and many may induce behavioral responses other than loss of bees. For example, beekeepers use smoke to calm bees and other chemicals to drive bees out of their hives. Bee populations are exposed to more than one contaminant in both the abiotic and biotic parts of an ecosystem, while ecosystems can be exposed to more than one contaminant in media such as air, soil, sediments, and water. Thus, characterizing ecosystem exposure involves both exposure of an ecosystem as well as within one. Similarly, both individual bees within a bee colony and the colony population itself are exposed and may respond to environmental contaminants.

Forager bees are obtained by screening hive entrances and vacuuming a minimum of 100 bees from each hive. Pollen is taken by scraper screens inserted under the hive. Dead and dying bees are caught in bottom-mounted traps, under the hive. Pollen traps were left on for 1-7 days. Dead bee traps were emptied approximately every 1-2 weeks, depending on the location. Hive air or atmospheres are sampled by inserting a 1/8 inch tube into the center of the hive box and of colony population and drawing air out and onto a multi-bed sorbent tube trap system. Typically, these samples were taken over a 6-10 hour period. Although the bee colony is the unit being studied, it is individual bees that are sampled to determine chemical exposures.

### 2.6 Colony Behavioral Assays

This study uses small survey hives, full-sized survey hives, and honey bee "condos" or electronic hives 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 2.6.0 and 2.6.1). 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), collect bioavailable contaminants, and respond by their behavior to changes in their environment.

Each bee leaving or entering a condo passes through an infra-red counter (Figure 2.6.2) mounted on the front of the condo box (Figures 2.6.0 and 2.6.1). 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 tray.



Figure 2.6.0 and 2.6.1. Honey bee condo. A. Closed. B. Flip-top was opened to reveal internal hive components.



Figure 2.6.2 Entrance mounted honey bee, bidirectional (ingress/egress) counter.

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, Cluster 3, 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 and 1999, where communication lines were available to the field sites (namely Churchville and sometimes at J-Field). The bee counter data acquisition software can communicate via the RS-232 serial port to a computer equipped to receive data. Where direct communications (e.g., telephone, wireless) to the hives at a field site are available and reliable, data summaries are forwarded via the Internet to all designated users (password protected) at 15 minute intervals. 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 Montana or the USACEHR Administrative Trailer at the APG/Edgewood site. Due to radio noise, wireless communications proved to be undependable at APG. The data from the Cluster 3 and J-Field PCs had to be manually transferred using Zip<sup>TM</sup> disks. A telephone connection between the Churchville site in Maryland and the UM server facilitated direct access to the Maryland reference site. An automatic generator/conditioned power source in the Administration Trailer at J-Field provided reliable power for 1999. There were no interruptions or data loss during the 1999 field season at J-Field.

# 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. Neural Networks have proven successful in predicting colony responses to weather and in flagging natural events such as swarming or robbing (Seccomb, 1998). Other deviations from the expected behavior occurred and were flagged by the ANNs. These deviations were not necessarily indicative of an adverse reaction or situation, but they provided a data subset that marked anomalous event days. These were the days when efforts should be focused to identify whether the cause of the deviation was natural (e.g., lack of food, disease) or from external influence such as toxic chemical exposure, which had to be verified by chemical sampling.

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; Inorganics in Bees and Pollen

Heavy metals and other inorganic elements of concern (Be, As and Se) were analyzed by inductively coupled plasma mass spectrometry (ICP/MS) by the chemical analysis laboratory at USACEHR.

**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). Again, this sampling method best assesses variation within the hive during different parts of the growing season and with forager bee population turnovers. Where feasible, sampling was conducted as soon as possible from any colony exhibiting a deviation from normal behavior, but only after visual inspection verified that the problem was not simply one of bee management (i.e., adequate nectar and pollen, disease and mite free). At

all locations, the ambient air was also sampled. Most of the electronic hives were sampled for these chemicals at least three times during the monitoring season, and survey hives were sampled at least two times during the growing season. The phytoremediation hives at J-Field were sampled several times during the spring bud-burst period. Honey bees gather resins from tree buds in the spring. The trees in the phytoremediation grove did not flower, and as such did not provide the bees with nectar or pollen. But, the bees did work the tree grove during the budburst period.

Detailed methods for sampling and analysis have been presented in our previous reports (Bromenshenk *et al.*, 1996, 1997, and 1999). Instrument calibration and the list of 20 organic compounds measured in 1999 appear in Section 4 of this report. 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. This improved our ability to quantify VOCs and SVOCs, although it does affect the ability to make precise comparisons to prior year's data (because of the improved instrumentation performance). However, the same approach was used in 1999 and 2000, so the 1998 through 2000 results can be directly compared.

**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 bee 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 scraper 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. QA/AC data relevant to recoveries and instrument calibration were supplied to us along with the sample results.

# 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 four field sites. Bees were monitored coming and going 200 times per second, and data was available every thirty seconds, providing a data set in as near real time as possible. As described earlier in this report, distance data delivery at best occurred every 15 minutes. Where communications lines were not available or reliable, data delivery was delayed by up to a week or more, since access onto some of the sites was restricted, sometimes for days. Without reliable communications lines, the only option was to transfer the data to a ZIP® disk at the field site and then either send it over the Internet or Federal Express® the disk to Montana.

These measurements were guided by two overall objectives:

- Real time monitoring of colony population dynamics to establish the relationship between weather, food resource availability, and 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 shortas well as long-term 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 was 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 were again placed at J-Field and Churchville, and Cluster 3 was added, replacing Old O Field. 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 both 1998 and 1999, exposures 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.*, 1996, 1997, 1999). The 1997 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 includes total flight activity (TFA), inter-colony coefficient of variation (C.V.) of total flight activity, the percentage of bees returning to the colonies at each site at the end of each day (PRC), the net loss (NL) of bees at the end of each day, and the adjusted net loss (ANL) of bees at the end of each day.

Tier 2 analysis applied Tier 1 analysis methods to individually compare the colonies at a site. The PRC, NL, and ANL methods were all expected to demonstrate greater fluctuations for individual colonies than observed in the Tier 1 results. Normalized Total Activity (NTA) was calculated as the total activity for each colony divided by the total activity for the seven colonies at the site.

Tier 3 flight activity analyses included all data analysis methods that operate on short-term flight activity data. The UM Siteview Data Analysis Package (SITEVIEW) offers many analysis options for Tier 3 data.

Flight activity data collected from spring through fall of 1999 was summarized and compared with data from the 1998 field monitoring seasons. This report focuses on comparing the 1999 and 1998 colony performance results. Comprehensive data summaries for 1996 through 1999 will be published in a Final Technical report scheduled for delivery in May, 2001. All of the colony performance data for all years and all sites can be provided on a CD, upon request).

Figures 3.3.0 and 3.3.1 present 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. Detailed summaries of colony performance and of events that occurred during the previous field monitoring seasons have been presented in previous annual reports (Bromenshenk *et al.*, 1997, 1998).

In general, as in previous years, flight activity at the APG sites— J-Field and Cluster 3—displayed similar day to day performance, usually reflecting seasonal activity trends as a consequence of colony responses to storms and other local weather conditions affecting both sites. Major or more regional weather conditions also affected Churchville, which was located 12 miles inland from APG-Edgewood. For example, on rainy days, flight activity often was disrupted at all three sites. As expected, the two APG sites more closely matched each other in terms of weather-driven changes in flight activity, than did Churchville. All three sites showed a similar seasonal trend characterized by the highest flight activity during the spring and early summer (through July) months. Flight activity usually decreased during late July and early August, with activity picking up again by mid-August. In general, flight activity became more variable and began to decline by mid-September. By early October, flight at J-Field was increasingly sporadic; while, at Cluster 3 and Churchville, the bees stayed active through the third week of October. At Churchville, colonies 4 and 7 served as controls throughout the season. Colony 3 was intended as a control, but it absconded twice. The other four colonies plus several non-electronic colonies were used in toxicity assays beginning in early August. The effect of these trials on colony performance can be readily seen in all of the following figures. These trials are described and the results appear in an M.S. Thesis by Taylor (2001).

#### **Coefficients of Variation:**

Coefficients of variation of flight activity among the colonies at each site for the 1999 monitoring season were plotted and compared. As seen in Figure 3.3.2 and Table 3.3.0, in 1999, prior to mid-September when the queens start shutting down egg-laying, the C.V.s for flight activity at J-Field and Cluster 3 ranged from a low of 15% at each site to highs of approximately 60% at Cluster 3 and 75% at J-Field. Higher C.V.s of 30-100% at Churchville and 50-100% at Fort Missoula were induced by experimental trials that were conducted at these two sites as part of the ongoing calibration trials for acute contact toxicity (Churchville) and to profile chemical exposures, uptake, and fate dynamics (Fort Missoula). The Churchville calibration trials began in early August and continued through the end of the 1999 field season. To date, the lowest seasonal C.V. of 15-50% was observed at the Churchville reference site in 1997, when these condos were left more or less undisturbed - except for occasional inspections and chemical sampling. The coefficients of variation for the 1998 sites are presented in Figure 3.3.3.

Low coefficients of variation indicated that the colonies at a site were well matched in terms of the size of the forager force and the number of foraging trips made each day from each hive. Because the size and activity of the forager force is more or less related to population size, a low CV implied that colony population sizes were also similar at a site. Higher C.V.s indicated that the colonies varied more in flight activity, number of foraging trips, and presumably population size.

Some variation in colony population size and activity levels is expected. However, the colonies at each site were selected to provide a similar distribution of colony population size among the hives just prior to deployment at each site. Increased coefficients of variation indicate a greater range of bee population sizes and activity levels at a specific site, compared to other sites in the same region. Upon inspection, sites with high C.V.s usually had one or more weak bee populations or some particularly strong colonies. By itself, the C.V. simply assesses whether the honey bee population foraging performance is well matched among the hives at a site, or whether it is highly variable. Under normal situations, one expects to see relatively low C.V.s. Sites with high C.V.s warrant inspection and possible chemical sampling to address the reason for the high between hive variability.

#### **Total Daily Flight Activity:**

As shown by Figures 3.3.0 and 3.3.1, total daily flight activity at Cluster 3 and J-Field remained vigorous through September 15, when the weather changed, and flight was curtailed for a couple of days. Following this event, flight activity dropped off rapidly and became quite variable at J-Field. Flight activity at Cluster 3 also dropped at this time, but to a much lesser degree. Bees at Cluster 3 remained active through October 22. At Churchville, three colonies (3, 4, and 7) were used as controls, the others, beginning in early August, were manipulated via a series of contact toxicity experiments. The flight activity of the control colonies at Churchville exhibited a mid-September disruption of flight induced by weather changes, as was also seen at the two APG sites. However, there was no overall, long-term reduction in flight activity at Churchville. Like the bees at Cluster 3, the Churchville control colonies continued to forage through October 22. Flight activity dropped off sooner at J-Field, probably because of a lack of forage (i.e., the colonies had to be fed to sustain them).

In previous years, power failures, usually following thunderstorms, often produced data gaps because the electronic systems went off-line. This was a particular problem at J-Field, where access to the site is often denied. In 1999, the USA CEHR administration trailer was moved onto the J-Field site. This trailer had a diesel generator and conditioned electrical power. As before, there were frequent power disruptions from storms, electrical surges, brownouts, and physical damage to lines that occurred during the removal activities. However, the generator system worked flawlessly, as can be seen from the plot of total flight activity for 1999. A small data gap occurred in early August for two hives that were accidentally unplugged by people walking across the site.

The high variability in overall flight activity as well as data gaps clearly evident in the plots of total flight activity for Churchville in 1999 are a consequence of acute contact toxicity trials conducted at that site in late summer and early fall. This provide graphic visualization evidence of the ability of the real time monitoring system to reveal the presence of stressors that alter flight dynamics. These experiments are detailed in Taylor (2001). Subsets of colonies were dosed with different levels of a highly toxic pesticide (contact toxicity trials) to determine the response as measured by the bee counters. Systematic trials at different concentrations of each chemical are explained in Taylor's thesis (2001) and field log book, and the results were statistically significant. Data gaps occurred when colonies were taken off-line or exchanged during the trials. These gaps were verified by the log book. Exactly what was done with each colony each day is well understood and documented in her thesis.

#### Percent Return to Colony (Figures 3.3.4 and 3.3.5):

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 3.3.1). This metric provided an indication of the general health of the colonies at each site and of possible exposure to toxic chemicals. The percent return can be compared to a known mean and standard deviation based on six years of data from multiple sites and hives.



**Figure 3.3.0** 1999 APG site by site comparisons of adjusted total daily flight activity (entering + exiting) corrected for number of 30 second sampling periods collected per day. Fort Missoula is in a different geographical and climatic zone from the rest of the sites. Cluster 3 and J-Field closely parallel each other in terms of daily flight throughout most of the season.

A PRC of 100% indicated that on a given day, as many bees returned to the hive as left on their foraging trips. PRCs somewhat less than 100% were expected as a consequence of forager bees dying of old age, being blown off course, overtaken by predators, accidentally killed (e.g., car windshield), or returning to the wrong hive. Sudden or severe reductions in the numbers of bees returning to the hive on a given day would indicate a high probability of exposure of the foraging bees to toxic chemicals in air, food, or water. Other than swarming, which is easy to identify by examining the flight pattern profile of the hive on the day of the event (as much as 50-70% of the population may leave the hive in less than 30 minutes), no stressor other than exposure to a toxic substance, including disease, produces a sudden or severe decrease in the PRC.

Similarly, if the daily return rates fall off and then continue to drop over a period of several days, inspection of the colony and collection of samples would be warranted to determine whether the

colony was being impacted by a pathogenic disease or mites or if it had been exposed to toxic chemicals.

Monitoring for this type of change is similar to using a control chart approach. Sudden deviations in the PCR that exceed 1 SD of the mean are said to exceed a warning limit and warrant inspection, those that exceed two SD are said to be out of compliance and warrant additional action and, if possible, correction. A systematic bias of three or more readings in a row, either below or above the mean, also constitute a warning. Some of these events will reflect the normal progression of the colony. For example, in the spring, bees that survive the winter have will have a relatively short life span left, and as such the PCR values tend to be lower. The work of Taylor (2001) indicates that the PCR will reflect long term impacts to forager populations following exposure to toxic chemicals at different concentrations. Most importantly, after the hive mounted traps stop catching an excess of dead and dying bees, the bee



**Figure 3.3.1** 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. J-Field and Old O Field are both located on the lower peninsula of APG-Edgewood and are subjected to similar climatic conditions. These two sites tend to parallel each other in terms of daily flight. Fort Missoula is in a different geographical and climatic zone.

counters continued to show reduced flight performance - both in terms of overall activity and percent returns.



**Figure 3.3.2** Site by site comparisons of coefficients of variation among colonies for flight activity throughout the 1999 spring, summer, fall season. The spike at the beginning of the Cluster 3 monitoring period reflects positioning and repositioning of the bees and equipment at this site (the site was moved a few hundred yards, resulting in some equipment resets and some initial drifting by bees). The high CVs for Churchville were mostly a consequence of toxicity assays conducted at the site, beginning in August.



**Figure 3.3.3** Site by site comparisons of coefficients of variation among colonies for flight activity 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.



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**Figure 3.3.4** Cumulative Percent Return by site, spring, summer, fall, 1999. The effects of hive 3 absconding twice at Churchville and of the toxicity assays can be seen by the occasional percent return values falling below the 90% axis. Overall, return rates best at Cluster 3 and for the Churchville controls (96% or better), slightly poorer at J-Field.



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**Figure 3.3.5** Cumulative Percent Return by site, spring, summer, fall, 1998. Overall return rates were above 96%, dipping to 92 on occasion. The May spike at Churchville shows the effects of food shortages, rain, and robbing.



**Figure 3.3.6** Site by site comparison of Adjusted Net Loss for 1999. Net loss is corrected for number of samples collected per day. The dose-response toxicity assays at Churchville are reflected in the high/low spikes shown, as were the trials at Fort Missoula.



**Figure 3.3.7** Site by site comparison of Adjusted Net Loss for 1998. Net loss is corrected for number of samples collected per day. Churchville sustained floral (food) resource shortages in May and early June.

#### Table 3.3.0

#### 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 Churchville and 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	1999
Churchville	CV	92%-100%	94%-98%	94%-105%	96%-100%
Cluster 3	CL3	-		-	95%-102%
J-Field	JF	-	92%-98%	92%-103%	90-104%
Fort Missoula	FM	-	94%-108%	90%-104%	94-106%

Generally, for J-Field and the control colonies at the Churchville reference site, the sitewide PRC results stayed within the range of 90% to 102% during the foraging season. Values greater than 100% were thought to be mainly a result of bees drifting from one colony into another. In earlier years, warping of the doors sometimes allowed outgoing bees to bypass the counters. Before the 1999 field season, the white opaque covers on the counter assemblies were replaced with clear Lexan<sup>™</sup>. With clear covers, the counters could easily be inspected for any visible signs of bee "leakage"; more or less eliminating this source of error in 1999. Return rates for Cluster 3 in 1999 were among the best seen at any site over a five-year period. As in previous years, the return rates at J-Field tended to be slightly lower than at the reference sites at Churchville and Missoula, with the exception of Fort Missoula in 1998. However, Fort Missoula was used for acute pesticide toxicity testing in 1998. Although the PRCs during these test trials were not included in the computation of Fort Missoula PRC s summaries for that year, the exposures of these hives to a pesticide earlier in the summer may have had some carryover effect.

Table 3.3.1 provides a comparison of Tier 1 values (coefficients of variation) for each site for 1996, 1997, 1998, and 1999. In 1997, C.V.s at both MD sites were the same. In 1998, C.V. values for the Maryland sites were lower at J-Field than the at the reference site. Overall, the C.V.s were somewhat higher in 1998 than in 1997. In 1998, the highest C.V.s for flight activity were observed at the Churchville reference site, in part because of severe floral (food) shortages in early summer. C.V.s of 30-140% at the Fort Missoula research site reflected the numerous experiments conducted at the site over the three-year period, especially replacements of colonies after the dose/response trials.

#### **Table 3.3.1**

#### 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 and Churchville have been removed from this data set. Sites not monitored in a given year are indicated by a dash in the appropriate cell.

	C.V.			
Site	1996	1997	1998	1999
Churchville CV	50%-90%	15%-50%	30%-100%	30-100%
Cluster 3 CL3	-	-	-	15%-75%
J-Field JF	-	15%-50%	20%-80%	15%-60%
Fort Missoula FM	-	30%-140%	20%-100%	50-100%

C.V.s at Churchville in 1999 were higher than at Cluster 3 or J-Field. As at Fort Missoula, the higher C.V.s at Churchville in 1999 were due in part to the conduct of dose/response systems calibration trials and other experiments conducted in the beeyard. The C.V.s for J-Field and for Cluster 3 for 1999 were as low as any measured at any APG site over the five-year study period (including sites monitored in earlier years that are not listed in this table).

#### **Adjusted Net Loss:**

The most important finding of the PRC analyses (summarized in Table 3.3.0) was that over all sites and all years, 90% or more of the bees leaving the hives at each site returned during each day. The best sites displayed site wide averages of 94% or better of the bees returning each day. J-Field consistently displayed slightly lower returns (90-92%). Based on six 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.

Our methyl parathion trials clearly show short term depressions in return rates far exceed 8%. Therefore, based on the PRC metric there was no evidence of any acute toxicity at any of these sites.

This finding should not be taken to mean that there were no toxic chemicals at these sites. Some chemicals may be more toxic to other plants or animals than to bees, and toxicity is a function of the dose received. A toxic chemical concentrated in a small hot spot such as an area of bare soil would have a low probability of being encountered by the bees or taken back to the hive. Our chemical analysis data indicates the presence of hot spots. For example, at J-Field, occasional bee and pollen samples showed elevated levels of lead, but the dose concentration or exposure

period apparently was not long enough to result in a change in the PCR for the colony in question.

The ANL (Table 3.3.2) provides an additional way of examining the data reflected by the percentage of bees returning to each colony (PRC). A net loss is demonstrated by a positive number; and a net gain by a negative number. As reported in the previous annual report, variations in adjusted net loss calculated at the end of each day improved 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 by one or more of the counters. For 1999, the counter assemblies were fitted with clear Lucite<sup>™</sup> coverings, so that any entrapment of bees can be easily seen and corrected. For 1999, there was somewhat higher net loss of bees at J-Field; although, all values fell between the range of -4 to +6 that we have documented at APG over the last several years.

#### Table 3.3.2

#### 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 and Churchville have been removed from this data set. Sites not monitored in a given year are indicated by a dash in the appropriate cell. Positive values indicate a net loss; negative values a net gain.

	ANL			
Site	1996	1997	1998	1999
Churchville CV	0 to +4	0 to +3	-2 to +3	0 to +2
Cluster 3 CL3	-	-	-	-1 to +3
J-Field JF	-	+1 to +5	-1 to +4	0 to +5
Fort Missoula FM	-	-4 to +3	-2 to +5	-4 to +3

#### 3.4 Results of Tier 2 Evaluations of Flight Activity Data

Daily flight activity data for each individual colony at each site throughout the field season were compared to that of other colonies at the site. These plots constitute the day to day history or diary of each and every colony. Any experienced beekeeper can "read" these color plots and immediately rank colonies according to the size of the forager population. The seasonal growth dynamics of the forager force are clearly illustrated. Deviations from expected performance can be visually discerned. The normalized flight activity color maps provide the full richness of the real time data sets, profiling the dynamics of each colony at each site. This information is lost in the summary statistics present in the C.V. summary tables. For those sites that were part of a multi-year continuing study, this same data is presented for the previous year. Figures 3.4.0 - 3.4.6 present total daily flight activity for the sites under study in 1999 and 1998.

Variations in flight activity can be quantified as C.V.s among hives (Tier 1) and graphically visualized for individual hives (Tier 2) by using normalized flight activity color maps for each site as displayed in Figures 3.4.0-3.4.6. 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. Differences in colony performance can easily be contrasted and compared via these visualizations; and seasonal changes in each colony's flight dynamics can be tracked.

Colonies at the Churchville reference site displayed the most obvious variation in total flight activity in 1999 (Figure 3.4.0). The total flight activity of control hives 4 and 7 look similar to those of colonies at Cluster 3 (Figure 3.4.4). Churchville colony 3, that absconded twice, clearly can be distinguished by the effect on overall flight activity. Also, the dose-response, acute toxicity trials began in August can easily to seen given the performance of colonies 1,2,5, and 6.

Colonies at the Churchville reference site displayed some obvious variance in total flight activity during 1998 as shown in Figure 3.4.1. 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.

The 1999 flight activity data for J-Field (Figure 3.4.2) profiles a site where all of the colonies were very strong through mid-July. So strong, that some of the hive swarmed. The long-term effect of swarming can be seen in the plots for colonies 6 and 7. Colony 6 showed little decrease in overall performance. Colony 7 dropped off in flight activity in the fall. Field inspection notes indicate that the overall bee population in this colony remained strong, but flight levels were somewhat lower than in the other colonies. It was also noted that this hive was closest to the USACEHR trailer. As such, it would have received more shading from the west sun in the afternoon than received by the other colonies. Other real time flight data systems have shown in earlier tests that overall bee flight patterns (when the bees begin and end flight) is in part due to the amount of light (e.g., solar radiation) striking the hive. A short-term power outage in early August can be seen by the data gap for hives 5 and 6. The outage occurred when someone walked across the site, stepping on and disconnecting the power cords to these two hives.

In 1998, (Figure 3.4.3), two colonies (#3 and #4) at J-Field displayed a higher than average contribution to the site-wide total flight activity. While strong, vigorously flying colonies were common at this site both in 1996 and 1997, in 1998 the colonies demonstrated somewhat decreased overall flight activity levels compared to the earlier years, with overall flight activity levels increasing again in 1999.

In 1999, Cluster 3 colonies started the trials in good condition and continued good flight activity until late in the fall (i.e. end of October) (Figure 3.4.4). Hives 1-4 were somewhat stronger than hives 5-7. Hive 4 displayed the highest activity levels throughout the study period; hive 5 the lowest. But, overall, these colonies performed well. No bee populations nor queens were lost at this site during the study; nor were any bee declines documented. The hive to hive variability, as noted earlier, displayed some of the lowest overall C.V.s measured at any APG site over the last several years. Because this site was set up with electronic hives for the first time in 1999, there is no colony performance data with which to compare this site to previous years. In 2000, this site was again fitted with electronic hives. Those comparisons will appear in the Yr 2000 annual report. Also, six survey hives were placed at Cluster 3 in 1997; so chemical exposure data is available for comparison to 1999 levels (Section 4 of this report).

The Fort Missoula colonies in 1999 and in 1998 (Figures 3.4.5 and 3.4.6) reflected different geographical and climatic influences, compared to the Maryland colonies. In general, the colonies in Montana grew to large population sizes and exhibited higher overall flight activity. This is in part due to a more sustainable floral (food) resource and in part to our longer day length. In Maryland, most of the nectar flows, from which bees make honey, occur before the end of June. In Montana, bees generally encounter several nectar flows over the spring and summer, beginning in April and extending into August. Also, in Maryland, the dawn to dusk period is several hours shorter than in Montana in the summer. It is not surprising to see bees still flying after 10:00 p.m. in Montana; whereas the bees will have returned to the Maryland hives by 8 or 9 p.m. at the latest. A variety of trials were conducted at the Fort Missoula experimental site in 1999, mostly addressing chemical uptake and recovery by our sampling systems.

In 1998, on July 20 some of the Fort Missoula colonies were exposed to methyl parathion placed in the hive as Crisco patties. Additional trails were conducted on August 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, the flight activity levels gradually decreased for the treated colonies. These experiments with time specific plots (1-2 week intervals) were published in the 1998 annual report and are covered along with the 1999 trials in the thesis by M.A. Taylor, 2001.

In general, colonies at all of the Maryland sites displayed good levels of total flight activity during the 1999 flight season. Flight numbers were generally as high or higher than in previous years and more consistent among hives at the APG sites. At Cluster 3 and at Churchville, the bees continued to forage later into the fall (late October) than in previous years. Colonies at the Churchville reference site showed good flight activity until early August, when doseresponse/toxicity trials were initiated. This produced in treated (dosed) colonies lower levels of flight activity and a higher degree of variability than observed in the previous years. The percent return Figures (3.4.7-3.4.9) for J-Field, Cluster 3, and Churchville provide a colony by colony comparison for return rates within and among sites for 1999. Good return rates mean that there were little or no losses of forager bees. The 1998 percent return charts were published in the 1998 annual report and are not repeated here.

Percent bees returned by end of day for the Churchville reference colonies, 1999 (Figure 3.4.7), exhibit the effects of the toxicity assay trials begun in August. The control colonies 4 and 7 show good return rates with some drifting of bees into these colonies during and after the dose trials. The treated colonies displayed low return rates (85% or less) and/or drifting and robbing (the white horizontal bars clearly evident in late August and late September. The J-field colonies (Figure 3.4.8) show relatively consistent return rates that were greater than 90%, with some hives dropping for short periods below 85%, mainly during the swarm season in July. The Cluster 3 colonies (Figure 3.4.9) displayed some drifting (values greater than 100% return) when the colonies were first placed on the site in July. This more or less disappeared within 3-4 days of deployment. Colony 5 tended to recruit or accumulate excess bees, especially in September. Overall, percent return rates were somewhat higher (usually 95% or better than at J-Field).

#### 3.5 Results of Tier 3 Evaluations of Flight Activity Data

A main focus of the 1998 and 1999 year's data analysis efforts was refining the application of methods first 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 during both years was due to starting data collection as early as April or May, before the primary swarm season for honey bees (usually May-June).

#### 3.6 Overall Discussion and Conclusion: 1999 Colony Performance

During 1999, the colonies at Cluster 3 and J-Field, in terms of total flight activity and within site coefficients of variation day ranked among the best observed since the honey bee biomonitoring was begun in 1996. This was also true for the control colonies at Churchville. With the exception of the colonies subjected to toxic doses of methyl parathion, none of the colonies displayed acute losses of bees.

However, as in previous years, the percent return rate (PCR) for foragers coming back to the hive each day, at J-Field fell somewhat below that of the other sites. Other factors, such as poorer nutrition may have played a role, although the colonies were provided with supplemental food. As is reported in Section 4 and 5 of this report, the colonies at J-Field were subjected to high levels of more kinds of VOCs than at any other site. Also, the colonies at this site were occasionally exposed to elevated levels of lead (as seen in bees and in pollen), and manganese and strontium concentrations were significantly (statistically) enriched in bees and pollen. Therefore, we speculate that it is likely that some of the dwindling of the colonies might have be a result of a low level toxicity from the cumulative effects of these chemicals.

With respect to the Boundary hives, one of the two colonies at Cecil County and at Jones Farms exhibited some problems. At Cecil County, queen egg-laying was low and the population lagged considerably behind that of other Boundary sites. At Jones Farm, one of the queens was either lost or stopped laying. Despite the disruption of egg-laying, this colony maintained satisfactory population size until late season, and was eventually re-queened. In 1998, these two sites, as well as Tower Farm and Silver Lake lost one of each pair of bee colonies. Both in 98 and 99, these sites exhibited higher exposure levels to volatile organic compounds than the other Boundary sites (Section 4).

The methyl parathion toxicity trials (M.A. Taylor, 2001) re-enforced the observations of other investigators that collecting dead bees in front of the hive is of little value for determining bee losses and that traps mounted on the hive, at best, only collect a portion of the total bees lost. Traps are most useful at the time that the toxic chemical first appears within the hive. They are not good at quantifying bees lost in the field.

One of the most significant findings of Taylor's work is that long after an acute mortality event had ceased, based on dead and dying bees caught by traps mounted under the dosed hives, the effect of the event continued to impact the size and activity of the forager bee population as documented by the bee counters - sometimes up to several weeks. This effect was not seen in her control colonies. In fact, most of them increased in size and flight activity.

Overall flight activity combined with the percent of bees returning to the hive provided not only a means of identifying a possible toxic exposure event, but also provided the ability to assess the effect on total flight activity, to identify the effect of such a toxic event within an hour, and to determine the duration of the effect and to follow population recovery, if any. Although some effects are unknown and yet to be determined, many events were predictable in how they might play out.

Obviously, each toxic event has its own dose-response relationship and toxicity response and the conditions under which exposure occurs are not uniform. However, the flight activity and PRC metrics, as verified by Taylor's research (2001), can and do quantify the effects of a exposure to a toxic substance, as well as those of other stressors. In other words, the system can detect a change if it occurs.

Further examination of the data and possibly inspection of the hive and chemical sampling is warranted in order to provide a weight of evidence with respect to probable cause and effect—toxic dose, disease, weather, bee management. However, some responses such as a sudden loss of bees in the field with greatly reduced return rates at the hive are almost always going to be from bee poisoning. The few other causes such as a severe storm or fire that caught large numbers bees in the field (which rarely happens since bees sense incoming storms and fire and return quickly to the hive) and vacating the hive because of swarming or mites are easy to identify by an experienced or trained bee manager.



**Figure 3.4.0** Total flight activity for colonies at the Churchville reference site, 1999. Hotter colors denote higher activity. Plots reflect dose-toxicity calibration trials beginning in August at this site (i.e., changes in bee activity in response to trials). Colonies 4 and 7 were controls. Colony 3 absconded twice. Colonies 1,2,5, and 6 were used in dose-response trials.



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**Figure 3.4.1** Total flight activity for colonies at the Churchville reference site, 1998. Hotter colors denote higher activity. The effects of heavy rains and food resource shortages are reflected in the May through early June flight activity.



**Figure 3.4.2** Total flight activity for colonies at the J-Field Site, 1999. Hotter colors denote higher activity. Colonies 6 and 7 swarmed and re-queened with minimal effect on overall flight activity. Floral (food) resources became limiting by late September at J-Field. The August data gap for hives 5 and 6 was caused by someone stepping on the power cords and disconnecting the hives.



**Figure 3.4.3** Total flight activity for colonies at the J-Field Site, 1998. Hotter colors denote higher activity. Colonies 3 and 4 had the highest flight activity. Data gaps are due to either rain holding bees in their hives or power outages. This problem was corrected in 1999 by using an automated electrical generator.



**Figure 3.4.4** Total flight activity for colonies at the Cluster 3 Site, 1999. Hotter colors denote higher activity. Colony 4 had the highest flight activity throughout the season; colony 5 the lowest. Overall flight activity was good and continued late into the season (i.e., October). No colonies swarmed, absconded, or lost a queen at this site. All completed the field trials with a queen and good bee populations.



New and Improved Methods for Biomonitoring, 1999 Annual Report

**Figure 3.4.5** Total flight activity for colonies at the Fort Missoula Site, 1999. Hotter colors denote higher activity. Very strong hives. Counter 2 (colony 2) was not used in 1999.



**Figure 3.4.6** Total flight activity for colonies at the Fort Missoula Site, 1998. Hotter colors denote higher activity. The data show the effects of acute toxicity trials initiated on July 20 and August 7. Some of the colonies served as controls, others were dosed at different levels with methyl parathion.



**Figure 3.4.7** Percent Bees Returned by end of day for Churchville Reference Colonies, 1999. Colonies 4 and 7 were seasonal controls. The effects of the toxicity assay trials begun in August are shown by low return rates (85% or less) and by drifting and robbing (the white horizontal bars clearly evident in late August and late September.



**Figure 3.4.8** Percent Bees Returned by end of day for J-Field Colonies, 1999. Overall return rates exceeded 90%, with only occasional drifting (values in excess of 100%)



**Figure 3.4.9** Percent Bees Returned by end of day for Cluster 3 Colonies, 1999. Most return rates exceeded 95%. Some drifting is evident when the site was first established in July. Colony 5 drew in bees in September.

#### SECTION 4 CHEMICAL SAMPLING OF VOLATILE AND SEMIVOLATILE ORGANIC COMPOUNDS

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

- Measure chemical agents in the ambient air as well as those in the air inside beehives 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 along three transects extending into 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) as measured in honey bee colony populations.

For the 2000 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) in a manner consistent with EPA's TO Methods for the Determination of Toxic Organic Compounds in Ambient Air. Ambient air and hive air sampling are based on Compendium Method To-17, Determination of Volatile Organic Compounds in Ambient Air Using Active Sampling onto Sorbent Tubes. TO-17 is an alternative to canister-based sampling and analysis methods, (Compendium Methods TO-14 and TO-15) and to the previous sorbent-based methods that were first formalized as EPA Methods T0-1 and T0-2 (EPA/600/4-89/017). All of these EPA methods cover sampling at one location, storage and transport of samples, and analysis at another, typically, more favorable site. TO-17 was released January 1999.

For 1999, we quantified 54 compounds from the EPA Method 502 Volatile Organic Compound list. Of the 54 compounds we analyzed, we report the twenty found above detection limits and in amounts of some significance (Table 4.0.0). Ambient air was sampled at each site at some distance from the bee hives, while the hive air samples were taken from within each hive. These samples reflect compounds found in the ambient air, in or on the bees and any substances that they bring back to the hive, as well as those already present in the hive, including pheromones and waxes as detailed in Smith et al.(2002). The remainder were below detection limits or present infrequently and at levels so low as to be of questionable concern. These results are not presented in this report. The concentration of all compounds, including those below detection limits or of questionable certainty, are reported in a comprehensive spreadsheet database that can be provided upon request.

This chapter presents the results of the 1999 monitoring of VOCs and SVOCs and, where possible, compares them to the results from 1997, and 1998. Because of improved instrumentation and software at UM's analytical laboratory, this report evaluates and discusses 20 VOCs, up from the nine that we have focused on in previous reports. J-Field was sampled all three years; Cluster 3 was evaluated with survey hives in 1997, was not surveyed in 1998, and was brought on-line with electronic hives and full-sampling in 1999.

# Table 4.0.0EPA Method 502 Volatile Organic CompoundsReported for APG, 1999

Halogenated Organics	Petroleum Fuel Residues
cis-Dichloroethene	*Benzene
1,1-Dichloroethene	*Toluene
1,2-Dichloroethane	<i>m</i> , <i>p</i> -Xylene
Trichloromethane	o-Xylene
*Perchloromethane	*Ethylbenzene
*Trichloroethene	Styrene
1,1,1-Trichloroethane	*Naphthalene
*Perchloroethene	-
1,1,2,2-Tetrachloroethane	Tear Gas Residues
Perchloroethane	*Acetophenone
Bromobenzene	*
*1,4-Dichlorobenzene	

\* Compounds reported in 1998

#### 4.1 Sampling Frequency

Chemical sampling frequencies for VOCs and SVOCs varied depending on individual locations and specific study objectives, as described in Section 2.4 of this report. At J-Field, a more intensive sampling schedule was followed early in the field season to investigate the potential transfer of VOC/SVOC compounds to bees in the phytoremediation grove. Samples were taken from 15 standard-size hives surrounding the phytoremediation grove of hybrid popular trees, during March and April when the trees were undergoing bud burst. A set of electronic condos was placed at J-Field in June to continue the combined chemical exposure and real-time effects biomonitoring of the installation restoration activities at J-Field.

Based on the results of the initial 1998 boundary survey, we decided to deploy hives in 1999 to again assess bioavailable VOCs and SVOCs at the ten primary Boundary Sites (plus Cluster 13) (Table 4.1.0 and Figure 4.1.0). Two complete rounds of samples were obtained in 1999 - one in July and one in September. The 1999 September round was important since in 1998 we were

unable to obtain a complete late-season sample. By fall of 1998, many of the Boundary hives had reduced populations and four sites had lost one of the two sample colonies.

In July of 1999, a set of instrumented, electronic beehives were placed near Bush River Cluster 3 to conduct biomonitoring of exposures as well as real-time monitoring of effects during the remediation/removal activities that were being conducted. After several weeks of foraging activity, monthly samples were collected from this set of condos. Sample dates were August 17, September 14 and October 13. The results from this work follow up on the chemical exposure results obtained from pairs of survey hives that were placed at three locations within the Cluster 3 site during the 1997 field season.

Reference hives at Churchville were deployed as in previous years. An initial round of samples was drawn from an instrumented condo cluster of seven hives on June 24. A second round was acquired from the condos just before four of the seven colonies were used for methylparathion contact toxicity experiments. A final round of samples from Churchville was collected from three control hives on September 24. Beginning in August and extending through September, a series of hives were used at Churchville in experimental trials intended to provide information relative to calibrating our real-time biomonitoring system for exposures to toxic compounds. This work was reported by M.A. Taylor in her Thesis Report for a M.S. in Ecology, 2002. In addition to taking ambient and hive air samples for volatile organics determinations, whole bees and pollen were sampled for trace elements and heavy metals (i.e., inorganics). This sampling was conducted twice—in the early summer following the spring rains and nectar flow, and again in late summer or fall. The inorganics results appear in Section 5 of this report.

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 4.1.1.

# Table 4.1.01999 APG Boundary Study Sites (see also Figure 4.1)

#### Transect Hub - Youth Center (YC)

Hives:99028 & 99076Location:Youth Center, APGMap coord:HC29D06

#### Northeast Transect (extra site)

Hives:	99029 & 99035
Location:	APG Cluster 13
Map coord:	HC29F05

#### Northeast Transect - (3 mi; OP)

Hives:	99079 & 99039
Location:	Otter Creek Point Estuary/State Park
Map coord:	HC24H11

#### Northeast Transect - (9 mi; LO)

Hives:	99074 & 99077
Location:	Lohr's Orchard
Map coord:	HC19B08

#### Northeast Transect - (21 mi; CC)

Hives:99037 & 99038Location:Conowingo Orchard, Cecil CountyMap coord:Harford County Index Map, 48mm from right edge, 39mm from top edge<br/>(about the "w" in Rowlandsville Rd)

#### West, Northwest Transect - (3 mi; JF)

Hives:	99032 & 99069
Location:	Jones Farm Fruit Stand/Truck Garden
Map coord:	HC24A12

#### West, Northwest Transect - (9 mi; TF)

Hives:	99071 & 99040
Location:	Tower Hill Farm
Map coord:	HC22H03

### West, Northwest Transect - (21 mi; SV)

Hives:	99002 & 99036
Location:	Fairview Manor Landscaping Firm, Shawsville
Map coord:	HC07E02

#### West, Southwest Transect - (3 mi; RI)

Hives:	99030 & 99048
Location:	Rumsey Mansion Private Residence (Rumsey Island)
Map coord:	HC28C06

#### West, Southwest Transect - (9 mi; SL)

Hives:	99046 & 99075
Location:	Silver Lake Drive Private Residence
Map coord:	BC29G09

## West, Southwest Transect - (21 mi; CA)

Hives:	99042 & 99044
Location:	Clyburn Arboretum
Map coord:	BC34F01



**Figure 4.1.0** Primary Boundary Study sites sampled in 1999 and J-Field. The transects originate at Youth Center (Y.C.) on the APG-Edgewood peninsula and extend to the Clyburn Arboretum in Baltimore county (WSW transect), Shawsville in Harford County (WNW transect), and Cecil County in Pennsylvania (NE transect). Bee monitoring sites are located approximately 3, 9, and 21 miles from Youth Center.

#### Table 4.1.1

# Hive- and Ambient-Air Sampling Dates, 1999 APG Biomonitoring Applications, On-Site and Boundary Studies

Site (Colony ID)	Sampling Dates
J-Field (On-Site)	
JFA1	3/11, 4/13, 4/27, 10/16
JFA2	3/11, 4/13, 4/27, 10/16
JFA3	3/11, 4/13, 4/27, 10/16
JFAAir	3/11, 4/13, 4/27, 10/16
JFB1	3/11, 4/13, 4/27, 10/16
JFB2	3/11, 4/13, 4/27, 10/16
JFB3	3/11, 4/13, 4/27, 10/16
JFBAir	3/11, 4/13, 4/27, 4/28, 10/16
JFC1	3/11, 3/19, 4/13, 4/27, 10/16
JFC2	3/11, 3/19, 4/13, 4/27, 10/16
JFC3	3/11, 4/13, 4/27, 10/16
JFCAir	3/11, 4/13, 4/27, 10/16
JFD1	3/11, 3/23, 4/13, 4/27, 10/16
JFD2	3/20, 3/23, 4/13, 4/27, 10/16
JFD3	3/11, 4/13, 4/27, 10/16
JFDAir	3/11, 3/23, 4/13, 4/27, 10/16
JFE1	3/11, 3/23, 4/13, 4/14, 4/27, 10/16
JFE2	3/11, 4/13, 4/14, 4/27, 10/16
JFE3	3/11, 4/13, 4/14, 4/27, 10/16
JFEAir	3/11, 3/23, 4/13, 4/14, 4/27, 10/16
JF1 (#026)	6/19, 8/6, 9/10
JF2 (#030)	6/19, 8/6, 9/10
JF3 (#034)	6/19, 8/6, 9/10
JF4 (#035)	6/19, 8/6, 9/10
JF5 (#041)	6/19, 8/6, 9/10
JF6 (#049)	6/19, 6/28, 8/6, 9/10
JF7 (#050)	6/19, 8/6, 9/10
JFCondo Air	6/19, 6/28, 8/6, 9/10
Grove Air 1	3/11, 4/13, 4/28
Grove Air 2	3/11, 4/13, 4/28
Grove Air 3	3/11, 4/13, 4/28
Grove Air 4	3/11, 4/13, 4/28

# Table 4.1.1 (continued) Hive- and Ambient-Air Sampling Dates

#### Cluster 3 (On-Site)

8/17, 9/14, 10/13
8/17, 9/14, 10/13
8/17, 9/14, 10/13
8/17, 9/14, 10/13
8/17, 9/14, 10/13
8/17, 9/14, 10/13
8/17, 9/14, 10/13
8/17, 9/14, 10/13

#### **Churchville Reference Site (Off-Site)**

CV1	6/24, 8/3
CV2	6/24, 8/3
CV3	6/24, 8/3
CV4	6/24, 8/3
CV5	6/24, 8/3
CV6	6/24, 8/3
CV7	6/24, 8/3
CVSH1 (#029)	9/24
CVSH2 (#038)	9/24
CVSH3 (#044)	9/24
CV Air	6/24, 8/3, 9/24

#### **Boundary Study Pivot Point (On-Site)**

Youth Center 1 (#028)	7/23, 9/14 7/23, 9/14 7/23, 9/14
Youth Center 2 (#076)	
Youth Center Air	
Northeast Transect	
Cluster 13 Hive 1 (#029)	7/23, 9/14
Cluster 13 Hive 2 (#035)	7/23, 9/14
Cluster 13 Air	7/23, 9/14
011 D 1 (1000)	7/22 0/25

7/23, 9/25
7/23, 9/25
7/23, 9/25

Lohr's Orchard 1(#074)	7/27, 9/14
Lohr's Orchard 2 (#077)	7/27, 9/14
Lohr's Orchard Air	7/27, 9/14
# Table 4.1.1 (continued) Hive- and Ambient-Air Sampling Dates

Conowingo Orchard 1 (#037)	7/27, 9/24
Conowingo Orchard 2 (#038)	7/27, 9/24
Conowingo Orchard Air	7/27, 9/24
West, Northwest Transect	
Jones Farm 1 (#032)	7/23, 9/14
Jones Farm 2 (#069)	7/27, 9/14
Jones Farm Air	7/23, 9/14
Tower Hill Farm 1 (#071)	7/26, 9/24
Tower Hill Farm 2 (#040)	7/27, 9/24
Tower Hill Air	7/26, 9/24
Farview Manor 1 (#002)	7/27, 9/24
Farview Manor 2 (#036)	7/27, 9/24
Farview Manor Air	7/27, 9/24
West, Southwest Transect	
Rumsey Mansion 1 (#030)	7/23, 9/25
Rumsey Mansion 2 (#048)	7/27, 9/25
Rumsey Mansion Air	7/23, 9/25
Silver Lake Drive 1 (#071)	9/25
Silver Lake Drive 2 (#046)	7/27, 9/25
Silver Lake Drive Air	7/26, 9/25
Clyburn Arboretum 1 (#042)	7/27, 9/25
Clyburn Arboretum 2 (#044)	7/27, 9/25
Clyburn Arboretum Air	7/27, 9/25

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#### 4.2 Sampling Methods

JAG box, multi-bed sampling trains were introduced in the 1998 field season. These consisted of a series of three tubes, a drying tube to adsorb water molecules, a guard tube to remove the larger terpene and carbohydrate molecules that impair GC/MS desorption processes, and finally a carbotrap sampling tube (Figure 4.2). The introduction of the JAG box sampling trains greatly decreased the loss of samples due to moisture penetrating the sample tubes. Sample tubes were sealed in individual vials and refrigerated, until shipped, to retard loss of volatile contaminants. Samples from APG were air expressed under frozen gel packs, with trip blanks, to the University of Montana labs. Once in Missoula, they were stored in a dedicated 4 °C sample refrigerator until analyzed.

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.

Throughout 1999, 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.

For the purposes of this report, the concentrations of all organic compounds are expressed as ppt per volume of air. In the digital database, the concentrations are listed both as ppt per unit of air or as ng/m<sup>3</sup> of air. These values can be compared to EPA human risk and other agency (e.g., OSHA) benchmark values to assess the significance to human exposures. Ambient air values can be more directly related to published risk threshold values than hive air samples, keeping in mind that these air samples are 6-10 hour averages taken periodically throughout the spring, summer, and fall. They are not continuous values, nor has any source or plume modeling been conducted.

The concentrations of chemicals in the hive atmospheres reflect the cumulative exposure of the colony from air and from all other sources or media. Where another medium is involved, such as contaminated water, whether this would constitute a human risk would depend on whether a person drinks the same water as the bee, eats plants or animals exposed to this water, as well as the amount and rate of consumption. For example, bees might obtain contaminated water from a pond used by waterfowl that might be acquired and consumed by a hunter. As such, the bee could provide a warning of the presence of the contaminant in water in addition assessing air quality.

Each ambient air sample provides a point specific monitor of the air quality. Each hive air sample provides a area or distributed measure of air quality and of all other source media in the vicinity (forage range) of the colony. The odds of a chemical(s) being detected improve with the hive samples, the ability to compare the data to threshold values to human health has to be restricted to comparisons with the air samples, since no protocol has been agreed upon from comparing cumulative exposures from multiple sources to a human health risk benchmark that assumes air is the primary exposure route.



**Figure 4.2.0** Schematic diagram of the 1999, multi-bed 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 the upper left.

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.

**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 4.2.0). 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 100 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 4.2.1** 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 1999, 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 Outline of Chemical Results

For purposes of reporting and discussing the results of the chemical sampling, APG-area sample locations will be broken into the following groups: *J-Field, Bush River Cluster 3, Churchville*, *and the Boundary study sites.* Since we conducted sampling at the J-field, Cluster 3, and Churchville sites during previous field seasons, we include some comparative summaries as well in this report. A complete tabulation of all 56 contaminants measured is found in the Excel spreadsheet that can be requested in support of this report.

For each location we present tables that indicate the highest **chemical 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.**" 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 hives 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).

Tables have been constructed in two panels – one reflecting the nine compounds that have been tracked since 1997 and the other representing the additional twelve contaminants detected and quantified during the 1999 study. These Tables present the results on a site by site basis, compare sites and years, and document the highest values recorded for each chemical at any site or year. Because of the large number of these Tables and to facilitate comparisons among them, they (the 4.4 series) have been placed at the end of this section.

## 4.4 J-field Results

The results of the 1999 field sampling of J-Field are summarized in Tables 4.4.1 through 4.4.4. Tables 4.4.0 and 4.4.1 document VOC maxima and means in ambient air and in the air inside both the phytoremediation and condo (electronic) hives for the nine compounds that we have followed in our beehive biomonitoring since the initial field work at J-Field in 1997. Tables 4.4.2 and 4.4.3 provide the maxima and means for the 11 additional contaminants added in 1999. A multiyear comparison for the original nine contaminants is provided in Table 4.4.4.

The multiyear, comparative values in Table 4.4.4 suggest that exposure of the bees to the BTEX suite of petroleum contaminants (benzene, toluene, ethylbenzene) at J-Field during 1999 were higher (benzene) or in same range as in previous years (toluene, ethylbenzene). BTEX compounds in the air inside beehives either equals or exceeds, often by several-fold, the levels in ambient air. Also, in 1999 BTEX compounds tended to be highest in the C set of hives (Table 4.4.0). These hives were closest to the main access road, between the road and the P-1 and P-9 monitoring wells, west of the phytoremediation grove, and south of the Prototype Building. However, the mean values for the BTEX compounds at J-Field during the 1999 field season

were no higher than those found in the boundary survey hives that were located in urban airsheds with prolific vehicle emission sources.

The presence of naphthalene in ambient air was higher in 1999 than in 1998 or 1997. Napthalene was highest in beehives in 1997 (both mean and maxima), dropped off somewhat in 1998, and displayed a higher maxima in 1999 compared to 1998. Since naphthalene and aliphatic derivatives of naphthalene are strongly associated with persistent residuals of diesel fuel, we believe that it presence may be in part a result of the operation of diesel-powered construction equipment on site.

Mean levels of chlorinated organics for the 1999 field season were down, both inside beehives and in the ambient air, with respect to TCM and TCE; and were similar or somewhat lower compared to previous years for PCE and DCB. Isolated "hits" were recorded for PCA (perchloroethane), a compound utilized as a smoke obscurrent, the largest being 234 ppt maxima noted in Area B at J-Field. Isolated hits were also recorded for 1,1-choloroethene, trichloromethane, 1,1,1-trichloroethane (2923 ppt in the set D hives), and 1,1,2,2tretrachloroethane (347 ppt in the set A hives). The most significant trends to emerge with the 1999 field season's results are the high contaminant exposures for honeybee foragers to cis-1,2dichloroethene (3942 ppt in the set D hives).

Styrene was found at quite high levels in beehive air (1163-5484 ppt maxima) at J-Field. While styrene is listed as a contaminant by the US EPA, it is also biogenically produced by a number of plant species. We have observed an elevated level of styrene in hives located in areas free from organic contamination, and we have found styrene associated with propolis gathered by the bee foragers (unpublished data, D.C. Jones). However, the levels at J-Field greatly exceed those at Cluster 3 or the Churchville reference site. There may be other anthropogenic sources of styrene at J-Field, such as the floats on the docks leading into some of the marshy areas.

Overall, J-Field displayed high levels of the largest numbers of VOCs in hive atmospheres and the ambient air, compared to Cluster 3 or the Churchville reference.

#### 4.5 Bush River Cluster 3 Results

Honey bee biomonitoring was performed at the Bush River Cluster 3 site in 1997 and 1999 and is scheduled again for 2000. In 1997, three pairs of hives were deployed within the actual site, where capping operations occurred in 1999. In order to monitor these capping operations, without being in the way of the heavy machinery being use, an instrumented cluster of seven condos was placed adjacent to the Cluster 3 site in 1999. The colonies were put in place shortly after remediation activity was begun on the site.

The results of chemical sampling from Cluster 3 are contained in two tables – Table 4.4.5 lists the concentrations of the original set of nine contaminants reported in previous years (1977 for

this site), while Table 4.4.6 reports the concentrations of the additional 11 compounds added in 1999.

BTEX contaminants in both ambient air and the air inside the beehives were common at Cluster 3 site, although the levels tended to be somewhat lower than at J-Field. Since toluene exceeds the benzene concentrations at this location, a fuel source is possible. The recorded benzene levels in 1999 were about 3 times the US EPA cancer risk level (31 ppt), posing an inhalation exposure for personnel working the area. The remaining BTEX and naphthalene levels at Cluster 3 are similar to those seen at some of the Boundary sites, and probably typify urban airshed levels for this region.

Organochlorine contaminants show a similar pattern to that discussed for J-Field above. Mean honey bee exposures were greatest for TCM, PCE, and bromobenzene. The bromobenzene (179 ppt) maximum inside beehives was observed at this site. As expected, mean and maxima values for TCM and PCE were also elevated. One compound, cis-1,2-dichloroethene, was somewhat higher in the ambient air at Cluster 3 (mean=20; maximum=55 ppt) than at J-Field. However, it was much lower in the air inside the beehives at Cluster 3 than at J-Field. The highest ambient air level of this compound was measured at the Churchville reference site (233 ppt maximum).

Overall, levels of chlorinated contaminants from the original list on 9 compounds showed a decrease in the 1999 field samples compared to the 1997 samples. Except for benzene, the BTEX compounds tended to occur at similar levels in 1997 and 1999. Benzene in both ambient and hive air was higher at Cluster 3 in 1999.

In summary, in 1999, Cluster 3 tended to rank somewhat below J-Field for exposures to VOCs, but was more like J-Field than the Churchville reference in terms of the kinds of compounds detected and the levels.

#### 4.6 Churchville Results

Samples were collected from the Churchville site in June, August and September 1999. Table 4.4.7 contains multiple year comparisons of the concentrations of the original nine contaminants; Table 4.4.8 lists the concentrations of the additional 11 contaminants.

The BTEX suite of compounds show a distribution typified by a wealth of mobile vehicle emissions. The most prevalent member is toluene, the usual signature of a gasoline-dominated source. Toulene levels, both in ambient air and inside beehives were usually highest at Churchville, although not much higher than at J-Field or at Cluster 3. *However, the mean toluene level observed for Churchville is somewhat lower than is typical of many urban airsheds, probably due its rural location.* 

As in previous years, DCB levels at Churchville in hive atomospheres were higher than at Cluster 3 or J-Field. But, the ambient air maximum for DCB was seen at J-Field. Mean

perchloroethane inside beehives was highest at Churchville, but the highest maximum value was clearly in the ambient air at J-Field. PCE was lowest in both ambient and hive air at Cluster 3 in terms of mean and maximum values.

Comparing the list of nine VOC compounds reported from 1996 through 1998, the highest levels of DCB (46 ppt maximum) and toluene (1643 ppt maximum) at Churchville were seen in hive atmospheres in 1999. For the other seven compounds, a higher value was seen in ambient or hive air during a previous year.

Overall, the kinds and levels of VOCs at Churchville more closely resembled those at Cluster 3 than at J-Field.

#### 4.7 Boundary Study Results—Youth Center

The concentrations of the volatiles and semivolatiles in the boundary study are shown in Tables 4.4.9 through 4.4.22. Concentrations for the individual sites for the original 9 contaminants are shown in Tables 4.4.9 through 4.4.12. Tables 4.4.13 through 4.4.16 hold the concentrations for the additional contaminants at the individual sites. Directional and radial summaries for the original components are given in Tables 4.4.17 and 4.4.18, and for the additional compounds in Tables 4.4.19 and 4.4.20.

It should be noted, that for the purposes of the Boundary Study, the Youth Center (YC) acts as the pivot point of three sampling transects extending into the communities near APG-Edgewood. During prior years, Youth Center was the site in the Canal Creek (upper post) study area that most often had somewhat elevated levels of some of the nine VOCs that we have been following using bee colonies. Unlike Cluster 3 and J-Field, this site has not been documented as a landfill or disposal site.

Because it is centrally located in the upper Edgewood post area, and because it seemed to reflect either an undocumented point source or an area source of some of the VOC chemicals of concern, YC became a reference point to establish as the anchor for a survey to see if there were any gradients of exposure extending from this central area of the upper post out into the surrounding communities.

For the purposes of the following results and discussion, keep in mind that the overall comparisons and statistical evaluation of VOCs in the Boundary study include Youth Center, and an additional site at Cluster 13 in the Lauderick Creek study area. The known landfills, where APG restoration activities are ongoing (J-Field and Cluster 3) are not included in the statistical analysis or comparisons, although J-Field values are listed in several of the Tables to facilitate comparisons by the reader.

Overall, YC and Cluster 13 tend to resemble Cluster 3, but differ greatly from J-Field, especially with respect to the concentrations of chlorinated organic compounds.

For the list of nine previously reported VOCs, levels of both chlorinated organic contaminants and the BTEX compounds were similar in 1999 to those of the previous two years, with the exception of DCB, which showed a significant decrease in concentrations in 1999. Overall, the levels of these VOCs at Youth Center were highest in 1996. In 1999, the concentrations of BTEX compounds at Youth Center had a chemical signature reflecting gasoline related sources, with highest levels occurring for toluene, and lower levels of benzene, ethyl benzene, and xylenes.

#### 4.8 Overall Comparison of Results Among the 1999 Study Sites

Comparisons of the sampling sites on Aberdeen Proving Ground and the off-base sites are provided in Tables 4.4.21 and 4.4.22 for the original 9 contaminants, and in Tables 4.4.23 and 4.4.24 for the additional 11 contaminants.

These Tables demonstrate that BTEX contaminants are ubiquitous at all sites. While naphthalene has been present at high levels in several hives, overall it is not a substantial contributor to the overall hydrocarbon emissions. A similar gasoline related source signature of the BTEX compounds was observed for many of the off-base sites in the boundary study. Most compounds had similar concentrations along the three directional transects.

However, concentrations of TCM and trichloromethane were highest on the WSW transect. Concentrations of PCE, DCB, and perchloroethane were highest along the WNW transect, but were also relatively high on the WSW transect. Higher levels of contaminants along the WSW transect are not surprising, as this transect runs through the highly urbanized and industrialized area of greater Baltimore.

There was little evidence for off-base gradients of organic contaminants. The concentrations of 1,1,1-trichloroethane tended to decrease with distance from the base. On the other hand, concentrations of the BTEX compounds increased with increasing distance from APG. Similar to the APG sites, high concentrations of styrene were observed in the hives in the boundary study.

When contaminanted sites such as J-Field are included in the comparisons, concentrations of chlorinated organic compounds appear to be lower at the off-base sites than at the APG-sites. However, when J-Field is excluded from the comparisons, some of the off-post sites fall within the range of concentrations seen at APG upper post sites - and these sites include some of the most distant locations from APG.

Overall, concentrations of TCM, TCE, and DCB were similar for on- and off-base study hives. Finally, PCE and perchloroethane had slightly higher off-base levels than on APG. This was due to generally higher levels at all off-base sites, and could not be attributed to one particular site. The significance of the results for all of these sites is discussed in Section 4.10.

#### 4.9 Statistical Analysis of Volatile and Semi-volatile Organics Compounds in the Boundary Study

The Tables discussed in the previous section provide specific data for each site and for 20 chemical compounds. These Tables revealed specific information (such as which sites recorded the highest "hits" and the kinds of chemicals that occurred at elevated levels) and suggested trends that warranted further data analysis.

In 1998, some of the Boundary colonies were weak or lost by the end of the sampling period, which limited our ability to make statistical comparisons. The 1999 data set for the Boundary study provided a complete set of colonies for all sampling periods. Therefore, an analysis of variance was undertaken, with the goal to test for any spatial pattern dependent on direction or distance from the base.

The design is a multivariate analysis of variance by the General Linear Model (GLM) procedure using SPSS v. 8.0 software. It is a complete random design with 4 fixed factors (sample source, zone, heading, and month) and all interactions being included. The sample source is defined as the hive air versus the ambient air sampled. The zone is the distance from APG, with sampling sites at 3, 9 and 21 miles from the base, and a site on base, Youth Center, indicated as the 0 site. The 0 zone is not completely replicated because there is only one site that forms the base side for each geographical heading. The heading consists of three transects radiating in the directions NE, WNW, WSW from the base. The month represents the pooled July and the pooled September dates. This assumes for the purpose of the analysis that it is the season and not the exact day of sampling that is the focus of the study. Each factor was assumed to be fixed, that is, it is intended to represent only the intervals sampled and does not represent randomly sampled intervals from a larger distribution.

Prior to the analysis, the dependent variables-the 22 VOC concentrations-were subjected to a principal components analysis (PCA). The purpose of this analysis was to reduce the number of variables to a smaller subset of linear combinations. The results of the PCA analysis revealed 8 principal components which accounted for 75% of the variation among the original dependent variables. These 8 standardized principal components were then subjected to an analysis of variance by the GLM procedure.

The results of this statistical procedure are summarized in Table 4.9.0. The Wilks' Lambda Multivariate test is the first step of the analysis of variance used to identify which of the interactions between the independent variables- sample source, zone, heading, and month-showed a significant difference in at least one of the principal components. The significance value is the probability that there is at least one significant difference of the principal components. A 0.05 critical value (95% confidence level) was chosen. The observed power is a measure of whether or not a real difference will be missed. High values of observed power indicate a low likelihood that a real difference had been missed as a result of the analysis.

The results of Wilks' Lambda test show that zone and heading are the most important factors. When two-way interactions are considered, the zone\*heading, zone\*month, and heading\*month interactions are important, with zone and heading again being the dominant two factors. When three-way interactions are considered, the zone\*heading\*month interaction is the most meaningful with a significance value of <0.001 and an observed power of 0.998. Of all the interactions considered, this is the most important one because three of the four factors being considered are involved. There was no significant effect of sample source (ambient air versus hive air) on the spatial patterns of the levels and volatile and semivolatile compounds.

The next step in the analysis of variance was the Test of Between-Subjects Effects, which identifies which of the principal components contribute most to the factor interactions identified by the Wilks' Lambda test. Only two of the eight principal components (PC-1 and PC-4) played a significant role in explaining the variation in the data set. PC-1, which accounts for 17% of the total variation in the data set, strongly influences the three-way zone\*heading\*month interaction and to a lesser extent the zone\*heading interactions (Table 4.9.1). PC-4, which accounts for 9% of the total variation, influences the zone, and the zone\*heading, zone\*month, heading\*month and zone\*heading\*month interactions (Table 4.9.1).

Further examination of these two principal components showed that PC-1 was a weighted average of gasoline components, consisting primarily of benzene, ethylbenzene, and m,p- xylene and o-xylene with small contributions of 1,1-dichloroethene and 1,1,1-trichloroethane. PC-1 will be referred to as the BTEX compounds. PC-4 is a contrast between levels of benzene and perchloroethene, and 1,1-dichloroethene and toluene– as benzene and perchloroethene levels increase, dichloromethane and toluene levels decrease and vise-versa.

# Table 4.9.0

# MANOVA results for Wilk's Multivariate Test for Volatiles and Semi-Volatiles at Boundary Study Sites

Wilks' Lambda Multivariate Test Resu	lts	
Interaction	Significance	<b>Observed Power</b>
	(p<0.05)	$(\alpha = 0.05)$
One-way		
Sample Source	0.084	0.671
Zone	0.010 *	0.956
Heading	0.001 *	0.995
Month	0.140	0.581
Two-way		
Sample Source*Zone	0.641	0.435
Sample Source*Heading	0.859	0.308
Zone*Heading	<0.001 *	0.999
Sample Source*Month	0.199	0.512
Zone*Month	<0.001 *	0.997
Heading*Month	<0.001 *	0.999
Three-way		
Sample Source*Zone*Heading	0.260	0.660
Sample Source*Zone*Month	0.875	0.155
Sample Source*Heading*Month	0.210	0.501
Zone*Heading*Month	<0.001 *	0.998
Four-way		
Sample Source*Zone*Heading*Month	0.514	0.304

( "\*" indicates statistically significant effects)

#### Table 4.9.1

# Result of test of Between-Subjects effects for PC-1 and PC-4

Interaction term	Principal Component-1		Principal Component-4	
	p-value	<b>Observed Power</b>	p-value	<b>Observed Power</b>
One-way ANOVA				
Zone	n.s.		0.010	0.810
Heading	0.001	0.963	n.s.	
Two-way ANOVA				
Zone*Heading	0.018	0.807	0.002	0.960
Zone*Month	n.s.		0.001	0.968
Heading*Month	n.s.		0.002	0.925
Three-way ANOVA				
Zone* Heading*month	<0.001	0.999	0.006	0.899

("n.s." indicates non-significant effects [p>0.05])

To identify the source of variation within each principal component, the estimated marginal means, which are weighted averages of the variation in the principal component, were examined. The results of this analysis are shown in Figures 4.9.0 to 4.9.2. The major source of variation in the zone\*heading interaction for BTEX compounds was due to a high level of benzene, ethyl benzene, toluene, and m,p- and o-xylene at Tower Hill Farm (TF) and Shawsville (SV), two sites on the WNW transect (Figure 4.9.0; Tables 4.9.0 and 4.9.1).

Breaking this down further, plots of PC-1 Zone\*Heading\*Month show that there are greater differences in September than July (Figures 4.9.1 and 4.9.2). Overall, Figures 4.9.0 through 4.9.3 indicate that much of the seasonal variation in BTEX compounds was accounted for by sites located 9 and 21 miles off-base in the WNW direction— Tower Hill Farm and Shawsville respectively, and to some extent Jones Farm (July, 1999; 3 mi WNW), Silver Lake (July 1999; 9 mi WSW), and Rumsey Island (September 1999; 3 mi SW). The contribution of the latter three sites to the variation in BTEX levels can be explained by their close vicinity to two major highways in the area, I-40 and I-95 (Figure 4.1).



**Figure 4.9.0** Estimated marginal means of BTEX compounds, as a function of zone and heading in July and September. In the legend, N designates the NE, NW the WNW, and SW the WSW transect. **TF**=Tower Farm, **SV**=Shawsville.

Although, Tower Hill Farm and Shawsville are more rural sites, for the combined June and September sample periods, they contributed most to the variation in BTEX compounds. The Shawsville location was adjacent to a busy highway (23), with the hives only a few meters from the road. The Tower Farm hives were set back from a secondary highway and may also may have been affected by traffic on Highways I-147 and I-1.



**Figure 4.9.1** Estimated marginal means of BTEX compounds, as a function of zone and heading in July, 1999. In the legend, N designates the NE, NW the WNW, and SW the WSW transect. **JF**=Jones Farm, **SL**=Silver Lake.

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**Figure 4.9.2** Estimated marginal means of BTEX compounds, as a function of zone and heading in September, 1999. In the legend, N designates the NE, NW the WNW, and SW the WSW transect. **RI**=Rumsey Island, **TF**=Tower Farm, **SV**=Shawsville.



**Figure 4.9.3** Estimated marginal means of the contrast between benzene and PCE versus 1,1dichloroethene and toluene as a function of zone and heading in July through September, 1999. In the legend, N designates the NE, NW the WNW, and SW the WSW transect. **SL**=Silver Lake

The variation in principal component 4, the contrast between benzene and percholoroethene (PCE) and 1,1-dichloroethene and toluene levels, was mainly attributable to the Silver Lake site, 9 mi SW of the base (Figure 4.9.1). At this site low levels of benzene and PCE were contrasted with high levels of 1,1-dichloroethene and toluene, in July 1999, but not in September 1999 (Tables 4.9.0 and 4.9.1).

#### 4.10 Overall Discussion and Conclusions, Volatile Organic Compounds

In reviewing the overall results from the 1999 APG field sampling, it is clear that J-Field poses a higher risk for toxic exposures than the other field sites. Among the 20 organic contaminants that were followed, J-Field bee colonies exhibited the season's maximum in 18 of 20 cases and ambient J-Field ambient air samples exhibited the season's maximum in 16 of 20 cases. Clearly, the disposal activity at the J-Field site has left a legacy of possible exposures. The most serious acute (severe, short-term) exposures were attributable to 1,1,1-trichloroethane (TCA), which appeared at nearly 3 ppb in the hives and 0.5 ppb in the air. TCA held the highest seasonal average among organochlorine contaminants, as well, in J-Field ambient air. Two dichloroethenes (1,1 - and cis-1,2-) were most problematic among organochlorine mean concentrations for the bees, again at J-Field. Since there is restricted human access to J-Field, the levels are of most concern for ecosystem impacts. TCA is classified as a Group D substance, not classifiable as to human carcinogenicity (http://www.epa.gov/ttn/uatw/hlthef/trichlor.html). The OSHA PEL level for an 8-hour time weighted average is 35 ppm, well above the observed concentrations. Neither of the dichloroethylenes are addressed in the EPA United Air Toxics Website.

The most serious threat posed to humans by organic contaminants found during the 1999 APG field season is from benzene. Benzene levels measured at most sites are above the EPA's Cancer Risk Level (CRL). Benzene is a Group A, known human carcinogen of medium carcinogenic hazard (http://www.epa.gov/ttn/uatw/hlthef/benzene.html) with a CRL of 31 ppt. Mean ambient air concentrations of benzene were at 111 ppt even in the rural setting of the Churchville reference site. Any urban setting will show values well above those found at J-Field, Cluster 3 and Churchville. The airshed in which The University of Montana sits showed a 2-year mean of 464 ppt (Investigating Volatile Organic Compounds in an Urban Intermountain Valley Using a TD/GC/MS Methodology and Intrinsic Tracer Molecules, University of Montana, Ph.D. Thesis, Christopher L. Wrobel, 2000). The ubiquitous health risk posed by benzene exposures to the majority of the US urban population is not well recognized by public health officials.

Overall, there was no evidence of volatile organic contaminants moving off of APG into the surrounding communities. There is some evidence of VOCs moving from the communities onto APG (which can best be seen when concentration isopleths for each chemical are plotted - unpublished data, Bromenshenk et al., 2001).

Short of a catastrophic event such as an explosion and release of chemicals from an APG landfill or bunker, the health of a person living or working in the surrounding communities is more directly related to the levels of contaminants resultant from vehicles, gasoline and diesel, and nearby business/industrial sources in the community than it is to materials migrating from APG.

Finally, in a report to R.F. Weston, we compared the measured ambient air concentrations of volatile organics at APG in 1998 and 1999 to EPA Region 3 Human Risk Benchmark Concentrations (Bromenshenk et al., in review, 2001). Acknowledging that our values reflect

only a small subset of the total days of the year and that no modeling was attempted to try to generate a more representative value for long-term air quality, we found that not only benzene, as indicated in this report, but several other VOCs would have exceeded Region 3 RBCs. The significance of this finding remains to be determined, since no protocol exists for making direct comparisons of short term air concentrations to these RBCs. However, it does suggest that statements in both the Cluster 3 (General Physics Corp., 1998) and J-Field Human Risk Assessments (1998) concerning chemicals of potential concern may be correct with respect to the kinds of chemicals that appear on the short list of COPCs, but that the models used to estimate exposure concentrations may not be correct. Based on the models, none of the chemicals that we found to exceed Region 3 RBCs were thought to warrant air monitoring.

# J-Field Phytoremediation Hives' Atmosphere and Ambient Air (air) Maximum Volatiles and Semivolatiles by Hive Location, 1999 Values are ppt by volume

Compound	Set A	Set B	Set C	Set D	Set E	Overall
TCM	77	30	28	19	32	77
(air)	(74)	(24)	(42)	(75)	(8)	(75)
TCE	10	6	7	16	5	16
(air)	(16)	(4)	(10)	(5)	(1)	(16)
PCE	16	19	26	61	33	61
(air)	(19)	(16)	(9)	(17)	(23)	(23)
DCB	1	8	10	4	14	10
(air)	(10)	(7)	(0.1)	(5)	(5)	(10)
Benzene	496	353	549	407	340	549
(air)	(245)	(255)	(249)	(273)	(261)	(273)
<b>Toluene</b> (air)	1274	1201	1502	977	756	1502
	(417)	(604)	(468)	(464)	(309)	(604)
Ethylbenzene	312	175	662	163	413	662
(air)	(50)	(97)	(11)	(192)	(67)	(192)
Naphthalene	6	73	53	32	29	73
(air)	(64)	(32)	(0.5)	(53)	(63)	(64)
Acetophenone	29	161	117	109	·20	161
(air)	(115)	(83)	(6)	(99)	(111)	(115)

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# J-Field Phytoremediation Hives' Atmosphere and Ambient Air (air) Mean Volatiles and Semivolatiles by Hive Location, 1999 Values are ppt by volume

Compound	Set A	Set B	Set C	Set D	Set E	Overall
	(n = 12)	(n = 34)	(n =14)	(n = 14)	(n = 13)	(n= 87)
TCM	17	7	8	5	5	<b>8</b>
(air)	(21)	(6)	(16)	(17)	(4)	(13)
TCE	3	2	3	3	1	2
(air)	(7)	(1)	(4)	(2)	(1)	(3)
PCE	7	7	8	14	8	9
(air)	(10)	(5)	(6)	(10)	(9)	(8)
DCB	0.3	1	1	1	1	1
(air)	(6)	(2)	(0.1)	(2)	(1)	(2)
Benzene	234	113	270	229	155	200
(air)	(147)	(124)	(187)	(207)	(171)	(167)
<b>Toluene</b> (air)	551	458	509	496	326	463
	(217)	(217)	(264)	(245)	(122)	(213)
Ethylbenzene	74	40	97	74	80	73
(air)	(21)	(23)	(5)	(55)	(23)	(25)
Naphthalene	2	4	5	3	4	4
(air)	(31)	(4)	(0.3)	(19)	(13)	(15)
Acetophenone	10	11	13	15	6	11
(air)	(65)	(19)	(2)	(35)	(25)	(29)

# J-Field Phytoremediation Hives' Atmosphere and Ambient Air (air) Maximum Volatiles and Semivolatiles, Additional Chemicals by Hive Location, 1999 Values are ppt by volume

Compound	Set A	Set B	Set C	Set D	Set E	Overall
cis-1,2- Dichloroethene (air)	298 (9)	510 (49)	1459 (1)	3942 (21)	1472 (49)	3942 (49)
<b>1,1-Dichloroethene</b>	862	665	842	1434	2124	2124
(air)	(20)	(12)	(22)	(1)	(22)	(22)
<b>1,2-Dichloroethane</b> (air)	7	6	4	5	5	7
	(14)	(3)	(4)	(2)	(2)	(14)
<b>Trichloromethane</b> (air)	204	88	128	409	148	409
	(36)	(49)	(23)	(93)	(20)	(93)
<b>1,1,1-Trichloroethane</b> (air)	75	565	577	2923	83	2923
	(458)	(318)	(173)	(75)	(22)	(458)
1,1,2,2-Tetrachloroethane	347	44	11	26	95	347
(air)	(18)	(9)	(1)	(15)	(5)	(18)
Bromobenzene	2	153	39	3	13	153
(air)	(8)	(5)	(0.2)	(4)	(2)	(8)
Perchloroethane	0.3	234	4	1	0.4	234
(air)	(3)	(33)	(0)	(1)	(1)	(33)
<b>m,p-Xylene</b>	283	495	1781	328	252	1781
(air)	(23)	(166)	(28)	(230)	(178)	(23)
o-Xylene	134	3269	348	59	80	3269
(air)	(9)	(101)	(5)	(97)	(35)	(101)
Styrene	2041	3204	1163	3372	5484	5484
(air)	(9)	(1576)	(0.4)	(210)	(5)	(1576)

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# J-Field Phytoremediation Hives' Atosphere and Ambient Air (air) Mean Volatiles and Semivolatiles, Additional Compounds by Hive Location, 1999 Values are ppt by volume

Compound	Set A	Set B	Set C	Set D	Set E	Overall
	(n= 12)	(n= 34)	(n= 14)	(n= 14)	(n= 13)	(n= 87)
cis-1,2- Dichloroethene (air)	94 (3)	67 (11)	137 (0.3)	384 (5)	190 (1)	174 (4)
<b>1,1-Dichloroethene</b>	280	64	82	555	356	267
(air)	(4)	(4)	(5)	(1)	(3)	(3)
<b>1,2-Dichloroethane</b> (air)	2	1	2	3	1	2
	(5)	(1)	(2)	(2)	(2)	(2)
<b>Trichloromethane</b> (air)	55	20	45	74	33	45
	(15)	(15)	(11)	(39)	(10)	(18)
1,1,1-Trichloroethane	19	34	78	267	22	84
(air)	(113)	(60)	(58)	(34)	(12)	(55)
1,1,2,2-Tetrachloroethane	40	10	2	6	13	14
(air)	(7)	(2)	(0.3)	(4)	(1)	(3)
Bromobenzene	1	6	4	1	1	3
(air)	(3)	(1)	(0.1)	(1)	(1)	(1)
Perchloroethane	0.1	15	0.3	0.1	0.1	3
(air)	(1)	(4)	(0)	(0.2)	(0.1)	(1)
<b>m,p-Xylene</b>	72	95	215	112	74	114
(air)	(12)	(27)	(10)	(75)	(61)	(37)
o-Xylene	22	143	45	23	20	51
(air)	(6)	(20)	(2)	(28)	(10)	(13)
<b>Styrene</b>	382	1417	150	485	628	612
(air)	(5)	(211)	(1)	(46)	(1)	(264)

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#### J-Field Phytoremediation Hives' Atmosphere and Ambient Air (air) Comparison of 1999, 1998 and 1997 Maximum and Mean Volatiles and Semivolatiles Values are ppt by volume

Compound	1999 Max	1998 Max	1997 Max	1999 Mean (n = 87)	1998 Mean (n = 56)	1997 Mean (n = 24)
TCM	77	403	58	8	47	10
(air)	(75)	(101)	(78)	(13)	(34)	(20)
TCE	16	52	224	2	10	24
(air)	(16)	(80)	(36)	(3)	(20)	(18)
PCE	61	64	118	9	15	23
(air)	(23)	(226)	(38)	(8)	(34)	(23)
DCB	10	9	45	1	0.4	3
(air)	(10)	(9)	(23)	(2)	(0.9)	(6)
Benzene	549	218	170	200	<b>86</b>	55
(air)	(273)	(272)	(160)	(167)	(131)	(55)
<b>Toluene</b>	1502	618	1786	463	204	206
(air)	(604)	(738)	(285)	(213)	(243)	(163)
Ethylbenzene	662	1486	305	73	95	36
(air)	(192)	(234)	(45)	(25)	(68)	(32)
Naphthalene	73	43	135	4	5	18
(air)	(64)	(22)	(21)	(15)	(5)	(8)
Acetophenone (air)	161 (115)	369 (162)		11 (29)	55 (32)	

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## Cluster 3 Hives' Atmosphere and Ambient Air (air) Comparison of 1999 and 1997 Maximum and Mean Volatiles and Semivolatiles Values are ppt by volume

Compound	1999 Max	1997 Max	1999 Mean (n=21)	1997 Mean (n=35)
TCM	47	71	13	10
(air)	(85)	(68)	(32)	(19)
TCE	3	21	1	4
(air)	(2)	(16)	(1)	(4)
PCE	54	158	18	11
(air)	(46)	(35)	(19)	(15)
DCB	12	91	2	10
(air)	(1)	(13)	(0.3)	(14)
Benzene	237	114	93	39
(air)	(222)	(157)	(136)	(67)
Toluene	633	1641	311	227
(air)	(458)	(1045)	(315)	(284)
Ethylbenzene	184	470	46	44
(air)	(122)	(90)	(62)	(34)
Naphthalene	45	54	4	7
(air)	(2)	(27)	(1)	(15)
Acetophenone (air)	137 (6)		15 (4)	

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## Cluster 3 Hives' Atmosphere and Ambient Air (air) Maximum and Mean Volatiles and Semivolatiles, Additional Compounds, 1999 Values are ppt by volume

Compound	1999 Max	Hive ID	1999 Mean (n=21)
<b>cis-1,2-</b> Dichloroethene (air)	282 (55)	9904803 10/13/99	54 (20)
<b>1,1-Dichloroethene</b>	150	9904203	11
(air)	(0.5)	10/13/99	(0.3)
<b>1,2-Dichloroethane</b> (air)	13	9904203	1
	(7)	10/13/99	(3)
<b>Trichloromethane</b> (air)	114	9904203	17
	(44)	10/13/99	(23)
<b>1,1,1-Trichloroethane</b> (air)	471	9904603	50
	(55)	10/13/99	(20)
1,1,2,2-Tetrachloroethane	3	9902703	0.4
(air)	(2)	10/13/99	(1)
Bromobenzene	179	9904202	26
(air)	(0.2)	9/14/99	(0.1)
<b>Perchloroethane</b>	1	9904203	0.3
(air)	(1)	9/14/99	(0.3)
<b>m,p-Xylene</b>	241	9904702	62
(air)	(154)	9/14/99	(94)
o-Xylene	1504	9904702	153
(air)	(62)	9/14/99	(30)
Styrene	541	9904602	71
(air)	(99)	9/14/99	(53)

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## **Churchville Hives' Atmosphere and Ambient Air** (air) **Comparison of 1999, 1998, 1997 and 1996 Maximum and Mean Volatiles and Semivolatiles** Values are ppt by volume

Compound	1999 Max	1998 Max	1997 Max	1996 Max	1999 Mean (n=17)	1998 Mean (n=18)	1997 Mean (n=19)	1996 Mean (n=19)
TCM	25	93	66	79	4	32	16	18
(air)	(14)	(86)	(15)	(52)	(8)	(29)	(13)	(34)
TCE	2	0	21	2564	1	0	5	183
(air)	(4)	(0)	(18)	(17)	(2)	(0)	(9)	(6)
PCE	19	38	183	70	7	14	16	18
(air)	(20)	(11)	(48)	(36)	(11)	(4)	(24)	(26)
DCB	46	9	16	23	6	1	4	5
(air)	(2)	(0)	(65)	(12)	(1)	(0)	(32)	(5)
Benzene	170	167	<b>8</b> 6	275	78	92	37	67
(air)	(132)	(147)	(110)	(106)	(111)	(93)	(72)	(186)
<b>Toluene</b>	1643	421	409	1422	662	218	101	283
(air)	(581)	(93)	(896)	(328)	(416)	(44)	(472)	(204)
Ethylbenzene	146	171	72	74	64	49	19	18
(air)	(43)	(10)	(274)	(22)	(32)	(6)	(140)	(17)
Naphthalene	16	4130	50	253	2	479	6	19
(air)	(1)	(2)	(13)	(12)	(1)	(1)	(7)	(1)
Acetophenone (air)	112 (28)	52 (0)			11 (10)	9 (0)		

#### **Table 4.4.8**

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#### Churchville Hives' Atmosphere and Ambient Air (air) Maximum and Mean Volatiles and Semivolatiles, Additional Compounds, 1999 Values are ppt by volume

Compound	1999 Max	Hive ID	1999 Mean (n= 17)
cis-1,2- Dichloroethene (air)	839 (233)	990CV701 6/24/99	149 (118)
1,1-Dichloroethene	128	9903605	18
(air)	(3)	8/3/99	(1)
<b>1,2-Dichloroethane</b> (air)	2	9903802	1
	(1)	8/3/99	(1)
Trichloromethane	70	9902902	20
(air)	(81)	8/3/99	(40)
<b>1,1,1-Trichloroethane</b>	826	9904403	55
(air)	(7)	9/24/99	(6)
1,1,2,2-Tetrachloroethane	4	9903803	1
(air)	(0.3)	9/24/99	(0.2)
Bromobenzene	86	990CV401	6
(air)	(0.3)	6/24/99	(0.1)
<b>Perchloroethane</b> (air)	232	990CV401	14
	(0.3)	6/24/99	(0.2)
<b>m,p-Xylene</b>	217	9902802	105
(air)	(152)	8/3/99	(61)
o-Xylene	118	990CV401	44
(air)	(142)	6/24/99	(17)
Styrene	9239	990CV301	1594
(air)	(145)	6/24/99	(78)

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# Youth Center (Pivot Point Boundary Study) Hives' Atmosphere and Ambient Air (air) Comparison of 1999, 1998, 1997 and 1996 Maximum and Mean Volatiles and Semivolatiles Values are ppt by volume

Compound	1999 Max	1998 Max	1997 Max	1996 Max	1999 Mean (n=4)	1998 Mean (n=18)	1997 Mean (n=19)	1996 Mean (n=19)
TCM	47	57	68	69	13	23	26	39
(air)	(40)	(22)	(21)	(53)	(25)	(14)	(8)	(49)
TCE	2	0	50	355	1	0	13	96
(air)	(1)	(0)	(1)	(67)	(1)	(0)	(0.4)	(39)
PCE	21	28	18	158	9	10	10	53
(air)	(28)	(17)	(22)	(148)	(15)	(6)	(10)	(84)
DCB	0.3	13	6	23	0.1	2	2	9
(air)	(5)	(0)	(6)	(20)	(2)	(0)	(2)	(13)
Benzene	164	144	77	468	96	109	32	206
(air)	(180)	(178)	(131)	(382)	(129)	(121)	(59)	(476)
<b>Toluene</b>	796	778	233	861	511	268	140	364
(air)	(458)	(102)	(345)	(593)	(247)	(79)	(166)	(417)
Ethylbenzene	78	172	47	58	50	39	20	27
(air)	(2)	(26)	(74)	(42)	(1)	(12)	(34)	(29)
Naphthalene	0.4	4	5	26	0.3	1	2	8
(air)	(25)	(36)	(15)	(8)	(13)	(12)	(6)	(8)
Acetophenone (air)	7 (64)				3 (34)			

## Boundary Study Hives' Atmosphere and Ambient Air (air) Northeast Transect, 1999 Maximum and Mean Volatiles and Semivolatiles Values are ppt by volume (n= 4)

Compound	CL 13	CL 13	OP	OP	LO	LO	CC	CC
	Max	Mean	Max	Mean	Max	Mean	Max	Mean
TCM	4	1	22	13	21	16	8	2
(air)	(26)	(16)	(1)	(1)	(2)	(1)	(3)	(2)
TCE	2	1	3	1	1	0.3	1	1
(air)	(2)	(2)	(1)	(1)	(1)	(0.5)	(0.4)	(0.2)
PCE	21	10	16	10	15	7	8	5
(air)	(12)	(7)	(8)	(6)	(14)	(9)	(1)	(0.4)
DCB	6	2	0.3	0.1	0.2	0.1	1	0.4
(air)	(1)	(1)	(0.5)	(0.3)	(2)	(1)	(2)	(1)
Benzene	171	119	175	140	121	68	145	119
(air)	(201)	(148)	(158)	(127)	(156)	(100)	(10)	(1)
<b>Toluene</b>	2122	1055	1502	712	1194	587	5110	1937
(air)	(419)	(300)	(692)	(490)	(1254)	(765)	(207)	(109)
Ethylbenzene	2292	1112	767	443	643	283	834	405
(air)	(428)	(263)	(935)	(537)	(575)	(393)	(9)	(7)
Naphthalene	27	7	1	1	1	0.4	1	1
(air)	(3)	(3)	(0.4)	(0.3)	(9)	(4)	(10)	(5)
Acetophenone	106	33	14	5	6	3	4	38
(air)	(13)	(12)	(10)	(5)	(29)	(17)	(2)	(19)

## Boundary Study Hives' Atmosphere and Ambient Air (air) West, Northwest Transect, 1999 Maximum and Mean Volatiles and Semivolatiles Values are ppt by volume (n= 4)

Compound	JF	JF	TF	TF	SV	SV
	Max	Mean	Max	Mean	Max	Mean
TCM	55	20	40	17	32	11
(air)	(101)	(55)	(34)	(19)	(0.4)	(0.4)
TCE	1	1	3	2	3	1
(air)	(1)	(1)	(2)	(2)	(0.4)	(0.4)
PCE	29	12	48	24	75	37
(air)	(25)	(23)	(23)	(12)	(3)	(3)
DCB	17	6	3	2	3	1
(air)	(11)	(6)	(0.2)	(0.1)	(0.1)	(0.1)
Benzene	132	83	374	210	515	339
(air)	(124)	(120)	(371)	(247)	(387)	(387)
<b>Toluene</b>	939	602	1168	1072	1446	1137
(air)	(472)	(429)	(716)	(457)	(618)	(618)
Ethylbenzene	1330	653	1516	1061	1398	965
(air)	(923)	(660)	(1292)	(773)	(748)	(748)
Naphthalene	4	2	1	1	1	1
(air)	(25)	(13)	(0.3)	(0.3)	(8)	(8)
Acetophenone	16	5	106	62	23	8
(air)	(65)	(33)	(7)	(7)	(6)	(6)

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## Boundary Study Hives' Atmosphere and Ambient Air (air) West, Southwest Transect, 1999 Maximum and Mean Volatiles and Semivolatiles Values are ppt by volume (n= 4)

Compound	RI	RI	SL	SL	CA	CA
	Max	Mean	Max	Mean	Max	Mean
TCM	12	7	72	26	47	20
(air)	(7)	(4)	(10)	(6)	(7)	(4)
TCE	2	1	0.2	0.2	1	1
(air)	(1)	(1)	(1)	(1)	(1)	(0.4)
PCE	10	7	6	3	49	25
(air)	(15)	(13)	(1)	(1)	(46)	(25)
DCB	4	2	1	0.2	1	0.2
(air)	(4)	(2)	(0)	(0)	(1)	(0.3)
Benzene	262	158	104	75	138	129
(air)	(206)	(186)	(213)	(120)	(114)	(112)
<b>Toluene</b> (air)	1525	732	3557	1440	345	307
	(1464)	(924)	(297)	(181)	(419)	(256)
Ethylbenzene	1007	633 <sup>-</sup>	1592	721	473	230
(air)	(1338)	(1034)	(211)	(106)	(592)	(298)
Naphthalene	32	<b>8</b>	1	1	1	1
(air)	(1)	(1)	(0.3)	(0.3)	(1)	(0.3)
Acetophenone	126	39	<b>8</b>	3	17	8
(air)	(10)	(6)	(1)	(1)	(4)	(2)

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# Youth Center (Pivot Point Boundary Study) Hives' Atmospheres and Ambient Air (air) Maximum and Mean Volatiles and Semivolatiles, 1999 Additional Compounds Values are ppt by volume (n= 4)

Compound	1999 Max	Hive ID	1999 Mean
cis-1,2- Dichloroethene (air)	0 (1)		0 (0.3)
<b>1,1-Dichloroethene</b>	106	9902801	54
(air)	(0.3)	7/23/99	(0.3)
<b>1,2-Dichloroethane</b> (air)	1	9902801	1
	(1)	7/23/99	(1)
<b>Trichloromethane</b> (air)	30	9902801	15
	(18)	7/23/99	(12)
<b>1,1,1-Trichloroethane</b>	8	9907601	4
(air)	(44)	7/23/99	(25)
1,1,2,2-Tetrachloroethane	0.3	9902801	0.2
(air)	(0.7)	7/23/99	(0.4)
Bromobenzene	5	9902801	1
(air)	(1)	7/23/99	(0.4)
<b>Perchloroethane</b>	45	9902801	11
(air)	(0.3)	7/23/99	(0.2)
<b>m,p-Xylene</b>	91	9902801	60
(air)	(3)	7/23/99	(30)
o-Xylene	3189	9902802	813
(air)	(3)	9/14/99	(2)
Styrene	32209	9907602	10063
(air)	(1)	9/14/99	(1)

## Boundary Study Hives' Atmosphere and Ambient Air (air) Northeast Transect, 1999 Maximum and Mean Volatiles and Semivolatiles. Additional Compounds Values are ppt by volume (n= 4)

Compound	CL 13	CL 13	OP	OP	LO	LO	CC	CC
	Max	Mean	Max	Mean	Max	Mean	Max	Mean
cis-1,2- Dichloroethene (air)	0 (0)	0 (0)	0 (0)	0 (0)	0.6 (0)	0.2 (0)	0 (0.2)	0 (0.1)
1,1-Dichloroethene	1	1	24	6	521	175	230	84
(air)	(23)	(12)	(1)	(1)	(1)	(0.4)	(4)	(3)
1,2-Dichloroethane	1	1	1	1	2	1	1	1
(air)	(9)	(5)	(1)	(1)	(1)	(1)	(0.3)	(0.2)
Trichloromethane	110	44	11	8	27	12	18	7
(air)	(86)	(46)	(13)	(7)	(9)	(5)	(34)	(19)
1,1,1-Trichloroethane	29	9	57	29	40	16	15	7
(air)	(48)	(29)	(18)	(10)	(53)	(36)	(2)	(2)
1,1,2,2-Tetrachloroethane	2	1	2	1	0.1	0.1	0.3	0.2
(air)	(4)	(3)	(0.3)	(0.2)	(2)	(1)	(1)	(0.4)
Bromobenzene	6	1	2	1	0.3	0.2	3	1
(air)	(0.1)	(0.1)	(0.1)	(0.1)	(0.4)	(0.2)	(0.4)	(0.2)
<b>Perchloroethane</b> (air)	19	5	22	11	0.3	0.2	0.2	0.1
	(0)	(0)	(0.2)	(0.1)	(0.3)	(0.2)	(0.1)	(0.1)
<b>m,p-Xylene</b>	492	330	118	79	74	36	161	87
(air)	(21)	(14)	(200)	(114)	(50)	(43)	(9)	(8)
<b>o-Xylene</b>	268	162	58	35	18	12	76	41
(air)	(8)	(5)	(83)	(52)	(26)	(18)	(2)	(1)
Styrene	7953	3764	2748	2195	774	257	10099	3524
(air)	(11)	(7)	(52)	(46)	(36)	(19)	(0.4)	(0.3)

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## Boundary Study Hives' Atmosphere and Ambient Air (air) West, Northwest Transect, 1999 Maximum and Mean Volatiles and Semivolatiles. Additional Compounds Values are ppt by volume (n=4)

Compound	JF	JF	TF	TF	SV	SV
	Max	Mean	Max	Mean	Max	Mean
<b>cis-1,2-</b> Dichloroethene (air)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<b>1,1-Dichloroethene</b>	2	1	474	151	229	97
(air)	(0.4)	(0.4)	(0.3)	(0.3)	(1)	(1)
<b>1,2-Dichloroethane</b> (air)	2	1	3	1	2	1
	(12)	(7)	(3)	(2)	(1)	(1)
<b>Trichloromethane</b>	87	42	95	38	25	14
(air)	(2)	(2)	(33)	(21)	(7)	(7)
1,1,1-Trichloroethane	17	9	43	22	24	16
(air)	(5)	(5)	(16)	(9)	(3)	(3)
1,1,2,2-Tetrachloroethane	0.2	0.2	0.4	0.2	0.4	0.3
(air)	(18)	(9)	(1)	(0.4)	(0.2)	(0.2)
Bromobenzene	5	1	11	5	0.2	0.1
(air)	(8)	(4)	(0.2)	(0.2)	(0.4)	(0.4)
Perchloroethane	63	16	70	42	0.1	0
(air)	(6)	(3)	(0)	(0)	(0)	(0)
<b>m,p-Xylene</b>	301	158	423	241	303	182
(air)	(188)	(136)	(325)	(174)	(62)	(62)
o-Xylene	107	48	183	103	131	52
(air)	(69)	(53)	(123)	(66)	(10)	(10)
<b>Styrene</b>	3238	1143	15159	5496	384	240
(air)	(55	(28)	(107)	(55)	(0.1)	(0.1)
# Boundary Study Hives' Atmosphere and Ambient Air (air) West, Southwest Transect, 1999 Maximum and Mean Volatiles and Semivolatiles. Additional Compounds Values are ppt by volume (n= 4)

Compound	RI	RI	SL	SL	CA	CA
	Max	Mean	Max	Mean	Max	Mean
cis-1,2- Dichloroethene (air)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<b>1,1-Dichloroethene</b>	0.4	0.3	57	19	155	40
(air)	(1)	(1)	(1)	(1)	(0.3)	(0.2)
<b>1,2-Dichloroethane</b>	2	1	1	1	1	1
(air)	(1)	(1)	(0.4)	(0.4)	(1)	(1)
<b>Trichloromethane</b>	71	28	38	14	108	29
(air)	(142)	(73)	(5)	(3)	(25)	(14)
<b>1,1,1-Trichloroethane</b>	35	21	49	20	19	13
(air)	(35)	(21)	(7)	(4)	(2)	(2)
1,1,2,2-Tetrachloroethane	3	1	0.4	0.3	1	1
(air)	(0.4)	(0.3)	(0.1)	(0)	(1)	(0.3)
Bromobenzene	2	1	1	1	1	1
(air)	(0.1)	(0.1)	(0)	(0)	(0.3)	(0.2)
Perchloroethane	57	24	3	1	15	4
(air)	(0.1)	(0.1)	(0)	(0)	(0)	(0)
<b>m,p-Xylene</b>	202	132	211	72	89	43
(air)	(173)	(134)	(53)	(27)	(90)	(45)
o-Xylene	104	59	37	37	37	17
(air)	(85)	(65)	(2)	(2)	(36)	(18)
Styrene	6905	3075	230	86	492	251
(air)	(155)	(83)	(7)	(4)	(65)	(33)

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# Boundary Study Hives' Atmosphere and Ambient Air (air) Directional Summary of Off-Base Transects, 1999 Maximum and Mean Volatiles and Semivolatiles Values are ppt by volume

Compound	NE Max	NE Mean n=12	WNW Max	WNW Mean n=12	WSW Max	WSW Mean n=12
TCM	22	10	55	16	72	18
(air)	(3)	(1)	(101)	(25)	(10)	(5)
TCE	3	1	3	1	2	1
(air)	(1)	(1)	(2)	(1)	(1)	(1)
PCE	16	9	75	24	49	12
(air)	(14)	(5)	(23)	(13)	(46)	(13)
DCB	1	0.2	17	3	4	1
(air)	(2)	(1)	(11)	(2)	(4)	(1)
Benzene	175	109	515	211	262	121
(air)	(158)	(86)	(387)	(213)	(206)	(139)
<b>Toluene</b> (air)	2122	1073	1446	937	3577	826
	(1254)	(455)	(716)	(500)	(1464)	(454)
Ethylbenzene	834	377	1516	893	1592	528
(air)	(935)	(312)	(1292)	(727)	(1338)	(479)
Naphthalene	1	1	4	1	32	3
(air)	(10)	(3)	(25)	(4)	(1)	(1)
Acetophenone	38	4	106	25	126	17
(air)	(29)	(8)	(65)	(15)	(10)	(3)

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# Boundary Study Hives' Atmosphere and Ambient Air (air) Radial Summary of Off-Base Sites, 1999 Maximum and Mean Volatiles and Semivolatiles Values are ppt by volume

Compound	3 Mi Max	3 Mi Mean n=12	9 Mi Max	9 Mi Mean n=12	21 Mi Max	21 Mi Mean n=12
TCM	55	13	72	20	47	11
(air)	(101)	(20)	(34)	(9)	(7)	(2)
TCE	3	1	3	1	3	1
(air)	(1)	(1)	(2)	(1)	(1)	(0.3)
PCE	29	10	48	11	75	22
(air)	(25)	(14)	(23)	(7)	(46)	(9)
DCB	17	3	3	1	3	1
(air)	(11)	(3)	(2)	(0.4)	(2)	(1)
Benzene	262	127	374	118	515	196
(air)	(206)	(144)	(371)	(156)	(387)	(167)
<b>Toluene</b> (air)	1525	682	3557	1033	5110	1127
	(1464)	(614)	(1254)	(468)	(618)	(328)
Ethylbenzene	1330	576	1592	688	1398	533
(air)	(1338)	(743)	(1292)	(424)	(748)	(351)
Naphthalene	32	4	1	1	1	1
(air)	(25)	(5)	(9)	(2)	(10)	(2)
Acetophenone	126	16	106	23	38	7
(air)	(65)	(15)	(29)	(8)	(6)	(9)

# Boundary Study Hives' Atmosphere and Ambient Air (air) Directional Summary of Off-Base Transects, 1999 Maximum and Mean Volatiles and Semivolatiles. Additional Compounds Values are ppt by volume

Compound	NE Max	NE Mean n=12	WNW Max	WNW Mean n=12	WSW Max	WSW Mean n=12
cis-1,2- Dichloroethene (air)	1 (0.2)	0.2 (0)	0 (0)	0 (0.1)	0 (0)	0 (0)
<b>1,1-Dichloroethene</b> (air)	521	88	<b>474</b>	83	155	20
	(1)	(1)	(1)	(1)	(1)	(1)
<b>1,2-Dichloroethane</b> (air)	2	1	3	1	2	1
	(1)	(1)	(12)	(3)	(1)	(1)
<b>Trichloromethane</b>	27	9	95	31	108	24
(air)	(34)	(10)	(33)	(10)	(142)	(30)
<b>1,1,1-Trichloroethane</b>	57	17	43	16	49	15
(air)	(53)	(16)	(16)	(6)	(35)	(19)
1,1,2,2-Tetrachloroethane	2	0.4	0.4	0.2	3	1
(air)	(2)	(1)	(18)	(3)	(0.4)	(0.2)
Bromobenzene	2	0.7	11	2	2	1
(air)	(0.2)	(0.2)	(8)	(2)	(0.3)	(0.1)
Perchloroethane	22	4	70	19	57	10
(air)	(0.3)	(1)	(6)	(1)	(0.1)	(0)
<b>m,p-Xylene</b>	161	67	423	194	211	82
(air)	(200)	(55)	(325)	(124)	(173)	(69)
o-Xylene	76	29	183	68	108	38
(air)	(83)	(20)	(123)	(413)	(85)	(28)
Styrene	10999	1992	15159	2293	6905	1138
(air)	(52)	(22)	(107)	(83)	(155)	(40)

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# Boundary Study Hives' Atmosphere and Ambient Air (air) Radial Summary of Off-Base Sites, 1999 Maximum and Mean Volatiles and Semivolatiles. Additional Compounds Values are ppt by volume

Compound	3 Mi Max	3 Mi Mean n=12	9 Mi Max	9 Mi Mean n=12	21 Mi Max	21 Mi Mean n=12
<b>cis-1,2-</b> <b>Dichloroethene</b> (air)	0 (0)	0 (0)	1 (0)	0.1 (0)	0 (0.2)	0 (0)
<b>1,1-Dichloroethene</b>	24	2	521	115	230	74
(air)	(1)	(1)	(1)	(1)	(4)	(1)
<b>1,2-Dichloroethane</b> (air)	2	1	3	1	2	1
	(12)	(3)	(3)	(0.3)	(1)	(1)
<b>Trichloromethane</b>	87	26	95	21	108	17
(air)	(142)	(40)	(33)	(10)	(34)	(13)
1,1,1-Trichloroethane	57	20	49	19	24	12
(air)	(48)	(18)	(53)	(16)	(3)	(2)
1,1,2,2-Tetrachloroethane	3	1	0.4	0.2	1	1
(air)	(18)	(3)	(2)	(0.4)	(1)	(0.3)
Bromobenzene	5	1	11	2	3	1
(air)	(8)	(1)	(0.4)	(0.2)	(0.4)	(0.3)
<b>Perchloroethane</b>	63	17	70	14	15	1
(air)	(6)	(1)	(0.3)	(0.1)	(0.1)	(0)
<b>m,p-Xylene</b>	301	123	423	116	303	104
(air)	(188)	(141)	(325)	(81)	(90)	(38)
o-Xylene	107	47	183	51	131	37
(air)	(85)	(57)	(123)	(23)	(36)	(3)
<b>Styrene</b>	6905	2138	15159	1946	10099	1338
(air)	(155)	(52)	(107)	(26)	(65)	(11)

# Summary of Hives' Atmosphere and Ambient Air (air) Comparison of APG Sites Versus Off-Post Sites, 1999 Daily Maximum Volatiles and Semivolatiles Values are ppt by volume

Compound	1999 APG Max	1999 APG Site	1998 APG Max	1998 APG Site	1997 APG Max	1997 APG Site	Off- Post Mean	Off-Post Site
TCM	77	J-Field	403	J Field	209	Canal Ck	72	SL
(air)	(85)	(CL 3)	(186)	(DF1)	(83)	(NG)	(101)	(JF)
TCE	16	J-Field	52	J Field	224	J Field	3	CV+
(air)	(16)	(J-Field)	(80)	(J Field)	(44)	(BR3)	(4)	(CV)
PCE	61	J-Field	369	DF 1	158	Cluster 3	75	SV
(air)	(46)	(CL 3)	(226)	(J Field)	(81)	(O Field)	(46)	(CA)
DCB	12	CL 3	67	DF 1	91	Cluster 3	46	CV
(air)	(10)	(J-Field)	(308)	(O Field)	(23)	(J Field)	(11)	(JF)
Benzene	549	J-Field	722	DF 4/5	282	Bush R 10	515	SV
(air)	(273)	(J-Field)	(275)	(Westwd)	(165)	(O Field)	(387)	(SV)
<b>Toluene</b>	2122	CL 13	1320	DF 1	3156	Bush R 10	5110	CO
(air)	(604)	(J-Field)	(1222)	(DF 2/3)	(625)	(O Field)	(1464)	(RI)
Ethylbenzene	2292	CL 13	5424	O Field	512	O Field	1592	SL
(air)	(428)	(CL 13)	(1063)	(O Field)	(431)	(Bush R 9)	(1338)	(RI)
Naphthalene	73	J-Field	309	Carroll Is	135	J Field	32	RI
(air)	(64)	(J-Field)	(40)	(DF 4/5)	(79)	(Bush R 5)	(25)	(JF)
Acetophenone (air)	161 (115)	J-Field (J-Field)	582 (162)	DF 1 (J Field)			126 (65)	RI (JF)

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# Summary of Hives' Atmosphere and Ambient Air (air) Comparison of APG Sites Versus Off-Post Sites, 1999 Highest Mean Volatiles and Semivolatiles Values are ppt by volume

Compound	1999 APG Mean	1999 APG Site	1998 APG Mean	1998 APG Site	1997 APG Mean	1997 APG Site	Off- Post Mean	Off-Post Site
TCM	13	CL 3/ YC	48	J Field	27	Canal Ck	26	SL
(air)	(32)	(CL 3)	(123)	(D Field)	(37)	( Up Edgwd)	(55)	(JF)
TCE	2	J-Field/YC	52	J Field	24	J Field	2	TH
(air)	(3)	(JF)	(80)	(J Field)	(18)	(J Field)	(2)	(CV/TH)
PCE	18	CL 3	40	D Field	23	J Field	37	SV
(air)	(19)	(CL 3)	(34)	(J Field)	(28)	(Old O Field)	(25)	(CA)
DCB	6	CL 13	7	Cluster 13	10	Cluster 3	6	CV/JF
(air)	(10)	(J-Field)	(32)	O Field	(7)	(Old O Field)	(6)	(JF)
Benzene	200	J-Field	121	D Field	66	Old O Field	339	SV
(air)	(167)	(J-Field)	(159)	(Westwd)	(78)	(Old O Field)	(387)	(SV)
<b>Toluene</b>	1055	CL 13	355	Carroll Is	305	Bush R.	1937	CP
(air)	(315)	(CL 13)	(514)	D Field	(284)	(Cluster 3)	(924)	(RI)
Ethylbenzene	1112	CL 13	274	O Field	58	Old O Field	1061	TH
(air)	(263)	(CL 13)	(208)	(D Field)	(48)	(Old O Field)	(1034)	(TH)
Naphthalene	7	CL 13	46	Carroll Is	18	J Field	8	RI
(air)	(15)	(J-Field)	(12)	Yth Ctr	(15)	(Cluster 3)	(13)	(JF)
Acetophenone (air)	33 (34)	CL 13 (YC)	55 (32)	J Field (J Field)			62 (33)	TH (JF)

# Summary of Hives' Atmosphere and Ambient Air (air) Comparison of APG Sites Versus Off-Post Sites, 1999 Daily Maximum Volatiles and Semivolatiles, Additional Compounds Values are ppt by volume

Compound	1999 APG	1999 APG	1999 Off-Post	1999 Off-Post
	Max	Site	Max	Site
cis-1,2- Dichloroethene (air)	3942 (55)	J-Field (CL 3)	839 (223)	CV (CV)
<b>1,1-Dichloroethene</b>	2124	J-Field	521	LO
(air)	(23)	(CL 13)	(4)	(CO)
<b>1,2-Dichloroethane</b>	7	J-Field	3	TH
(air)	(14)	(J-Field)	(12)	(JF)
Trichloromethane	409	J-Field	95	TH
(air)	(93)	(J-Field)	(142)	(RI)
1,1,1-Trichloroethane	2923	J-Field	826	CV
(air)	(458)	(J-Field)	(53)	(LO)
1,1,2,2-Tetrachloroethane	347	J-Field	4	CV
(air)	(18)	(J-Field)	(18)	(JF)
Bromobenzene	179	CL 3	86	CV
(air)	(8)	(J-Field)	(8)	(JF)
Perchloroethane	234	J-Field	232	CV
(air)	(33)	(J-Field)	(6)	(JF)
<b>m,p-Xylene</b>	1781	J-Field	423	TH
(air)	(154)	(CL 3)	(325)	(TH)
o-Xylene	3269	J-Field	183	TH
(air)	(101)	(J-Field)	(142)	(CV)
Styrene	32209	Youth Center	15159	TH
(air)	(1576)	(J-Field)	(155)	(RI)

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# Summary of Hives' Atmosphere and Ambient Air (air) Comparison of APG Sites Versus Off-Post Sites, 1999 Highest Mean Volatiles and Semivolatiles, Additional Compounds Values are ppt by volume

Compound	1999 APG	1999 APG	1999 Off-Post	1999 Off-Post
	Mean	Site	Mean	Site
cis-1,2- Dichloroethene (air)	384 (20)	J-Field (CL 3)	149 (118)	CV (CV)
<b>1,1-Dichloroethene</b>	555	J-Field	175	LO
(air)	(12)	(CL 13)	(3)	(LO)
<b>1,2-Dichloroethane</b>	3	J-Field	1	all sites
(air)	(5)	(J-Field/CL 13)	(7)	(JF)
<b>Trichloromethane</b>	74	J-Field	42	JF
(air)	(46)	(CL 13)	(73)	(RI)
1,1,1-Trichloroethane	267	J-Field	55	CV
(air)	(113)	(J-Field)	(36)	(LO)
1,1,2,2-Tetrachloroethane	40	J-Field	1	several sites
(air)	(7)	(J-Field)	(9)	(JF)
Bromobenzene	26	CL 3	6	CV
(air)	(3)	(J-Field)	(0.4)	(SV)
Perchloroethane	15	J-Field	42	TH
(air)	(4)	(J-Field)	(0.2)	(CV / LO)
<b>m,p-Xylene</b>	330	CL 13	241	TH
(air)	(94)	(CL 13)	(174)	(TH)
<b>o-Xylene</b>	813	Youth Center	103	TH
(air)	(30)	(CL 3)	(123)	(TH)
Styrene	10063	Youth Center	5496	TH
(air)	(211)	(J-Field)	(83)	(RI)

# SECTION 5 CHEMICAL ANALYSIS OF TRACE ELEMENT IN FORAGER BEES AND POLLEN

#### 5.1 Overview of General Methods and Results

#### **General Procedures:**

Trace elemental analyses of live bees, dead bees, and pollen from the 1999 season were conducted for samples that had been slowly dried over a two-week period in a 45 °C forced-air oven to minimize losses of volatile elements such as arsenic and lead. The samples were then ground to pass through an 80-mesh size and packaged for ICP-MS determination at the USACEHR Laboratories at Fort Detrick, MD. Elements determined were As, Ba, Be, Bi, Cd, Co, Cr, Cs, Cu, Ga, Mn, Ni, Pb, Rb, Se, Sr, Ti, U, V, and Zn (Tables 5.6 through 5.11).

Bees and pollen were sampled at sites both on-post and off-post in the area of Aberdeen Proving Ground-Edgewood area. Four sites were surveyed on the Aberdeen Proving Grounds: J-Field, Cluster 13, Cluster 3, and the Youth Center. At J-Field, groups of three full-size hives (non-electronic) were placed at five locations surrounding a grove of hybrid popular trees. Because these trees were part of an ongoing phytoremediation project, these full-sized hives were termed the phytohives. These five locations were labeled A through E. At the B location, seven smaller electronic hives, used as part of the bee-based real-time biomonitoring of this site, were also sampled. These hives were called the J-Field condos. A similar set of seven electronic hives or condos were deployed at Cluster 3.

For the Boundary study, pairs of small survey hives were placed at Youth Center and at Cluster 13 on the Edgewood area post and at locations at 3, 9 and 21 miles from Youth Center, along off-post transects extending into Baltimore county (WSW transect), Harford county (NW transect), and Cecil County (NNE transect). Cluster 3, provided an additional, on-base, site near the NNE transect.

Live bees were obtained by screening off hive entrances and then capturing forager bees returning to the hive with a vacuum sampling system. Dead bees were obtained from traps fitted underneath the condo hives.

Live bees were sampled and any dead bees were taken from the traps, starting in May and ending in early November. At J-Field, the phytohives were sampled on 5/10/99 and 11/2/99, and the condo hives on 6/22 and 11/2. Cluster 3 hives were sampled on 11/8/99, with the exception of a single measurement in August. The hives at the Churchville reference site were sampled on 8/2/99 and 11/8/99. Finally, the boundary study hives were sampled twice, in the fall only, between 10/6/99 and 10/16/99.

Pollen samples were taken from the phytohives at J-Field on 6/15/99, and from the condo hives at J-Field on 8/13/99 and 9/13/99. The pollen sampling dates for the other sites were: Cluster 3, 9/20/99; Churchville, 8/10/99 and 9/25/99; and boundary study sites, 9/20/99.

#### **Results:**

Table 5.5.0 summarizes the results for J-Field by hive type and area. A comparison of trace elements and heavy metal levels between the hives at J-Field, Cluster 3, and Churchville is given in Table 5.5.1. Finally, the results of the boundary study are summarized in Table 5.5.2 (full summary for each trace element and site), Table 5.5.4 (directional summary for live bees and pollen), Table 5.5.5 (radial summary for live bees), and Table 5.5.6 (radial summary for pollen). Because of the large number and size of these tables, the 5.5 series of Tables appears at the end of this section.

Based on samples taken from the J-Field hives between May through November 1999, there were no seasonal patterns in element levels in forager bees or pollen.

There also were no detectable levels of Be, Bi, Cs, and U in live bees, and Ti was only found at very low levels (usually below detection limits, with the exception of off-post samples from Otter Point containing a mean of 3.3 ppm Ti). Elements found at low-ppm levels were As, Cd, Co, Cr, Ga, Se, Sr, and V. Levels of trace elements were similar in forager bees and pollen for As, Ba, Cr, Ga, Rb, and V. Levels were higher in forager bees compared to pollen for Cd, Co, Cu, Mn, and Se. Levels of Ni were higher in pollen compared to forager bees at the off-base Boundary Survey Study sites, but the opposite pattern was found for the other sites. Levels of Pb were generally higher for pollen, except for the Condo hives at J-Field, were levels in forager bees and pollen at the off-base Boundary Survey Study sites, but lower for pollen at all the other study sites.

Levels for Cu were within or at the lower end of the range that is normally observed for bees (~25 ppm). An outlier was found for Cu in live forager bees from a Condo hive at J-Field (184 ppm). This value was deleted from the data set for the final analysis, since this high a value has never been observed during six years of biomonitoring at APG. Zinc levels of forager bees were within the normal range (80-120 ppm), and there were no differences between the APG and the off-base sites. Cadmium levels in live bees tissue and pollen tended to be higher at J-Field compared to Cluster 3 and Churchville sites, but were similar to levels observed at some of the off-base boundary study sites (section 5.2). Levels of Ba, Cd, Cu, and Mn in pollen were highest at the Clyburn Arboretum in Baltimore (section 5.3). This can be explained by the rural character of the sampling sites in the NW and NNE directions, and the higher industrial and urban activity in the WSW direction from the base. Pb Concentrations were high in some of the pollen and bee samples from J-Field. Overall, lead appeared in some, but not all of the samples, making it highly variable. But the levels were high enough to warrant reporting of lead exposure

to some of the hives (namely, bees from the J-Field condo set by the USACEHR administration trailer, B location) and pollen from the phytohives at location E. Lead levels at Cluster 3 were somewhat enriched, averaging about twice those of the Boundary Sites, but considerably lower than those at J-Field.

Overall, there was a distinct site-related distribution of Mn levels in forager bees as well as pollen (section 5.4). Mn levels were highest for forager bees at J-Field, second highest at Cluster 3, and lowest at all of the off base sites. Similarly, strontium levels were higher for the APG sites compared to the off-base sites (section 5.4). Levels of Sr were highest at the J-Field hives, second highest at Cluster 3, and lowest at the off-base sites. Similar to Mn, there were no statistically significant differences in Sr levels between the off-base sites.

### 5.2 Statistical Comparison of Trace Element Contaminants at J-field and Cluster 3 of Aberdeen Proving Ground and the Churchville Reference Site

In the statistical tests described in this section and sections 5.3 and 5.4, values below the detection limit, indicated as "BDL" in the summary tables, were replaced by a very small value. Thus, all data below the detection limit of 0.001 ppm were replaced by a value of 0.0005 ppm. Furthermore, in all the statistical tests, the data was checked for normality and equal variance . The data were normally distributed for some of the trace elements, but not for others. However, the use of the original data in the statistical tests was justified by the fact that the requirement of equal variances was always met (Personal communication, Dr. Colin Henderson, University of Montana).

A principal component analysis was conducted to study the effects of study location on the levels of trace elements in forager bees and pollen. The fixed factor in the study design was site, consisting of the phytohives at J-Field, Cluster 3, and Churchville. The dependent variables were the elements that had levels above the detection limit. The elements considered in this analysis were As, Ba, Cd, Co, Cr, Cu, Ga, Mn. Ni, Pb, Rb, Se, Sr, V, and Zn. The results of Wilks' Lambda multivariate test are shown in Table 5.2.0. The Wilk's statistic indicate that there is a statistically significant effect of sampling site on trace element levels for both live bee tissue and pollen (p<0.05). Moreover, the variation in trace element levels in both forager bees and pollen could be explained by one principal component (or root), explaining 78 and 90 % of the observed variance respectively.

A further analysis of these two principal component is shown in Table 5.2.1. The results suggest a contrast between the levels of Ba and V versus Cr, Rb, Se, and Zn in forager bees. In general as the levels of Ba and V decrease as the levels of Cr, Rb, Se, and Zn go up (Table 5.2.1). For pollen this relationship is more complex as there are 11 trace element that contribute significantly to this principal component (Table 5.2.1), and no clear pattern was discovered when trace element levels were compared between sites. However, these results suggest that some of the trace elements covary with each other.

#### Table 5.2.0

### MANOVA Results for the Comparison of Trace Element Levels in Live Bee Tissue and Pollen The sites compared are J-field, Cluster 3, and Churchville; shown are the Wilks statistic and canonical correlations.

Effect	Live Bee Tissue			Pollen		
	Wilks (F)	D.F. $\mathbf{v}_1, \mathbf{v}_2$	p-value	Wilks (F)	D.F. v <sub>1</sub> ,v <sub>2</sub>	p-value
Site	9	30, 66	<0.001	17	30, 6	0.001
	Eigenvalu e	%	Cum. %	Eigenvalu e	%	Cum. %
Root 1	7.0	78	78	243	90	90

#### **Table 5.2.1**

# MANOVA Results for the Comparison of Trace Element Levels in Live Bee Tissue and Pollen The sites compared are J-field, Cluster 3, and Churchville;

shown are the standardized discriminant function coefficients by element. Only trace element with coefficients >0.750 are shown in the table.

Trace Element	Live Bees, Root 1	Pollen, Root 1
As		
Ba	- 0.8	- 5.1
Cd		- 2.7
Со		
Cr	1.0	
Cu		- 2.6
Ga		5.2
Mn		- 2.2
Ni		- 3.1
Pb		0.9
Rb	1.2	- 2.3
Se	0.8	0.8
Sr		- 4.3
v	- 1.1	
Zn	1.5	1.2

Finally, a univariate comparison between the sampling sites was carried out for each of the trace metals, and tested for site differences using a one-way ANOVA (Figures 5.2.0 and 5.2.1). Arsenic levels tended to be higher for Cluster 3 than at the other two sites, for both live bees and pollen, whereas cadmium levels were highest at J-Field (Figure 5.2.0). On the other hand, levels of chromium and selenium were lowest at J-Field hives, both for live bees and pollen (Figures 5.2.0 and 5.2.1). Rubidium levels were highest at J-Field for live bees, but lowest for pollen (Figure 5.2.0 and 5.2.1). Strontium levels in pollen were slightly elevated at J-Field (Figure 5.2.1). Manganese levels were statistically significantly higher at J-Field compared to the two other sites, both in live bees tissue and in pollen (Figures 5.2.0 and 5.2.1). Zinc levels were lowest in forager bees from the J-Field hives, and highest the Churchville reference site (Figure 5.2.0). These results also suggest that there is source of manganese at J-Field (see section 5.4), and possibly for cadmium. A possible cause for lower Zn levels in forager bee tissue at J-Field is a higher level of environmental stresses, since, Zn is a regulated element in bees. Interestingly, Se showed a similar pattern as Zn.



Figure 5.2.0 Trace element levels of As, Cd, Cr, and Se in forager bees and pollen at from hives at J-Field, Cluster 3 and Churchville (reference site). Error bars represent 1 SE. Statistically significant

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differences within sample type and per trace element are indicated as "\*" (one-way ANOVA, P<0.05).

**Figure 5.2.1** Trace element levels of Rb, Sr, Mn, and Zn in forager bees and pollen at from hives at J-Field, Cluster 3 and Churchville (reference site) in 1999. Error bars represent 1 SE. Statistically significant differences within sample type and per trace element are indicated as "\*" (one-way ANOVA, P < 0.05).

#### 5.3 Spatial Patterns of Trace Elements in the Boundary Study Sites

#### Live Bee Tissue

To study the spatial patterns of trace element levels in live bees in the boundary study, a multivariate analysis of variance (MANOVA) was carried out. The MANOVA addressed the effects of heading direction and distance, indicated as "zone", on trace element levels, as well as possible correlations between different trace elements. The trace elements considered in the MANOVA were those observed in levels well above the detection limit of 0.001 ppm; i.e., As, Ba, Cd, Co, Cr, Cu, Ga, Mn. Ni, Pb, Rb, Se, Sr, V, and Zn. The experimental design of the MANOVA was similar to the design for the volatile and semivolatile organic compounds (section 4.1).

The fixed variables were heading, with three transects in the directions slight east of due North (NNE), Northwest (NW), and slightly south of due west (WSW) from Aberdeen Proving Ground-Edgewood, and zone, with sites 0, 3, 9 and 21 mi from the base (Figure 5.3.0). Note that the on-base site, Youth Center at 0 mi, functions as the pivot point for all three directional transects, and that all three directional transects were completely replicated. The dependent variables were the 15 trace elements described above.

The results of the Wilk's Lambda multi-variate test indicated that there were statistically significant interactions between heading and zone for trace element levels in live bees (Table 5.3.0). The effects of heading and zone individually were statistically significant also (Table 5.3.0). Further analysis shows that three principal components explained 82 % of the variation observed in the zone\*heading interaction, and that almost 80 % of the variation in heading and zone was explained by one principal component for each variable (Table 5.3.0).

A breakdown of these principal components for live bees is shown in Table 5.3.1. The first root in the interaction between zone\*heading, root 1, is a contrast between the levels of As, Ba and Cd with the levels of Cr, Cu, and Mn. This contrast is mainly driven especially by the Rumsey Island site, where levels of As, Ba, and Cd were relatively low, and the Cr, Cu, Mn, levels high (Figure 5.3.0). Another contribution to this root are Tower Hill Farm and Jones Farm, where opposite trends were observed for some of the trace element in the groups described above (Figure 5.3.0). The second root in the interaction between zone\*heading, root 2, is the contrast between the levels of As, Sr, and Zn with the levels of Cr and Mn. No clear pattern in trace element levels stands out to explain this interaction, suggesting that the interaction is quite complex. The last and third root in the interaction between zone\*heading, root 3, is a contrast between the levels of As and Rb with Sr. This interaction agrees with the observation that high levels of As and Rb levels in forager bee tissue generally correspond with low levels of Sr and vice versa (Figure 5.3.0).

#### Table 5.3.0

# MANOVA Results of the Comparison

of Trace Element Levels in Live Bee tissue at the Boundary Study Sites;

shown are Wilk's statistic and canonical correlations.

Effect	Live Bees					
	Wilks (F)	D.F. $v_1, v_2$	p-value			
Zone by Heading	2	90, 153	0.001			
Heading	.2	30, 52	0.002			
Zone	7	45, 78	<0.001			
	Eigenvalue	%	Cum. %			
Zone by Heading						
Root 1	2.3	33	33			
Root 2	1.9	28	61			
Root 3	1.5	21	82			
Heading						
Root 1	2.5	79	79			
Zone						
Root 1	15.4	79	79			

# Table 5.3.1

# MANOVA Results of the Comparison of Trace Element Levels in Live Bee Tissue at the Boundary Study Sites; Shown are the standardized discriminant function coefficients by element. Only trace element with coefficients >0.75 are shown in the table.

Trace Element	Z	one by Headin	g	Heading	Zone
	Root 1	Root 2	Root 3	Root 1	Root 1
As	0.8	- 1.3	<b>-</b> 1.1	1.7	
Ba	1.1				
Cd	0.8			·	- 0.8
Со					1.0
Cr	- 1.5	1.8		- 0.8	1.4
Cu	- 1.2			- 0.8	1.0
Ga				1.6	
Mn	- 1.0	1.2			
Ni					
Pb					
Rb			- 1.0	1.2	- 2.0
Se				- 0.8	
Sr		- 0.9	1.4	- 1.2	
v					
Zn	<b></b>	- 1.0			



**Figure 5.3.0** Geographic outline of the boundary study with the mean levels of selected trace elements for live forager bee tissue (in ppm), 1999.

The effects of heading and distance from Aberdeen Proving Ground on the levels of several trace elements in bee tissue were also statistically significant (Tables 5.3.0 and 5.3.1). The effect of heading was mainly due to the contrast between levels of As, Ga, and Rb versus levels of Cr, Cu, and Se. There was a strong pattern for As, Ga and Rb based on heading direction, with the highest levels in bee tissue occurring in the NNE transect. However, the contrast of As, Ga, and Rb with levels of Cr, Cu, and Se was more difficult to pin down, and no specific patterns emerged (Figure 5.3.0; Table 5.9). Finally, there was a statistically significant effect of zone on the levels of several trace elements in bee tissue (Table 5.3). This effect was attributed to the contrast in between levels of Cd and Rb versus Co, Cr, and Cu (Table 5.3). Again further

examination of the spatial patterns of these elements did not reveal any clear patterns, suggesting a rather complex interaction between the different trace elements.

#### **Pollen:**

A multi-variate analysis of variance was attempted for the levels of trace elements in pollen to address spatial patterns of and correlations between elements. However, we were not able to trap pollen at all sites on all dates (i.e., pollen trapping success depends on the bees bringing pollen back to the hive). As such, several sites had only one pollen value. Because of these singular cells, a complete multi-variate analysis of variance for pollen values was not possible. A two-way analysis of variance was carried out for each element individually, with heading and zone as the dependent factors.

There were significant effects of zone for Ba, Cd, Cu, and Mn levels in pollen (Table 5.3.2). Although the direction with respect to the base did not influence trace element levels in pollen, there were statistically significant Zone\* Heading interactions for Ba, Cd, and Mn (Table 5.3.2). Further analysis shows that this interaction term is caused by two trends. Levels of Ba, Cd and Cu and Mn were highest at Clyburn Arboretum, a site located near downtown Baltimore on the WSW transect, and Mn levels were highest in the WSW transect compared to the other two (Figure 5.3.1). Opposite trends were found in the NNE and NW transects, with decreasing levels of Ba, Cd, and Mn as the distance from the base increased (Figure 5.3.1). The higher levels at Clyburn Arboretum were probably due to high traffic and industrial activity in this direction from the base, whereas the N and NW transect had a more rural character (Figure 5.3.1).

# Table 5.3.2Two-way ANOVA Results for the Comparisonof Trace Element Levels in Pollen at the Boundary Study Sites.Statistically significant effects are indicated as "\*".

Trace Element	Zone by	Heading	Неа	ding	Zone		
	F-value	F-value p-value		p-value	F-value	p-value	
Ba	3.57	0.017 *	1.97	0.169	5.68	0.006 *	
Cd	7.56	<0.001 *	1.51	0.248	8.97	0.001 *	
Cu	1.60	0.204	0.48	0.628	9.24	0.001 *	
Mn	7.42	<0.001 *	0.92	0.417	26.99	<0.001 *	



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**Figure 5.3.1** Geographic outline of the boundary study with the mean levels of selected trace elements for pollen (in ppm), 1999.

#### 5.4 Concentration Gradient Analysis for Manganese and Strontium

The previous statistical analysis, described in sections 5.2 and 5.3, as well as previous APG biomonitoring reports, indicated that manganese and strontium levels were elevated and clustered on the Aberdeen Proving Ground-Edgewood area compared to the off-base sites. Because the initial statistical comparisons revealed that these two inorganic elements were associated with location effects, further examination of the spatial patterns of these two elements was warranted. Mn and Sr levels were compared among all sites that were monitored in 1999. In addition, a one-way ANOVA test with Bonferroni post-hoc test was carried out (using SPSS 6.0). Although there were some differences in Mn and Sr levels in bees from the full-size phytohives and the smaller condo hives at J-field, both hive types were pooled for this analysis, in order to increase the sample size. Similarly, data from all three headings in the boundary study were pooled by distance.

The ANOVA results for manganese indicate that Mn levels in live forager bees and pollen are significantly higher at J-Field compared to the other APG and off-base sampling sites  $(F_{7,108}=6.89, P<0.001 \text{ and } F_{7,56}=3.76, P=0.002 \text{ respectively}; Figure 5.4.0)$ . Moreover, Mn levels in live bees tended to be somewhat higher at Cluster 3 and Youth Center on APG compared to the off-base sites, although these differences were not statistically significant. Dispersion mapping of Mn concentrations (Bromenshenk, unpublished, 2001) revealed that Mn was somewhat elevated at sites near the waters of Chesapeake Bay, even at some of the off-post sites, when compared to more distant Boundary Sites.

These data indicate that a source of Mn is present in the J-Field area on Aberdeen Proving Ground and that presence of Mn may be related to distance from the Bay. There are several possible explanations for the presence of Mn. A natural, local source of Mn could be the soil chemistry at J-Field. A second natural source of Mn at J-Field and other sites could be the salt marches facing Chesapeake Bay, which are close to the hive locations. Mn levels tend to be higher in salt marshes compared to both seawater and fresh water (Personal communication, Dr. Michael DeGrandpre, University of Montana). Thus, a natural source of Mn at J-Field cannot be excluded. A possible anthropogenic source of Mn at J-Field could be the use of Mn in pyro delay fuses and as an experimental fuel (Personal communication, Philip Rodacy, Explosives Unit, Sandia National Laboratory).

Similar to manganese, the ANOVA results also show that there is a source of strontium present at J-Field (Figure 5.4.1). Sr levels were significantly higher in both live forager bee tissues and pollen from the J-Field hives, compared to the other on- and off-base hives ( $H_7$ =67.38, P<0.001 and  $F_{7,56}$ =12.00, P<0.001 respectively). Sr levels in bee tissue and pollen from Cluster 3, the APG site closest to J-Field were somewhat higher than at other sites on Aberdeen Proving Ground and the off-base sites, but not statistically different from these sites. The most likely source for Sr at J-Field is the use of strontium nitrate in flares and strontium peroxide in tracer rounds. Since a portion of the strontium used for these purposes remains unburned, this can lead to local contamination with Strontium (Personal communication, Philip Rodacy, Sandia National Laboratory). Mapping of Sr concentrations (Bromenshenk, unpublished data, 2000) did not reveal patterns of Sr dispersion at off-post sites, as occurred with Mn.

Trace element levels in forager bee tissue and pollen were highly correlated for both Mn (r2=0.93, P<0.001, n=8) and Sr (r2=0.87, P=0.005, n=8).



**Figure 5.4.0** Manganese levels in live forager bees and pollen at Aberdeen Proving Ground, and off-base sampling sites, 1999. Error bars represent 1 SE. Letters indicate statistically significant differences within tissue type (one-way ANOVA, P < 0.05).



**Figure 5.4.1** Strontium levels in live forager bees and pollen at Aberdeen Proving Ground, and off-base sampling sites, 1999. Error bars represent 1 SE. Letters indicate statistically significant differences within tissue type (one-way ANOVA, P < 0.05).

# 5.5 Overall Discussion and Conclusion, Trace Elements and Heavy Metals

Lead and arsenic are heavy metals of concern to installation restoration managers at APG-Edgewood. Compared to the smelter areas of the west that we have previously investigated, the levels of these elements in bees at APG and in the levels surrounding it are low. With respect to arsenic, slightly higher arsenic levels were often seen off-post, especially at the orchard sites (Tables 5.5.0-5.5.). This is consistent with our findings that arsenic tends to be somewhat elevated in old orchards, probably as a result of the use of arsenical-based insecticides from the late 1980's through the 1940's.

At Cluster 3, in 1999 there was no evidence of elevated arsenic in bees and pollen compared to other sites. Cluster 13 showed no evidence of elevated arsenic, but did display somewhat

elevated levels of lead in bees (mean=8.5, about twice that of most other sites, with the exception of J-Field).

Lead levels were highest at J-Field in bees and pollen (Tables 5.5.0). Pollen from the E set of phytohives, on the north side of this site, was especially high in lead (mean=85 ppm). The highest lead values in bees were observed in the condo hives (mean=34.9 ppm), at the phytohive B location. Because bees forage wide areas and change the areas visited from hour to hour and day to day, the deployment of many hives in an area increases the chance of detection of a contaminant of concern. Which hive or hive set reveals the contaminant and on which day is a function of what is blossoming at the time that the bees were sampled and of where the bees are going. Keeping in mind that bees and pollen at J-Field were sampled on different dates, seeing the highest seasonal value of lead in pollen from the E set of hives and the highest level of lead in another set of nearby hives it is not an unexpected result.

Because it requires a specialized setup for low level determinations, mercury was not examined in this study. Of the toxic metals, cadmium was observed at low levels at APG (sub ppm) sites. It was somewhat elevated in bees at the Baltimore Arboretum.

Overall, this report provides additional information concerning trace elements and heavy metals at on- and off-post sites. It also provides a more rigorous and in depth statistical analysis of the inorganics results. Mn and Sr were singled out for further analysis based on the outcome of the preliminary multi-variate statistical analysis. These elements also have historically been shown to be in higher than expected concentrations at APG-Edgewood area sites. Although strontium is probably a result of the use of flares and tracers at APG, the source of the manganese is undetermined. Because it is also somewhat elevated on Rumsey island (which is just west of APG and near a state park) and otherwise tends to decrease with distance from APG-Edgewood, we suspect a natural source such as concentrated manganese in marshy areas. In 1999, we saw no evidence of off-post, long-range migration from APG-Edgewoord of any other trace element or heavy metal or of any VOC. The same was true in 1998. Because of this, we suspect a natural source of manganese that is associated with the APG peninsula and a military source of Sr that is restricted to on-site locations at APG. However, the high levels of these two inorganic chemicals at J-Field and to some extent at Cluster 3 warrant further investigation.

From a human health perspective, both Mn and Sr are listed in EPA Region 3's RBC tables for air quality (ng/m<sup>3</sup>). The RBC value for Sr is similar to that for iron, ranking among the least harmful of the inorganic chemicals included on the list. The RBC values for Mn are much lower, being about 1/6 that of mercury, although more than 100 fold higher than that of arsenic. The J-Field ecological risk assessment (1998) states that Mn is not thought to be very toxic from an ecological perspective. Neither of these chemicals appear in Ronald Eisler's Handbook of Chemical Risk Assessment, Health Hazards to Humans, Plants, and Animals (R. Eisler, 2000). With respect to bees, there appears to be no data available about the toxicity of either of these chemicals.

Strontium has not been recognized as a chemical of potential concern at APG. This appears to be an artifact of most contract laboratories conducting analysis only on the TAL (Target Analyte List) list of inorganic chemicals. That suite of chemicals does not include Sr, probably because it is uncommon at most U.S. sites, although that does not appear to be true of APG, and we speculate that it would appear at other Defense sites with firing ranges.

Whereas we can't estimate concentrations of trace elements and heavy metals in the air based on bee and pollen samples, our previous work in smelter areas suggest that the overall low levels of toxic elements found in the Maryland bee and pollen samples provide no reason to expect elevated levels in the ambient air.

With the possible exception of two pollen samples from J-Field, none of the toxic metals, for which we have dose-response information in the UM Toxics database, occurred at levels in bees or pollen that would be predicted to cause any acute toxicity to bees. Because previous and subsequent sampling of the pollen from the hives showing elevated lead at J-Field did not reveal any increase in lead, we speculate that some plants blossomed for a short period of time on one of the areas of J-Field where the soils are known to be contaminated by lead. As soon as the blossom was finished, these colonies found other sources of uncontaminated pollen and as such did not display any overt bee mortality.

In conclusion, the lead contamination at J-Field was detected, but only in a few pollen samples. As such, the bees were better at providing an integrated chemical characterization of inorganics at a site than locating small hot spots. However, this exposure scenario should be similar to that encountered by other wide ranging animals, especially birds and larger mammals. Mn and Sr, whether man-made or natural, is present at APG, especially J-Field. Available data does not show any evidence of long-range migration of any inorganics elements off-post. Sr is not mentioned in APG risk assessment documents. Although we found little information about Sr toxicity to humans, plants, or animals, it does not appear to be particularly toxic, and as such was not considered. Alternately, the omission of Sr may be because it is not included in the EPA TAL suite of inorganic chemicals.

Finally, a review of the Risk Assessment Documents for J-Field (1998) and Cluster 3 (1998) found that for J-Field, the dietary consumption of insects was taken into account when developing Ecological Effects Quotients for several birds and animals. Taking the Tree Swallow as an example, if we substitute the measured values of inorganic chemicals found in bees for the estimated values used in determining the Applied Daily Dose (ADD) from the ingestion of insects, the overall value for the contribution of insects to the diet of these insectivorous birds increased the Total ADD by five fold (Bromenshenk et al., 2001). Changes of that magnitude could significantly alter the resultant EEQs. EPA has been working toward better ways of estimating bioconcentration factors for insects. Our data would suggest that the current method may greatly underestimate the contribution from free flying insects. That generalization will not apply to all insects, but given the foraging habits of bees and the results of other pollution studies, we anticipate that the kinds of chemicals and the concentrations of

these chemicals are likely to be toward the high range compared to many other insects. As such, use the "bee model" and measured concentrations might provide a better assessment of a worst case exposure, integrated across the site in question.

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# Table 5.5.0Trace Elements in Forager Bees and Pollen for J -Field Hives,<br/>April through November 1999.Values are ppm by weight ± RSD ( %); n= 4 to 11.

As	Live Bees			Dead Bees			Pollen		
	Mean±RSD	Max	Min	Mean±RSD	Max	Min	Mean±RSD	Max	Min
PhytoA	0.3 ± 6.4	0.4	0.2	n.a.			0.3 ± 16.4	0.5	0.2
Phyto B	0.3 ± 14.6	0.5	0.2	n.a.			not present		
Phyto C	0.4 ± 35.7	0.8	0.2	n.a.			0.1 ± 13.5	0.2	0.1
Phyto D	0.2 ± 8.6	0.3	0.1	n.a.			0.1 ± 14.5	0.2	0.1
Phyto E	0.3 ± 8.4	0.3	0.2	n.a.			0.1 ± 7.9	0.1	0.1
Phyto-All	0.3 ±12.8	0.8	0.1	n.a.			$0.2 \pm 14.1$	0.5	0.1
Condo set	0.4 ± 6.2	0.5	0.2	0.4 ± 5.4	0.6	0.2	$0.2 \pm 13.4$	0.6	0.1

Ba	Live Bees			Dead Bees			Pollen		
	Mean±RSD	Max	Min	Mean±RSD	Max	Min	<b>Mean±RSD</b>	Max	Min
PhytoA	4.6 ± 4.1	6.0	3.2	n.a.			8.9±1.7	12.6	6.6
Phyto B	4.1 ± 20.4	8.8	0.6	n.a.			not present		
Phyto C	4.9 ± 3.7	6.4	4.0	n.a.			9.3 ± 1.1	9.7	8.9
Phyto D	$4.4 \pm 1.5$	8.2	2.5	n.a.			8.4 ± 2.8	9.8	7.5
Phyto E	6.6 ± 2.6	14.6	4.1	n.a.			8.2 ± 1.0	8.2	8.2
Phyto-All	5.0 ± 6.4	14.6	0.6	n.a.			8.8 ± 1.8	12.6	6.6
Condo set	11.9 ± 1.3	19.7	5.7	32.2 ± 2.7	88.6	5.5	$3.2 \pm 6.5$	4.4	1.5

Cd	Liv	e Bees		Dead	ead Bees		Po	ollen	
	Mean±RSD	Max	Min	Mean±RSD	Max	Min	Mean±RSD	Max	Min
PhytoA	0.8 ± 5.9	1.6	0.1	n.a.			0.4 ± 10.5	1.2	BDL
Phyto B	$0.8 \pm 20.0$	1.8	0.1	n.a.			not present		
Phyto C	0.6 ± 31.3	0.8	0.3	n.a.			0.1 ± 1.4	0.2	BDL
Phyto D	0.6 ± 2.6	1.2	BDL	n.a.			$0.03 \pm 3.4$	0.1	BDL
Phyto E	$0.7 \pm 3.0$	1.6	BDL	n.a.			BDL	BDL	BDL
Phyto-All	0.7 ± 10.8	1.8	BDL	n.a.			$0.2 \pm 4.6$	1.2	BDL
Condo set	$0.4 \pm 5.4$	0.7	0.2	0.2 ± 7.7	0.6	BDL	0.1 ± 7.5	0,6	BDL

Table 5.5.0 (continued) Trace Elements in Forager Bees and Pollen for J -Field Hives

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Co	Live Bees			Dead Bees			Pollen		
	Mean±RSD	Max	Min	<b>Mean±RSD</b>	Max	Min	Mean±RSD	Max	Min
PhytoA	1.3 ± 5.1	6.5	0.1	n.a.			0.8 ± 2.7	1.9	BDL
Phyto B	$0.3 \pm 3.2$	0.6	BDL	n.a.			not present		
Phyto C	0.4 ± 44.6	0.6	0.2	n.a.			$0.03 \pm 3.1$	0.1	BDL
Phyto D	$0.3 \pm 2.8$	0.6	BDL	n.a.			$0.3 \pm 3.1$	0.8	BDL
Phyto E	0.3 ± 2.9	0.7	BDL	n.a.			0.1 ± 5.7	0.1	0.1
Phyto-All	0.6 ± 9.0	6.5	BDL	n.a.			0.3 ± 3.2	1.9	BDL
Condo set	0.7 ± 6.1	1.8	0.3	0.4 ± 5.5	1.7	BDL	0.1 ± 8.2	0.6	BDL

Cr	Live Bees			Dead Bees			Pollen		
	Mean±RSD	Max	Min	Mean±RSD	Max	Min	<b>Mean±RSD</b>	Max	Min
PhytoA	0.2 ± 9.3	0.3	0.2	n.a.			0.3 ± 6.8	0.3	0.2
Phyto B	$0.2 \pm 2.7$	0.4	BDL	n.a.			not present		
Phyto C	0.4 ± 37.6	0.7	0.2	n.a.			$0.3 \pm 4.0$	0.3	0.2
Phyto D	$0.2 \pm 6.8$	0.3	0.2	n.a.			$0.2 \pm 4.3$	0.3	0.2
Phyto E	0.2 ± 5.4	0.3	0.2	n.a.			0.2 ± 5.7	0.2	0.2
Phyto-All	$0.2 \pm 10.3$	0.7	BDL	n.a.			$0.3 \pm 5.1$	0.3	0.2
Condo set	0.3 ± 9.4	0.5	0.2	$0.3 \pm 5.7$	0.6	0.2	0.3 ± 12.1	0.6	0.1

Table 5.5.0 (continued) Trace Elements in Forager Bees and Pollen for J -Field Hives

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Cu	Liv	e Bees		Dead	l Bees		Pe	ollen	
	<b>Mean±RSD</b>	Max	Min	Mean±RSD	Max	Min	Mean±RSD	Max	Min
PhytoA	18.7 ± 4.5	23.8	13.5	n.a.			11.1 ± 2.5	13.1	9.7
Phyto B	15.2 ± 19.2	21.6	4.1	n.a.			not present		
Phyto C	22.1 ± 1.7	31.6	16.5	n.a.			$15.6 \pm 4.1$	18.2	12.0
Phyto D	21.3 ± 2.2	25.8	15.1	n.a.			15.3 ± 3.3	22.1	9.9
Phyto E	19.0 ± 3.5	24.2	12.2	n.a.			9.8 ± 1.1	9.8	9.8
Phyto-All	19.0 ± 6.4	31.6	4.1	n.a.			13.6 ± 3.1	22.1	9.7
Condo set	25.9± 2.0	35.7	15.3	$23.7 \pm 3.3$	37.7	12.8	$5.9 \pm 6.2$	7.6	4.2

Ga	Live Bees			Dead Bees			Pollen		
	Mean±RSD	Max	Min	Mean±RSD	Max	Min	<b>Mean±RSD</b>	Max	Min
PhytoA	0.3 ± 5.0	0.4	0.2	n.a.			$0.5 \pm 3.1$	0.8	0.3
Phyto B	0.2 ± 2.3	0.5	BDL	n.a.			not present		
Phyto C	0.3 ± 13.3	0.4	0.2	n.a.			$0.5 \pm 2.5$	0.5	0.5
Phyto D	0.3 ± 4.8	0.5	0.1	n.a.			$0.5 \pm 2.7$	0.6	0.3
Phyto E	0.4 ± 2.8	0.9	0.2	n.a.			$0.5 \pm 2.2$	0.5	0.5
Phyto-All	$0.3 \pm 5.0$	0.9	BDL	n.a.			0.5 ± 2.7	0.8	0.3
Condo set	0.7 ± 2.3	1.0	0.4	1.8 ± 3.5	5.3	0.3	0.2 ± 8.8	0.3	BDL

Table 5.5.0 (continued) Trace Elements in Forager Bees and Pollen for J -Field Hives

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Mn	Live Bees			s Dead Bees			Pollen		
	Mean±RSD	Max	Min	<b>Mean±RSD</b>	Max	Min	<b>Mean±RSD</b>	Max	Min
PhytoA	397.4 ± 5.4	771.0	37.5	n.a.			$175.7 \pm 2.7$	200.0	137.0
Phyto B	326.1 ± 19.0	761.0	15.9	n.a.			not present		
Phyto C	270.8 ± 2.1	900.0	47.7	n.a.			128.7 ± 3.1	151.0	111.0
Phyto D	393.6 ± 1.7	789.0	26.4	n.a.			149.7 ± 3.7	222.0	112.0
Phyto E	350.7 ± 3.4	836.0	40.8	n.a.			241.0 ± 1.6	241.0	241.0
Phyto-All	354.6 ± 6.5	900.0	15.9	n.a.			$160.3 \pm 3.0$	241.0	111.0
Condo set	464.3 ± 2.4	794.0	113.0	238.0 ± 3.5	602.0	50.9	28.4 ± 6.0	52.3	13.9

Ni	Live Bees			Dead Bees			Pollen		
	Mean±RSD	Max	Min	Mean±RSD	Max	Min	Mean±RSD	Max	Min
PhytoA	$1.3 \pm 4.5$	2.6	0.4	n.a.			$2.3 \pm 1.6$	4.2	1.0
Phyto B	$1.3 \pm 19.2$	4.7	BDL	n.a.			not present		
Phyto C	$1.2 \pm 1.7$	3.1	0.3	n.a.	1		1.7 ± 4.4	2.2	1.1
Phyto D	0.7 ± 2.2	1.8	0.3	n.a.			$2.1 \pm 3.2$	2.7	1.8
Phyto E	$1.4 \pm 3.5$	3.3	0.3	n.a.			$3.9 \pm 2.1$	3.9	3.9
Phyto-All	1.2 ±7.3	4.7	BDL	n.a.			$2.2 \pm 3.0$	4.2	1.0
Condo set	34.1 ± 2.0	342	0.8	$0.8 \pm 4.8$	2.1	0.4	$0.9 \pm 9.4$	2.3	0.3

# Table 5.5.0 (continued) Trace Elements in Forager Bees and Pollen for J -Field Hives

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Pb	Live Bees			Dead Bees			Pollen		
	Mean±RSD	Max	Min	Mean±RSD	Max	Min	Mean±RSD	Max	Min
PhytoA	10.5 ± 3.6	32.5	2.5	n.a.			4.2 ± 1.8	5.4	1.9
Phyto B	6.0 ± 19.0	22.3	1.7	n.a.			not present		
Phyto C	8.7 ± 5.4	19.6	2.9	n.a.			$4.5 \pm 0.6$	5.3	4.0
Phyto D	5.8 ± 0.7	12.1	3.6	n.a.			$2.2 \pm 2.5$	29	1.7
Phyto E	5.4 ± 1.7	8.2	2.7	n.a.			<b>85</b> .0 ± 0.7	85.0	85.0
Phyto-All	7.2 ± 5.9	32.5	1.7	n.a.			$11.8 \pm 1.5$	85.0	1.7
Condo set	34.9 ± 1.4	271.0	1.7	$2.2 \pm 3.1$	5.4	0.2	$1.3 \pm 5.7$	8.9	1.3

Rb	Live Bees			Dead Bees			Pollen		
	Mean±RSD	Max	Min	<b>Mean±RSD</b>	Max	Min	Mean±RSD	Max	Min
PhytoA	6.8 ± 5.0	13.6	0.9	n.a.			11.6 ± 1.0	14.3	10.2
Phyto B	6.6 ± 19.9	11.5	1.3	n.a.			not present		
Phyto C	9.1 ± 2.1	20.5	4.1	n.a.			13.1 ± 1.4	15.1	9.9
Phyto D	8.0±1.0	11.3	4.7	n.a.			$14.7 \pm 2.5$	17.3	12.9
Phyto E	7.9 ± 2.3	13.2	4.2	n.a.			11.9±1.8	11.9	11.9
Phyto-All	7.5 ± 6.1	20.5	0.9	n.a.			13.0 ± 1.7	17.3	9.9
Condo set	7.4 ± 1.6	10.7	4.1	$10.8 \pm 2.7$	16.0	3.5	9.5 ± 5.5	9.5	2.3

Table 5.5.0 (continued) Trace Elements in Forager Bees and Pollen for J -Field Hives

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Se	Live Bees			Dead Bees			Pollen		
	Mean±RSD	Max	Min	Mean±RSD	Max	Min	Mean±RSD	Max	Min
PhytoA	0.4 ± 13.6	0.7	0.6	n.a.			0.2 ± 25.9	0.3	0.2
Phyto B	0.4 ± 11.0	0.6	BDL	n.a.			not present		
Phyto C	0.5 ± 36.7	0.9	0.3	n.a.			0.2 ± 16.5	0.3	0.2
Phyto D	$0.4 \pm 16.6$	0.5	0.2	n.a.			$0.2 \pm 31.2$	0.3	0.1
Phyto E	0.4 ± 23.4	0.6	0.2	n.a.			$0.2 \pm 16.7$	0.2	0.2
Phyto-All	0.4 ± 19.0	0.9	BDL	n.a.			$0.2 \pm 23.7$	0.3	0.1
Condo set	0.4 ± 15.4	0.6	0.2	0.5 ± 15.9	0.8	0.3	$0.2 \pm 26.0$	0.7	BDL

Sr	Live Bees			Dead Bees			Pollen		
	Mean±RSD	Max	Min	Mean±RSD	Max	Min	Mean±RSD	Max	Min
PhytoA	4.1 ± 4.2	5.6	3.2	n.a.			6.3 ± 1.3	8.8	4.8
Phyto B	3.4 ± 20.8	6.5	0.5	n.a.			not present		
Phyto C	4.8 ± 4.2	7.3	3.2	n.a.			9.7 ± 1.4	17.9	5.1
Phyto D	$4.1 \pm 1.1$	6.9	2.8	n.a.			4.9 ± 3.2	5.6	4.1
Phyto E	$5.2 \pm 2.4$	6.4	3.6	n.a.			5.1 ± 1.3	5.1	5.1
Phyto-All	4.3 ± 6.5	7.3	0.5	n.a.			6.8 ± 1.9	17.9	4.1
Condo set	8.3 ± 1.3	16.2	3.2	$10.6 \pm 3.0$	16.9	3.2	$5.8 \pm 5.6$	9.8	2.2

# Table 5.5.0 (continued) Trace Elements in Forager Bees and Pollen for J -Field Hives

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v	Live Bees			Dead Bees			Pollen		
	Mean±RSD	Max	Min	Mean±RSD	Max	Min	Mean±RSD	Max	Min
PhytoA	0.4 ± 6.4	0.6	0.3	n.a.			0.6 ± 5.3	0.7	0.6
Phyto B	0.5 ± 16.8	0.7	0.2	n.a.			not present		
Phyto C	0.7 ± 23.1	1.1	0.4	n.a.			0.7 ± 2.7	0.8	0.6
Phyto D	0.5 ± 3.6	0.6	0.4	n.a.			0.7 ± 4.1	0.9	0.6
Phyto E	0.5 ± 2.9	0.6	0.4	n.a.			0.5 ± 3.7	0.5	0.5
Phyto-All	0.5 ± 9.4	1.1	0.2	n.a.			0.7 ± 4.0	0.9	0.5
Condo set	0.6 ± 5.2	0.8	0.4	0.6 ± 3.2	0.8	0.4	0.5 ± 8.2	0.8	0.3

Zn	Live Bees			Dead Bees			Pollen		
	Mean±RSD	Max	Min	Mean±RSD	Max	Min	Mean±RSD	Max	Min
PhytoA	88.0 ± 4.9	109.0	58.5	n.a.			46.8 ± 2.7	65.7	36.9
Phyto B	82.6 ± 17.8	142.0	25.6	n.a.			not present		
Phyto C	86.3 ± 1.7	95.9	72.6	n.a.			122.4 ± 3.8	229.0	63.6
Phyto D	91.0 ± 1.8	115.0	69.9	n.a.			$50.5 \pm 2.8$	62.7	38.6
Phyto E	88.8 ± 2.7	116.0	63.6	n.a.			$66.4 \pm 0.9$	66.4	66.4
Phyto-All	87.6 ± 5.9	142.0	25.6	n.a.			72.6 ± 2.9	229.0	36.9
Condo set	109.6 ± 1.7	142.0	64.8	$105.0 \pm 3.5$	176.0	53.8	40.3 ± 5.5	143.0	15.4

Table 5.5.0 (continued) Trace Elements in Forager Bees and Pollen for J -Field Hives

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# Table5.5.1Trace Elements in Forager Bees and Pollen for J -Field, Cluster 3 and Churchville<br/>April through November 1999.Values are ppm by weight ± RSD ( %); n= 9 to 30.

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As	Live	Live Bees			l Bees		Pollen		
	Mean±RSD	Max	Min	Mean±RSD	Max	Min	Mean±RSD	Max	Min
J-fld: phyto	0.3 ±12.8	0.8	0.1	n.a.			$0.2 \pm 14.1$	0.5	0.1
J-fld:condo	0.4 ± 6.2	0.5	0.2	0.4 ± 5.4	0.6	0.2	0.2 ± 13.4	0.6	0.1
Cluster 3	0.4 ± 8.6	0.7	0.3	0.5 ± 3.9	0.8	0.4	0.4 ± 6.1	0.8	0.2
Churchville	0.3 ± 19.9	0.5	0.1	$0.2 \pm 4.4$	0.3	0.2	$0.2 \pm 3.6$	0.2	0.1

Ba	Live Bees			Dead Bees			Pollen		
	Mean±RSD	Max	Min	Mean±RSD	Max	Min	Mean±RSD	Max	Min
J-fld: phyto	5.0 ± 6.4	14.6	0.6	n.a.			8.8±1.8	12.6	6.6
J-fld:condo	11.9 ± 1.3	19.7	5.7	32.2 ± 2.7	88.6	5.5	$3.2 \pm 6.5$	4.4	1.5
Cluster 3	3.8 ± 1.8	6.2	3.0	6.9 ± 0.6	12.4	3.8	13.2 ± 1.1	31.6	2.2
Churchville	5.3 ± 15.6	15.6	5.3	$16.5 \pm 1.1$	21.0	12.1	6.7 ± 1.6	14.6	2.4

Cd	Live Bees			Dead	l Bees		Pollen		
	Mean±RSD	Max	Min	Mean±RSD	Max	Min	Mean±RSD	Max	Min
J-fld: phyto	0.7 ± 10.8	1.8	BDL	n.a.			$0.2 \pm 4.6$	1.2	BDL
J-fld:condo	0.4 ± 5.4	0.7	0.2	0.2 ± 7.7	0.6	BDL	0.1 ± 7.5	0.6	BDL
Cluster 3	0.3 ± 7.9	0.4	0.2	$0.2 \pm 6.1$	0.3	0.1	$0.03 \pm 2.1$	0.2	BDL
Churchville	0.2 ± 15.3	1.8	BDL	$0.2 \pm 3.5$	0.3	0.2	$0.03 \pm 0.6$	0.1	BDL

# Table5.5.1 (Continued)Trace Elements in Forager Bees and Pollen for J -Field,<br/>Cluster 3 and Churchville

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Co	Co Live Bees			Dea	d Bees		Pollen		
	Mean±RSD	Max	Min	Mean±RSD	Max	Min	Mean±RSD	Max	Min
J-fld: phyto	0.6 ± 9.0	6.5	BDL	n.a.			0.3 ± 3.2	1.9	BDL
J-fid:condo	0.7 ± 6.1	1.8	0.3	0.4 ± 5.5	1.7	BDL	0.1 ± 8.2	0.6	BDL
Cluster 3	0.9 ± 2.7	4.8	0.3	0.4 ± 3.9	0.5	0.2	$0.3 \pm 2.9$	0.9	BDL
Churchville	2.0 ±19.5	9.3	0.1	$0.5 \pm 2.2$	0.6	0.4	0.1 ± 2.6	0.3	BDL

Cr	Liv	Live Bees			l Bees		Pollen			
	Mean±RSD	Max	Min	Mean±RSD	Max	Min	Mean±RSD	Max	Min	
J-fld: phyto	$0.2 \pm 10.3$	0.7	BDL	n.a.			0.3 ± 5.1	0.3	0.2	
J-fid:condo	0.3 ± 9.4	0.5	0.2	0.3 ± 5.7	0.6	0.2	$0.3 \pm 12.1$	0.6	0.1	
Cluster 3	0.3 ± 11.9	0.4	0.2	0.3 ± 7.8	0.4	0.2	0.4 ± 4.9	0.4	0.3	
Churchville	0.3 ± 15.8	0.5	0.2	0.3 ± 3.6	0.3	0.3	0.4 ± 3.6	0.4	0.3	

Cu	Live Bees			Dea	d Bees		Pollen			
	Mean±RSD	Max	Min	Mean±RSD	Max	Min	Mean±RSD	Max	Min	
J-fld: phyto	19.0 ± 6.4	31.6	4.1	n.a.			13.6 ± 3.1	22.1	9.7	
J-fld:condo	25.9 ± 2.0	35.7	15.3	23.7 ± 3.3	37.7	12.8	$5.9 \pm 6.2$	7.6	4.2	
Cluster 3	18.9 ± 1.5	22.2	15.6	23.6 ± 2.1	27.9	20.4	8.2 ± 1.5	11.6	6.6	
Churchville	23.0 ± 14.4	44.1	17.2	23.4 ± 2.3	27.6	20.0	8.7 ± 2.0	10.6	7.6	

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Trace Elements in Forager Bees and Pollen for J -Field, Cluster 3 and Churchville

Ga	Liv	e Bees		Dead Bees			Pollen		
	Mean±RSD	Max	Min	Mean±RSD	Max	Min	Mean±RSD	Max	Min
J-fid: phyto	0.3 ± 5.0	0.9	BDL	n.a.			0.5 ± 2.7	0.8	0.3
J-fld:condo	0.7 ± 2.3	1.0	0.4	1.8 ± 3.5	5.3	0.3	0.2 ± 8.8	0.3	BDL
Cluster 3	$0.2 \pm 4.2$	0.4	0.1	$0.3 \pm 2.7$	0.5	0.2	0.6 ± 2.8	1.3	BDL
Churchville	0.3 ± 17.9	0.9	BDL	0.9 ± 2.4	1.2	0.7	0.4 ± 2.8	0.8	0.1

Mn	Liv	e Bees	Dea	d Bees		Pollen			
	Mean±RSD	Max	Min	Mean±RSD	Max	Min	<b>Mean±RSD</b>	Max	Min
J-fld: phyto	354.6 ± 6.5	900.0	15.9	n.a.			$160.3 \pm 3.0$	241.0	111.0
J-fld:condo	464.3 ± 2.4	794.0	113.0	238.0 ± 3.5	602.0	50.9	<b>28</b> .4 ± 6.0	52.3	13.9
Cluster 3	164.8 ± 2.3	183.0	146.0	159.2 ±2.4	292.0	89.3	20.0 ± 1.2	47.5	12.3
Churchville	123.9 ± 15.9	274.0	33.6	381.3 ± 1.2	453.0	315.0	$15.8 \pm 2.0$	18.0	14.3

Ni	Live Bees			Dead Bees			Pollen		
	Mean±RSD	Max	Min	Mean±RSD	Max	Min	Mean±RSD	Max	Min
J-fld: phyto	1.2 ±7.3	4.7	BDL	n.a.			$2.2 \pm 3.0$	4.2	1.0
J-fld:condo	34.1 ± 2.0	342	0.8	0.8 ± 4.8	2.1	0.4	0.9 ± 9.4	2.3	0.3
Cluster 3	1.2 ± 2.8	2.0	0.8	0.8 ± 3.2	1.0	0.6	$1.4 \pm 2.3^{\circ}$	1.7	0.9
Churchville	3.8 ± 15.3	24.7	0.4	$1.2 \pm 3.0$	2.2	0.4	1.0 ± 1.8	1.1	0.9

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Trace Elements in Forager Bees and Pollen for J-Field, Cluster 3 and Churchville

Pb	Liv	e Bees		Dead Bees			Pollen		
	Mean±RSD	Max	Min	Mean±RSD	Max	Min	Mean±RSD	Max	Min
J-fld: phyto	7.2 ± 5.9	32.5	1.7	n.a.			11.8 ± 1.5	85.0	1.7
J-fld:condo	34.9 ± 1.4	271.0	1.7	$2.2 \pm 3.1$	5.4	0.2	1.3 ± 5.7	8.9	1.3
Cluster 3	$4.2 \pm 1.8$	4.8	3,3	$2.6 \pm 1.1$	3.9	0.3	7.2 ± 1.4	16.2	3.7
Churchville	5.2 ±14.3	10.6	2.4	3.2 ± 1.3	4.2	2.1	3.9 ± 0.7	4.2	3.6

Rb	Live Bees			Dead Bees			Pollen		
	Mean±RSD	Max	Min	Mean±RSD	Max	Min	Mean±RSD	Max	Min
J-fld: phyto	7.5 ± 6.1	20.5	0.9	n.a.			13.0 ± 1.7	17.3	9.9
J-fld:condo	7.4 ± 1.6	10.7	4.1	10.8 ± 2.7	16.0	3.5	9.5 ± 5.5	9.5	2.3
Cluster 3	20.8 ± 1.6	28.7	7.4	8.2 ± 1.1	14.3	4.9	8.0 ± 1.4	9.1	6.9
Churchville	11.4 ± 14.9	21.4	5.9	13.0 ± 1.1	14.0	12.0	13.0 ± 1.6	15.3	10.4

Se	Live Bees			Dead Bees			Pollen		
	Mean±RSD	Max	Min	Mean±RSD	Max	Min	Mean±RSD	Max	Min
J-fld: phyto	0.4 ± 19.0	0.9	BDL	n.a.			0.2 ± 23.7	0.3	0.1
J-fld:condo	0.4 ± 15.4	0.6	0.2	0.5 ± 15.9	0.8	0.3	$0.2 \pm 26.0$	0.7	BDL
Cluster 3	0.6 ± 16.7	0.9	0.4	0.7 ± 12.2	1.1	0.4	0.3 ± 34.4	0.6	0.2
Churchville	0.6 ± 29.2	1.4	0.3	0.5 ± 14.7	0.6	0.3	0.3 ± 17.0	0.3	0.2

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#### Table 5.5.1 (continued)

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Trace Elements in Forager Bees and Pollen for J -Field, Cluster 3 and Churchville

Sr	Live Bees			Dead Bees			Pollen		
	Mean±RSD	Max	Min	Mean±RSD	Max	Min	Mean±RSD	Max	Min
J-fld: phyto	4.3 ± 6.5	7.3	0.5	n.a.			6.8 ± 1.9	17.9	4.1
J-fld:condo	8.3 ± 1.3	16.2	3.2	$10.6 \pm 3.0$	16.9	3.2	5.8 ± 5.6	9.8	2.2
Cluster 3	3.5 ± 1.8	7.7	2.3	6.1 ± 0.8	7.3	4.7	2.3 ± 0.9	3.6	0.3
Churchville	3.0 ± 15.9	8.0	0.9	<b>5.4 ±</b> 0.9	6.1	4.6	1.4 ± 1.3	2.1	1.1

v	Live Bees			Dead Bees			Pollen		
	Mean±RSD	Max	Min	Mean±RSD	Max	Min	Mean±RSD	Max	Min
J-fld: phyto	0.5 ± 9.4	1.1	0.2	n.a.			0.7 ± 4.0	0.9	0.5
J-fld:condo	0.6 ± 5.2	0.8	0.4	0.6 ± 3.2	0.8	0.4	0.5 ± 8.2	0.8	0.3
Cluster 3	0.6 ± 6.7	0.7	0.4	0.5 ± 2.8	0.6	0.4	0.6 ± 2.9	0.8	0.5
Churchville	0.6 ± 15.7	0.9	0.4	0.6 ± 2.3	0.6	0.5	0.6 ± 1.9	1.0	0.6

Zn	Live Bees			Dead Bees			Pollen		
	Mean±RSD	Max	Min	Mean±RSD	Max	Min	Mean±RSD	Max	Min
J-fld: phyto	87.6 ± 5.9	142.0	25.6	n.a.			72.6 ± 2.9	229.0	36.9
J-fld:condo	109.6 ± 1.7	142.0	64.8	105.0 ± 3.5	176.0	53.8	40.3 ± 5.5	143.0	15.4
Cluster 3	$108.0 \pm 1.5$	168.0	61.2	137.7 ± 1.4	173.0	108.0	54.2 ± 1.4	91.9	16.7
Churchville	121.8 ± 14.3	228.0	75.2	131.0 ± 1.8	148.0	121.0	43.0 ± 1.9	76.2	29.9

As		I	Live Bees			Pollen	
		Mean±RSD	Max	Min	Mean±RSD	Max	Min
Youth Center	APG	$0.2 \pm 6.5$	0.3	0.2	$0.3 \pm 6.7$	0.7	0.2
Cluster 13	APG	0.2 ± 22.4	0.4	0.2	0.2 ± 21.4	0.3	0.1
Otter Point	3 mi N	0.4 ± 18.1	0.5	0.3	0.3 ± 39.6	0.4	0.2
Lohr's Orchard	9 mi N	0.9 ± 36.6	1.8	0.4	0.5 ± 13.2	0.6	0.3
Cecil County	21 mi N	0.4 ± 12.9	0.5	0.3	$0.3 \pm 5.2$	0.3	0.3
Jones Farm	3 mi NW	0.5 ± 11.3	1.3	0.3	$0.3 \pm 3.5$	0.3	0.3
Tower Hill Farm	9 mi NW	0.3 ± 7.0	0.3	0.2	0.2 ± 13.1	0.2	0.1
Shawsville	21 mi NW	0.5 ± 14.2	0.6	0.5	0.3 ± 8.6	0.3	0.2
Rumsey Island	3 mi SW	0.3 ± 6.4	0.3	0.2	0.5 ± 9.7	0.9	0.1
Silver Lake Drive	9 mi SW	$0.4 \pm 20.0$	0.4	0.3	0.2 ± 2.5	0.2	0.2
Clb. Arboretum	21 mi SW	0.4 ± 3.6	0.4	0.3	0.3 ± 3.2	03	0.3

Table 5.5.2 Trace Elements in Forager Bees and Pollen Boundary Stud	y Hives, Fall 1999
Values are ppm by weight $\pm RSD$ (%); n= 4	

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Ba		I	Live Bees		Pollen			
		Mean±RSD	Max	Min	Mean±RSD	Max	Min	
Youth Center	APG	2.5 ± 1.2	3.8	1.2	$1.9 \pm 1.4$	2.7	1.6	
Cluster 13	APG	2.7 ± 3.2	3.8	1.7	2.7 ± 9.8	3.0	2.5	
Otter Point	3 mi N	4.2 ± 3.4	5.6	3.4	4.1 ± 3.3	4.5	3.6	
Lohr's Orchard	9 mi N	3.1 ± 10.0	3.5	2.4	3.1 ± 4.4	3.2	3.0	
Cecil County	21 mi N	2.8 ± 1.8	4.3	0.3	2.7 ± 0.7	3.2	2.2	
Jones Farm	3 mi NW	2.8 ± 2.6	4.3	2.2	$3.4 \pm 0.5$	3.4	3.4	
Tower Hill Farm	9 mi NW	3.3 ± 2.2	4.2	2.4	2.0 ± 1.8	2.8	1.1	
Shawsville	21 mi NW	3.2 ± 2.1	4.1	2.0	2.2 ± 1.7	2.2	2.2	
Rumsey Island	3 mi SW	$2.5 \pm 1.2$	4.0	1.6	1.9 ± 2.0	3.1	1.3	
Silver Lake Drive	9 mi SW	$2.5 \pm 3.1$	3.0	1.9	$2.3 \pm 2.0$	2.6	2.0	
Clb. Arboretum	21 mi SW	3.0 ± 1.6	7.3	0.5	$3.9 \pm 2.0$	4.0	3.7	

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Trace Elements in F	orager Bees	and Pollen	Boundary	Study
Hives, Fall 1999				

Cd		]	Live Bees			Pollen	
		Mean±RSD	Max	Min	Mean±RSD	Max	Min
Youth Center	APG	$0.3 \pm 3.8$	0.6	BDL	$0.2 \pm 3.7$	0.3	0.1
Cluster 13	APG	$0.2 \pm 30.5$	0.4	BDL	$0.2 \pm 12.7$	0.2	0.1
Otter Point	3 mi N	0.7 ± 9.0	1.1	0.5	0.9 ± 13.4	1.1	0.6
Lohr's Orchard	9 mi N	0.5 ± 48.6	0.9	0.2	0.4 ± 28.3	0.4	0.3
Cecil County	21 mi N	0.1 ± 14,4	0.4	BDL	BDL		
Jones Farm	3 mi NW	$0.8 \pm 10.2$	1.2	0.4	0.6 ± 0.9	0.6	0.6
Tower Hill Farm	9 mi NW	0.4 ± 2.9	0.7	0.3	0.1 ± 3.9	0.2	BDL
Shawsville	21 mi NW	0.5 ± 14.7	0.8	0.2	0.2 ± 4.7	0.2	0.1
Rumsey Island	3 mi SW	0.5 ± 4.5	0.9	0.3	0.2 ± 1.3	0.4	BDL
Silver Lake Drive	9 mi SW	0.4 ± 20.9	0.5	0.3	0.2 ± 4.7	0.2	0.2
Clb. Arboretum	21 mi SW	1.3 ± 4.2	2.1	0.1	0.5 ± 1.7	0.5	0.4

Со		J	Live Bees Pollen				
		Mean±RSD	Max	Min	Mean±RSD	Max	Min
Youth Center	APG	0.6 ± 2.3	1.8	BDL	0.1 ± 1.0	0.2	BDL
Cluster 13	APG	0.4 ± 30.2	0.8	BDL	1.5 ± 3.0	2.5	BDL
Otter Point	3 mi N	0.3 ± 23.3	0.4	0.1	0.1 ± 58.2	0.2	BDL
Lohr's Orchard	9 mi N	$1.2 \pm 24.5$	2.2	0.8	0.3 ± 35.6	0.3	0.2
Cecil County	21 mi N	0.4 ± 9.8	0.7	0.2	0.1 ± 2.9	0.1	0.1
Jones Farm	3 mi NW	0.4 ± 11.2	0.9	0.1	$0.2 \pm 0.6$	0.2	0.2
Tower Hill Farm	9 mi NW	0.6 ± 2.5	0.7	0.5	1.1 ± 3.8	2.0	0.2
Shawsville	21 mi NW	0.7 ± 8.4	1.3	0.2	0.2 ± 1.0	0.2	0.1
Rumsey Island	3 mi SW	0.5 ± 3.6	0.7	0.2	0.1 ± 3.9	0.2	0.1
Silver Lake Drive	9 mi SW	0.5 ± 14.0	0.7	0.3	$0.2 \pm 3.3$	0.2	0.1
Clb. Arboretum	21 mi SW	$1.5 \pm 2.3$	4.9	0.3	0.1 ± 0.8	0.2	BDL

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**Trace Elements in Forager Bees and Pollen Boundary Study Hives, Fall 1999** 

Cr		]	Live Bees			Pollen		
		Mean±RSD	Max	Min	Mean±RSD	Max	Min	
Youth Center	APG	$0.2 \pm 4.7$	0.2	0.2	0.3 ± 6.8	0.5	0.2	
Cluster 13	APG	$0.2 \pm 27.4$	0.4	0.2	$0.3 \pm 10.9$	0.3	0.3	
Otter Point	3 mi N	0.3 ± 28.1	0.3	0.2	$0.3 \pm 40.7$	0.3	0.3	
Lohr's Orchard	9 mi N	0.6 ± 44.8	1.1	0.4	0.5 ± 19.1	0.6	0.3	
Cecil County	21 mi N	$0.2 \pm 15.8$	0.4	0.2	0.4 ± 3.7	0.5	0.3	
Jones Farm	3 mi NW	0.3 ± 18.8	0.7	0.2	0.7 ± 4.6	0.7	0.7	
Tower Hill Farm	9 mi NW	$0.3 \pm 5.0$	0.4	0.2	$0.3 \pm 5.2$	0.3	0.3	
Shawsville	21 mi NW	0.4 ± 12.3	0.5	0.3	0.4 ± 1.3	0.5	0.3	
Rumsey Island	3 mi SW	0.5 ± 3.6	0.7	0.2	0.3 ± 7.1	0.3	0.2	
Silver Lake Drive	9 mi SW	$0.3 \pm 20.5$	0.4	0.2	0.4 ± 4.5	0.4	0.4	
Clb. Arboretum	21 mi SW	$0.3 \pm 4.3$	0,3	0.2	0.4 ± 6.3	0.5	0.2	

Cu		I	Live Bees			Pollen	
		Mean±RSD	Max	Min	Mean±RSD	Max	Min
Youth Center	APG	$16.7 \pm 2.2$	20.8	13.0	8.2 ± 2.3	8.8	7.7
Cluster 13	APG	15.4 ± 4.1	17.7	12.7	11.0 ± 5.4	11.2	10.8
Otter Point	3 mi N	15.5 ± 2.2	18.6	13.0	9.8 ± 3.1	10.4	9.1
Lohr's Orchard	9 mi N	$21.4 \pm 2.1$	24.1	17.7	9.9±1.6	11.2	8.6
Cecil County	21 mi N	18.7 ± 1.5	20.7	16.7	$10.3 \pm 2.0$	12.9	7.7
Jones Farm	3 mi NW	18.8 ± 1.5	23.7	14.6	12.0 ± 1.7	12.0	12.0
Tower Hill Farm	9 mi NW	$20.2 \pm 2.4$	22.0	19.0	8.6 ± 2.0	9.9	7.3
Shawsville	21 mi NW	19.7 ± 1.0	21.1	17.6	10.9 ± 3.2	11.3	10.4
Rumsey Island	3 mi SW	20.5 ± 1.6	22.7	18.7	9.3 ± 1.4	10.2	8.1
Silver Lake Drive	9 mi SW	$16.5 \pm 1.3$	19.5	1.3	9.8 ± 1.1	10.4	9.2
Clb. Arboretum	21 mi SW	23.7 ± 2.5	25.6	21.8	13.1 ± 2.3	14.3	11.9

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**Trace Elements in Forager Bees and Pollen Boundary Study Hives, Fall 1999** 

Ga		]	Live Bees Pollen			Pollen	
		Mean±RSD	Max	Min	Mean±RSD	Max	Min
Youth Center	APG	0.1 ± 0.7	0.2	BDL	0.1 ± 1.2	0.2	BDL
Cluster 13	APG	0.1 ± 20.9	0.2	BDL	$0.2 \pm 7.7$	0.2	0.1
Otter Point	3 mi N	0.2 ± 3.8	0.3	0.2	0.3 ± 8.8	0.3	0.2
Lohr's Orchard	9 mi N	0.2 ± 36.9	0.3	0.2	$0.2 \pm 17.2$	0.2	0.1
Cecil County	21 mi N	$0.2 \pm 8.5$	0.3	0.1	0.2 ± 5.7	0.2	0.1
Jones Farm	3 mi NW	$0.1 \pm 6.3$	0.2	BDL	$0.2 \pm 2.1$	0.2	0.2
Tower Hill Farm	9 mi NW	0.1 ± 2.5	0.2	BDL	0.1 ± 2.8	0.1	BDL
Shawsville	21 mi NW	0.2 ± 7.0	0.2	0.1	0.1 ± 1.9	0.1	BDL
Rumsey Island	3 mi SW	0.1 ± 2.7	0.2	BDL	$0.03 \pm 0.3$	0.1	BDL
Silver Lake Drive	9 mi SW	0.1 ± 6.8	0.1	BDL	0.1 ± 0.6	0.1	BDL
Clb. Arboretum	21 mi SW	0.1 ± 2.8	0,2	BDL	0.2 ± 4.9	0.2	0.2

Mn		Live Bees			Pollen		
		Mean±RSD	Max	Min	Mean±RSD	Max	Min
Youth Center	APG	133.2 ± 1.9	249.0	41.7	12.7 ± 2.1	18.4	9.9
Cluster 13	APG	70.8 ± 3.3	137.0	28.8	$20.3 \pm 5.4$	21.6	19.6
Otter Point	3 mi N	72.9 ± 2.0	102.0	56.8	31.3 ± 2.3	34.4	<b>28</b> .1
Lohr's Orchard	9 mi N	131.1 ± 1.8	163.0	96.3	19.1 ± 3.0	23.4	14.7
Cecil County	21 mi N	48.4 ± 1.1	66.6	41.9	12.8 ± 1.5	15.7	9.9
Jones Farm	3 mi NW	94.7 ± 1.8	143.0	63.7	39.0 ± 2.8	39.0	39.0
Tower Hill Farm	9 mi NW	121.3 ± 2.9	170.0	70.4	$15.2 \pm 2.3$	15.8	14.5
Shawsville	21 mi NW	113.5 ± 1.9	161.0	81.1	$16.6 \pm 2.4$	18.1	15.1
Rumsey Island	3 mi SW	183.6 ± 2.1	309.0	79.8	23.4 ± 1.8	29.9	19.8
Silver Lake Drive	9 mi SW	76.5 ± 1.0	118.0	48.5	15.6±1.1	16.1	15.1
Clb. Arboretum	21 mi SW	99.5 ± 3.2	216.0	2.4	33.4 ± 2.1	34.4	32.4

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**Trace Elements in Forager Bees and Pollen Boundary Study Hives, Fall 1999** 

Ni		Live Bees			Pollen		
		Mean±RSD	Max	Min	Mean±RSD	Max	Min
Youth Center	APG	1.2 ± 2.9	1.6	0.7	$1.6 \pm 1.7$	1.7	1.4
Cluster 13	APG	1.2 ± 11.2	3.5	0.3	$1.8 \pm 6.5$	2.1	1.5
Otter Point	3 mi N	0.5 ± 17.6	0.6	0.4	1.4 ± 12.1	1.5	1.3
Lohr's Orchard	9 mi N	1.3 ± 19.7	1.7	1.1	1.8±5.9	2.2	1.4
Cecil County	21 mi N	0.9 ± 6.0	1.1	0.6	1.6±1.6	1.6	1.5
Jones Farm	3 mi NW	0.6 ± 7.7	1.2	0.3	1.9±1.7	1.9	1.9
Tower Hill Farm	9 mi NW	$1.0 \pm 2.1$	1.5	0.6	1.8 ± 3.4	2.7	0.8
Shawsville	21 mi NW	1.0 ± 5.7	2.1	0.5	1.4±1.6	1.7	1.1
Rumsey Island	3 mi SW	1.1 ± 1.9	1.3	0.7	$1.6 \pm 2.2$	1.7	1.4
Silver Lake Drive	9 mi SW	1.1 ± 10.6	1.8	0.7	$2.0 \pm 2.0$	2.2	1.7
Clb. Arboretum	21 mi SW	2.3 ± 3.3	4.7	0.5	0.9 ± 2.1	1.1	0.7

Pb		Live Bees				Pollen		
		Mean±RSD	Max	Min	Mean±RSD	Max	Min	
Youth Center	APG	4.4 ± 1.4	5.3	3.3	6.4 ± 1.4	12.1	3.8	
Cluster 13	APG	8.5 ± 2.3	29.8	1.8	5.2 ± 4.7	8.4	3.4	
Otter Point	3 mi N	$2.5 \pm 3.5$	3.6	1.9	4.9 ± 3.6	5.0	4.8	
Lohr's Orchard	9 mi N	3.1 ± 9.0	3.3	3.0	$4.2 \pm 4.4$	5.0	3.4	
Cecil County	21 mi N	3.8 ± 2.4	6.5	2.6	4.3 ± 1.6	4.5	4.0	
Jones Farm	3 mi NW	3.4 ± 2.6	5.1	2.1	8.9 ± 1.9	8.9	8.9	
Tower Hill Farm	9 mi NW	3.4 ± 3.0	5.0	2.2	4.1 ± 2.0	4.1	4.0	
Shawsville	21 mi NW	3.7 ± 2.6	7.1	2.0	6.5 ± 2.7	8.1	4.8	
Rumsey Island	3 mi SW	3.9 ± 1.2	5.6	1.7	7.1 ± 2.0	9.9	5.7	
Silver Lake Drive	9 mi SW	5.6 ± 3.7	9.8	1.3	$3.5 \pm 2.1$	4.3	2.6	
Clb. Arboretum	21 mi SW	5.3 ± 3.1	12.9	1.5	$4.5 \pm 2.3$	5.4	3.5	

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Trace Elements in Forager Bees and Pollen Boundary Study Hives, Fall 1999

Rb		]	Live Bees			Pollen		
		<b>Mean±RSD</b>	Max	Min	Mean±RSD	Max	Min	
Youth Center	APG	16.7 ± 1.7	24.3	12.6	8.2 ± 0.9	15.1	5.6	
Cluster 13	APG	8.6 ± 3.8	12.8	6.4	10.6 ± 4,5	15.2	8.3	
Otter Point	3 mi N	$6.2 \pm 1.4$	7.9	4.9	10.1 ± 1.8	10.4	9.7	
Lohr's Orchard	9 mi N	14.2 ± 1.8	15.9	11.8	$10.0 \pm 1.6$	10.0	10.0	
Cecil County	21 mi N	6.1 ± 0.7	7.3	5.6	$10.1 \pm 1.1$	10.8	9,4	
Jones Farm	3 mi NW	$4.9 \pm 0.9$	6.0	3.7	10.0 ± 1.4	10.0	10.0	
Tower Hill Farm	9 mi NW	8.8 ± 1.7	9.7	7.6	9.3 ± 1.4	12.5	6.1	
Shawsville	21 mi NW	7.0 ± 1.2	7.9	5.5	10.6 ± 1.7	11.5	9.6	
<b>Rumsey Island</b>	3 mi SW	<b>8.8</b> ± 1.0	10.2	6.3	8.4 ± 1.8	8,6	8.2	
Silver Lake Drive	9 mi SW	$6.5 \pm 1.0$	8.0	4.9	9.3 ± 0.9	10.4	8.1	
Clb. Arboretum	21 mi SW	$6.8 \pm 2.9$	8.8	5.5	7.8 ± 1.9	8.6	6.9	

Se		Live Bees				Pollen		
		Mean±RSD	Max	Min	Mean±RSD	Max	Min	
Youth Center	APG	0.6 ± 23.6	0.6	0.5	0.3 ± 25.2	0.3	0.2	
Cluster 13	APG	0.3 ± 45.0	0.4	0.1	0.1 ± 22.5	0.2	BDL	
Otter Point	3 mi N	0.4 ± 45.1	0.5	0.3	0.3 ± 94.4	0.3	0.2	
Lohr's Orchard	9 mi N	0.9 ± 39.7	1.4	0.6	0.4 ± 42.5	0.5	0.3	
Cecil County	21 mi N	0.4 ± 19.5	0.7	0.2	0.2 ± 38.9	0.2	0.1	
Jones Farm	3 mi NW	0.5 ± 23.3	1.1	0.3	$0.2 \pm 18.8$	0.2	0.2	
Tower Hill Farm	9 mi NW	0.5 ± 21.5	0.5	0.4	$0.2 \pm 60.5$	0.2	0.2	
Shawsville	21 mi NW	0.6 ± 24.3	0.9	0.3	0.3 ± 46.1	0.3	0.2	
Rumsey Island	3 mi SW	0.4 ± 13.6	0.5	0.3	0.2 ± 22.8	0.3	0.1	
Silver Lake Drive	9 mi SW	0.6 ± 21.2	0.7	0.4	0.3 ± 26.9	0.3	0.2	
Clb. Arboretum	21 mi SW	0.3 ± 14.9	0.4	0.2	$0.2 \pm 24.2$	0.2	0.1	

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Table 5.5.2	(continued)
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Sr		Live Bees				Pollen			
		Mean±RSD Max Min		Mean±RSD	Max	Min			
Youth Center	APG	$1.7 \pm 1.3$	2.4	1.0	1.8 ± 1.1	2.4	1.5		
Cluster 13	APG	$2.0 \pm 5.0$	3.1	1.2	1.9 ± 5.8	2.0	1.8		
Otter Point	3 mi N	1.9 ± 4.6	2.0	1.6	2.1 ± 6.9	2.4	1.8		
Lohr's Orchard	9 mi N	1.8 ± 18.0	2.2	1.3	1.4 ± 8.2	1.5	1.2		
Cecil County	21 mi N	$2.4 \pm 2.4$	2.8	2.1	$2.3 \pm 0.6$	2.4	2.1		
Jones Farm	3 mi NW	2.1 ± 3.1	3.2	1.7	2.6 ± 1.4	2.6	2.6		
Tower Hill Farm	9 mi NW	1.7 ± 1.2	2.0	1.3	1.1 ± 1.3	1.3	0.8		
Shawsville	21 mi NW	$2.0 \pm 3.5$	2.5	1.6	1.9 ± 1.4	2.3	1.5		
Rumsey Island	3 mi SW	$2.4 \pm 0.8$	3.1	1.9	$1.8 \pm 2.3$	2.6	1.3		
Silver Lake Drive	9 mi SW	1.6 ± 4.9	1.8	1.4	$2.9 \pm 0.6$	4.1	1.6		
Clb. Arboretum	21 mi SW	$2.3 \pm 2.0$	3.1	1.3	$1.3 \pm 1.4$	1.5	1.0		

<b>Trace Elements in</b>	<b>Forager Bees</b>	and Pollen	<b>Boundary</b>	Study
Hives, Fall 1999	-		·	v

Ti		Live Bees			Pollen		
		Mean±RSD	Mean±RSD Max		Mean±RSD	Max	Min
Youth Center	APG	BDL			BDL		
Cluster 13	APG	BDL			BDL		
Otter Point	3 mi N	$\dot{2.3} \pm 0.2$	9.0	BDL	BDL		
Lohr's Orchard	9 mi N	0.3 ± 76.2	0.7	BDL	BDL		
Cecil County	21 mi N	0.1 ± 17.4	0.3	BDL	BDL		
Jones Farm	3 mi NW	$0.1 \pm 20.6$	0.4	BDL	BDL		
Tower Hill Farm	9 mi NW	BDL	BDL	BDL	BDL		
Shawsville	21 mi NW	0.1 ± 23.8	0.2	BDL	BDL		
Rumsey Island	3 mi SW	BDL			BDL	· · · · ·	
Silver Lake Drive	9 mi SW	BDL			BDL/		
Clb. Arboretum	21 mi SW	BDL			BDL		

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v		Live Bees			Pollen		
	· ·	Mean±RSD	Max	Min	Mean±RSD	Max	Min
Youth Center	APG	0.5 ± 2.1	0.5	0.4	$0.5 \pm 3.3$	0.8	0.4
Cluster 13	APG	$0.4 \pm 16.0$	0.6	0.4	0.5 ± 6.7	0.5	0.4
Otter Point	3 mi N	0.6 ± 18.7	0.7	0.4	$0.6 \pm 26.3$	0.6	0.5
Lohr's Orchard	9 mi N	0.9 ± 32.1	1.5	0.7	$1.0 \pm 11.0$	1.1	0.8
Cecil County	21 mi N	$0.4 \pm 10.5$	0.7	0,3	0.6 ± 1.2	0.7	0.4
Jones Farm	3 mi NW	0.5 ± 11.6	0.9	0.3	0.7 ± 3.0	0.7	0.7
Tower Hill Farm	9 mi NW	0.6 ± 4.0	0.8	0.4	$0.4 \pm 1.6$	0.4	0.4
Shawsville	21 mi NW	0.6 ± 9.6	0.7	0.5	0.8 ± 2.6	1.1	0.5
Rumsey Island	3 mi SW	$0.5 \pm 3.7$	0.5	0.4	0.5 ± 3.5	0.5	0.4
Silver Lake Drive	9 mi SW	$0.5 \pm 12.0$	0.7	0.4	0.7 ± 2.4	0.7	0.6
Clb. Arboretum	21 mi SW	0.5 ± 1.8	0.6	0.4	0.4 ± 1.5	0.5	0.3

Zn		Live Bees			Pollen		
		Mean±RSD	Max	Min	Mean±RSD	Max	Min
Youth Center	APG	99.4 ± 1.7	142.0	48.7	$222.5 \pm 1.8$	804.0	26.5
Cluster 13	APG	77.8 ± 4.3	132.0	39.5	90.5 ± 5.4	107.0	59.5
Otter Point	3 mi N	85.1 ± 3.4	108.0	63.3	$157.1 \pm 4.0$	257.0	57.2
Lohr's Orchard	9 mi N	115.9 ± 2.2	167.0	80.6	72.1 ± 1.6	86.7	57.4
Cecil County	21 mi N	109.0 ± 1.0	128.0	93.2	64.0 ± 1.0	92.4	35.5
Jones Farm	3 mi NW	143.0 ± 1.5	212.0	95.1	229.0 ± 2.7	229.0	229.0
Tower Hill Farm	9 mi NW	$119.0 \pm 2.6$	161.0	80.0	$50.2 \pm 1.2$	51.8	48.6
Shawsville	21 mi NW	112.8 ± 1.2	140.0	88.3	$134.0 \pm 2.5$	166.0	102.0
Rumsey Island	3 mi SW	$107.7 \pm 1.5$	137.0	74.1	51.5 ± 1.8	60.5	44.4
Silver Lake Drive	9 mi SW	102.1 ± 1.0	125.0	76.3	124.7 ± 1.3	170.0	79.3
Clb. Arboretum	21 mi SW	161.0 ± 2.8	215.0	115.0	93.3 ± 1.5	134.0	52.6

# New and Improved Methods for Biomonitoring, 1999 Annual Report, Working Copy Table 5.5.3 Directional Summary of Trace Elements in Forager Bees and Pollen in Boundary Study Hives. Fall 1999. Values are means in ppm by weight $\pm RSD$ (%). n=4.

Element		Live Bees			Pollen	
	Transect NNE	Transect NW	Transect WSW	Transect NNE	Transect NW	Transect WSW
As	0.6 ± 25.7	0.4 ± 10.8	0.4 ± 10.0	0.4 ± 19.3	0.3 ± 8.4	0.3 ± 5.1
Ba	3.4 ± 5.1	3.1 ± 2.3	$2.7 \pm 2.0$	3.3 ± 2.8	$2.5 \pm 1.4$	$2.7 \pm 2.0$
Be	BDL	BDL	BDL	BDL	BDL	BDL
Bi	BDL	BDL	BDL	BDL	BDL	BDL
Cd	0.4 ± 24.0	0.6 ± 9.3	0.7 ± 9.9	0.4 ± 13.9	0.3 ± 3.2	0.3 ± 2.6
Со	0.6 ± 19.2	0.6 ± 7.4	0.8 ± 6.6	$0.2 \pm 32.2$	0.5 ± 1.8	$0.1 \pm 2.7$
Cr	0.4 ± 29.6	0.3 ± 12.0	0.4 ± 9.5	$0.4 \pm 21.2$	0.5 ± 3.7	0.4 ± 6.0
Cs	BDL	BDL	BDL	BDL	BDL	BDL
Cu	18.5 ± 1.9	19.6 ± 1.6	$20.2 \pm 1.8$	$10.0 \pm 2.2$	$10.5 \pm 2.3$	$10.7 \pm 1.6$
Ga	0.2 ± 16.4	0.1 ± 5.3	0.1 ± 4.1	0.2 ± 10.6	$0.1 \pm 2.3$	0.1 ± 1.9
Mn	84.1 ± 1.6	109.8 ± 2.2	119.9 ± 2.1	$21.1 \pm 2.3$	$23.6 \pm 2.5$	24.1 ± 1.7
Ni	$0.9 \pm 14.4$	0.9 ± 5.2	1.5 ± 5.3	$1.6 \pm 6.5$	1.7 ± 2.2	$1.5 \pm 2.1$
Pb	3.1 ± 5.0	3.5 ± 2.7	4.9 ± 2.7	4.5 ± 3.2	$6.5 \pm 2.2$	5.0 ± 2,1
Rb	8.8 ± 1.3	6.9 ± 1.3	7.4 ± 1.6	10.1 ± 1.5	$10.0 \pm 1.5$	8.5 ± 1.5
Se	0.6 ± 34.8	0.5 ± 23.0	0.4 ± 16.6	0.3 ± 58.6	0.2 ± 41.8	$0.2 \pm 24.6$
Sr	2.0 ± 8.3	1.9 ± 2.6	2.1 ± 2.6	1.9 ± 5.2	$1.9 \pm 1.4$	$2.0 \pm 1.4$
Ti	0.9 ± 31.3	0.03 ± 23.8	BDL	BDL	BDL	BDL
U	BDL	BDL	BDL	BDL	BDL	BDL
v	0.6 ± 20.4	0.6 ± 8.4	0.5 ± 5.8	$0.7 \pm 12.8$	0.6 ± 2.4	$0.5 \pm 2.5$
Zn	$103.3 \pm 2.2$	124.9 ± 1.8	123.6 ± 1.8	97.7 ± 3.7	137.7 ± 2.1	89.8 ± 1.5

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#### New and Improved Methods for Biomonitoring, 1999 Annual Report, Working Copy Table 5.5.4

# Radial Summary of Trace Elements in Forager Bees in Boundary Study Hives. Fall 1999 Values are means in ppm by weight ± RSD (%). n= 4

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Element	Live Bees						
	0 miles	3 miles	9 miles	21 miles	J-Field		
As	0.2 ± 6.5	0.4 ±11.9	0.5 ±21.2	0.4 ±10.2	0.3 ±12.8		
Ba	2.5 ± 1.2	3.2 ± 2.4	3.0 ± 5.1	3.0 ±1.8	5.0 ±6.4		
Be	BDL	BDL	BDL	BDL	BDL		
Bi	BDL	BDL	BDL	BDL	BDL		
Cd	$0.3 \pm 3.8$	0.7 ± 7.9	$0.4 \pm 24.1$	0.6 ±11.1	0.7±10.8		
Со	0.6 ± 2.3	0.4 ±12.7	0.8±13.7	0.9 ±6.8	0.6 ±9.0		
Cr	0.2 ± 4.7	0.4 ±16.8	0.4 ±23.4	0.3 ±10.8	0.2 ±10.3		
Cs	BDL	BDL	BDL	BDL	BDL		
Cu	16.7 ±2.2	18.3 ±1.8	19.4 ±1.9	$20.7\pm\!\!1.7$	19.0 ±6.4		
Ga	$0.1 \pm 0.7$		0.1 ±15.4	0.2 ±6.2	0.3 ±5.0		
Mn	133.2 ± 1.9	117.1 ± 2.0	109.6 ± 1.9	<b>87</b> .1 ±2.1	346.6 ±6.5		
Ni	1.2 ± 2.9	0.7 ± 9.1	1.1 ±10.8	$1.4 \pm 5.0$	1.2 ±7.3		
Pb	4.4 ± 1.4	3.3 ± 2.4	4.0 ± 5.2	4.3 ±2.7	7.2 ±5.9		
Rb	16.7 ±1.7	6.6 ± 1.1	9.8 ± 1.5	6.6 ± 1.6	7.5 ± 6.1		
Se	0.6 ±23.6	0.4 ±27.3	0.7 ±27.5	0.4 ±19.6	0.4 ±19.0		
Sr	1.7 ± 1.3	2.1 ± 2.8	1.7 ± 8.0	$2.2 \pm 2.6$	4.3 ± 6.5		
Ti	BDL	0.8 ±10.4	0.1 ±76.2	0.1 ±13.7	0.5 ±9.4		
U	BDL	BDL	BDL	BDL	BDL		
V	0.5 ± 2.1	0.5 ±11.3	0.7±16.0	0.4 ± 7.3	BDL		
Zn	99.4 ± 1.7	111.9 ± 2.1	$112.3 \pm 1.9$	127.6 ± 1.7	87.6 ± 5.9		

#### New and Improved Methods for Biomonitoring, 1999 Annual Report, Working Copy Table 5.5.5

Radial Summary of Trace Elements in Pollen in Boundary Study Hives, Fall 1999 Values are means in ppm by weight ± RSD (%). n= 2

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Element	Pollen					
	0 miles	3 miles	9 miles	21 miles	J-Field	
As	0.3 ± 6.7	0.4 ±17.6	0.3 ± 9.6	0.3 ± 5.7	0.2 ± 14.1	
Ba	1.9 ± 1.4	3.1 ± 1.9	$2.5 \pm 2.7$	2.9 ± 1.5	<b>8.8</b> ± 1.8	
Be	BDL	BDL	BDL	BDL	BDL	
Bi	BDL	BDL	BDL	BDL	BDL	
Cd	$0.2 \pm 3.7$	0.6 ± 5.2	0.2 ±12.3	$0.2 \pm 3.2$	$0.2 \pm 4.6$	
Co	0.1 ± 1.0	0.1 ±20.9	0.5 ±14.2	0.1 ± 1.6	0.3 ± 3.2	
Cr	0.3 ± 6.8	0.4 ±17.5	0.4 ± 9.6	0.4 ± 3.8	0.3 ± 5.1	
Cs	BDL	BDL	BDL	BDL	BDL	
Cu	8.2 ± 2.3	10.4 ±2.1	9.4 ± 1.6	11.4 ±2.5	13.6 ± 22.1	
Ga	$0.1 \pm 1.2$	$0.2 \pm 3.7$	0.1 ± 6.9	$0.2 \pm 4.2$	$0.5 \pm 2.7$	
Mn	12.7 ± 2.1	31.2 ±2.3	$16.6 \pm 2.1$	20.9 ±2.0	$160.3 \pm 3.0$	
Ni	1.6 ± 1.7	1.6 ± 5.3	1.9 ± 3.8	1.3 ± 1.5	2.2 ± 3.0	
Pb	6.4 ± 1.4	7.0 ± 2.5	3.9 ± 2.8	5.1 ± 2.2	11.8 ± 1.5	
Rb	8.2 ± 0.9	9.5 ± 1.7	9.5 ± 1.3	9.5 ± 1.6	13.0 ± 1.7	
Se	0.3 ±25.2	0.2 ±45.3	0.3 ±43.3	0.2 ±36.4	$0.2 \pm 23.7$	
Sr	1.8 ± 1.1	$2.2 \pm 3.5$	1.8 ± 3.4	1.8 ± 1.1	1.8 ± 1.1	
Ti	BDL	BDL	BDL	BDL	BDL	
U	BDL	BDL	BDL	BDL	BDL	
V	0.5 ± 3.3	0.6 ±10.9	0.7 ± 5.0	0.6 ± 1.8	$0.7 \pm 4.0$	
Zn	222.5 ± 1.8	145.9 ± 2.8	82.3 ± 1.4	97.1 ± 1.7	72.6 ± 2.9	

#### SECTION 6.0 REPORTING

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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 USACEHR. 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 USACEHR.

#### SECTION 7.0 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 follow definitions developed by EPA's Risk Assessment Forum, a committee of EPA scientists.

Acute exposure. Short-term exposure to an organic or inorganic chemical of concern.

Acute toxicity. Sudden, moderate to severe bee kill.

**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.

Chronic Exposure. Long-term exposure to an organic or inorganic chemical of concern.

Chronic Toxicity. Low level, often long-term die off of bees.

**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

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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.

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**Ecosystem Exposure.** Exposure of an ecosystem to an organic or inorganic chemical or exposure of animals and plants in an ecosystem to chemicals impacting the ecosystem

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.

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**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.

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**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.

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