# Trapping TseTse

## Assessing the Use of Host Urine Baits for Sampling and Controlling Tsetse

Joe-Ann Moser

World Food Prize Foundation

Borlaug-Ruan Intern 2009

African Insect Science for Food and Health

Mbita Point Field Station, Kenya

June 5-August 4

# Table of Contents

# Acknowledgements

First and foremost, I would like to extend my sincerest gratitude to **Dr. Norman Borlaug**, **Mr. John Ruan**, **Ambassador Kenneth Quinn**, and the **World Food Prize Foundation** for the amazing opportunity that they bestowed upon me. Their establishment and continued support of the Borlaug-Ruan International Internship Program made possible my unforgettable experience in Kenya. I would also like to thank **Lisa Fleming**, Director of Youth and Education Programs of the World Food Prize Foundation, not only for her tireless efforts in the coordination of my internship, but also for her unwavering support as I embarked on my journey around the world.

I am extremely grateful to **Professor Christian Borgemeister**, Director General of Icipe, and **Dr. Baldwyn Torto**, Head of the Behavioral and Chemical Ecology Department, for graciously permitting me to research at the center for eight weeks this summer. I would sincerely like to thank my supervisor, **Dr. Maurice Omolo** not only for his constant guidance regarding my project, but also for his willingness in assisting in my experience of Kenya's many diverse cultural aspects as well.

I would like to thank **Mr. Onesmus Wanyama**, Head Research Assistant of the Behavioral and Chemical Ecology Department (BCED) at Icipe's headquarters in Nairobi, and **Mr. Edwin Rono** for teaching me the laboratory techniques I needed in order to carry out my analysis of the organic compounds extracted from goat urine samples. Thank you to **Mr. Jeremiah Opiyo** and **Mr. Basilio Njiru** at the Icipe Mbita Field Station for regularly assisting me in placing and collecting my tsetse traps and ensuring that the experimental setup ran smoothly throughout the entire duration of my work in the field, and to **Mr. William Owigo** for operating the boat that brought me to and from the research sites each and every day. My gratitude goes out to **Mr. Stanley Maramba Opiyo**, also of Mbita, for his assistance and never-ending patience as I labored through the daily task of counting and recording caught flies.

Thank you to **Carolyne Akal**, Executive Assistant to Professor Borgemeister, for her coordination of my internship, especially my travel arrangements and cultural excursions, while in the country. My sincerest appreciation goes to **Mary Etuku**, Mbita Guest Center Manager, and the rest of the staff for making my two month stay a most comfortable and enjoyable one. On many more than one occasion, they went out of their way to cater their services around my often bizarre work schedule.

From the bottom of my heart, I would like to thank **Mr. Simon Rohde** of Millard North High School for not only serving as my Twentieth Century World History teacher and faculty mentor for the World Food Prize Youth Institute, but also for all of the guidance and support he has offered to me in the pursuit of my ambitions. It is teachers like Mr. Rohde that make it possible for students like me to turn their most far-fetched visions into reality. Thank you.

Lastly, I would like to thank my parents, **Joerg** and **Ursula Moser**, and my sister, **Elena**, for their constant love and encouragement. Their unconditional support of my aspirations has allowed me to dream big from a very young age.

# Introduction

## Karibu

Meaning welcome, this first Swahili word that I learned upon my arrival at the Kenyatta International Airport in Nairobi, Kenya was, without a doubt, also the most important. I immediately knew that I had made the right decision in asking the World Food Prize to send me to Africa for two months during the summer. The ecstatic cries of "Karibu" did not end one day or even one week after I had arrived. They continued throughout my stay, a constant reminder that the hospitality did not simply end, but instead was a concept that had been ingrained into the lifestyle, fortified by the ever-present helping hands and smiling faces.

While I have lived in countless cities in quite a few countries-Germany, Taiwan, the United States, and Puerto Rico-during my eighteen years, my life has always been sheltered from the reality that is hunger and poverty, which are so rampant in many parts of the world. I have been fortunate enough to never know the lack of a necessity such as food, water, or easy access to reliable medical attention. Perhaps for this very reason, I had made it my mission to see first-hand the stories on television and in the newspapers that had ignited my curiosity, to witness the reality beyond the stereotypes and biases presented to those looking in from the outside.

Until the end of my junior year, I had only ever been able to read books, newspaper articles, and editorials about the most prevalent issues that the world currently faces. That is exactly what I did in the comfort of my own home, hungry for any sort of knowledge that would allow me to connect to people and places thousands of miles away. But at the same time, I knew without a doubt that the information I was receiving was not an accurate account of what was really happening. Yet, I was baffled how to solve this problem. And then I heard about the World Food Prize Youth Institute and the opportunities it provides for young students, who like me, are unsatisfied with not knowing the truth. I marveled at how I had been living in Omaha, which is so near to Des Moines, for five years without ever hearing about this wonderful opportunity, thankful that I had discovered it in time to participate the very last year that I would have been eligible.

I wasted no time in researching and writing the paper that would gain me my acceptance into the Youth Institute, an investigation into water scarcity and its negative role in food security in the Middle East. But even this research could not adequately prepare me for the humbling experience that was the 2008 Borlaug Dialogue. I sat in awe of each and every one of the speakers, men and women who had seen first-hand, who had dedicated their lives to this mission that they felt so passionately about. Even before the third day, when the past years Borlaug-Ruan Interns gave their presentations, I had no doubt in my mind whatsoever that I would be applying for an internship. The fascinating stories simply reassured my belief, and by the time I was heading back home, I had already dared to imagine myself winning one of the handful of coveted places.

I need not have worried because after weeks of anxious waiting, I was selected although my exact placement was still undetermined. My joy was surpassed a few days later when I received word that I would be spending my two months at the African Insect Science for Food and Health's field station in Mbita, Kenya. Getting to work on African sleeping sickness was a dream come true because it meshes impeccably with my plans for the future, which as of now include becoming a doctor specializing in infectious diseases working overseas for a non-profit organization such as Doctors Without Borders. Without a doubt, the scientific insight that I have gained in the last eight weeks will be invaluable as I prepare to attain this goal. Apart from the exposure to field work, something that caught me completely by surprise with its rigor and intensity, and unfamiliar laboratory techniques, I gained something much more valuable, insight into a slew of problems that will be truly invaluable in my attempt to be a part of the solution.

## Icipe-African Insect Science for Food and Health

African Insect Science for Food and Health, or Icipe, was founded in 1970 as the International Center for Insect Physiology and Ecology by the late Dr. Thomas R. Odhiambo, a world-renowned Kenyan entomologist who championed the creation of African institutes to solve the continent's most pressing scientific problems. This establishment was in direct response to "the need for alternative and environmentally-friendly pest and vector management strategies" (Building). As a research institution whose mission is to "help alleviate poverty, ensure food security and improve the overall health status of peoples of the tropics by developing and extending management tools and strategies for harmful and useful arthropods, while preserving the natural resource base through research and capacity building", Icipe is the only international institute working primarily on arthropods.

To achieve its mission, in 2003, Icipe adopted the 4Hs paradigm, which categorizes its research into four distinct divisions: environmental, human, plant, and animal health. This holistic and integrated approach is well-suited to improving the overall health of the community because it addresses interlinked problems. The other major component in this initiative is the Capacity Building Program, which includes the education of African nationals for leadership roles in insect science as well as the education of resource-limited communities in pest and vector management strategies.

### Mbita Point Field Station

In addition to its main campus in Nairobi, Icipe maintains five field sites in Kenya, one in Sudan, and a Biovillage Initiative in Ethiopia in order to validate research output with farmers. Located on the banks of Lake Victoria, the Thomas Odhiambo Mbita Point Field Station (MPFS) was established in 1980. It is Icipe's largest field site with 37 self contained rooms on slightly more than 60 acres of land (Meeting). Suba district, home to MPFS, with a population of 155,666 according to the last provincial census conducted in 1999, is also one of Kenya's most fiscally disadvantaged districts. The HIV/AIDS prevalence rate in this western Kenya district is 30% as

opposed to the national average of only 6.7% (One). Due to the station's influence, living conditions in the area have improved drastically. Current sectors of research include the malaria vector, crop and horticultural pests, and tick and tsetse fly prevention.

## Human African Trypanosomiasis and Nagana

African sleeping sickness, also known as Human African Trypanosomiasis (HAT) or Nagana, when affecting cattle and other livestock, is a parasitic disease that is one hundred percent fatal without treatment. After an epidemic swept through Africa in 1920, between 1931 and 1961, the annual number of recorded HAT cases was reduced by greater than 90% from more than 60,000 reported cases a year to less than 5,000 reported cases a year (Simarro *et al.* 2008). This was done through the systematic screening and treatment of millions of individuals across sub-Saharan Africa. Due in part to these promising results and the independence of many of these nations from their colonial rulers in the 1960s, efforts to monitor and contain the disease were reduced. A subsequent resurgence was seen across the continent and by the late 1990s, more than 30,000 cases were being reported every year (Omolo *et al.* 2009). According to the World Health Organization (WHO), major outbreaks have been observed in villages in the Democratic Republic of Congo, Angola, and southern Sudan as late as 2005 (see Figure 1). Prevalence in some of these areas has reached 50%, making African sleeping sickness the first or second leading cause of mortality, even more than malaria and HIV/AIDS, in those communities (African). In 2007, after a renewed campaign by the WHO, the number of new cases reported was only 10,769. This number does not, however, accurately reflect the actual number of people suffering with the disease, which was estimated to be between 50,000 and 70,000 in 2006 (Disease).

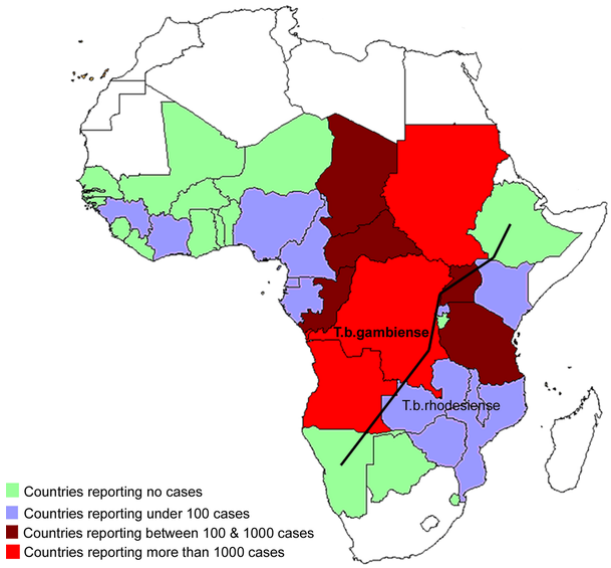



**Figure 1: Map of Africa Showing the Epidemiological Status of Countries Considered Endemic for the Disease**

Sleeping sickness is not only detrimental to human health, but the infection also substantially minimizes labor resources. And when over two-thirds of the continent's population consists of small-scale farmers, the inability to work also means the inability to provide for one's family. Perhaps even worse, many of these farmers depend on their livestock for survival on a daily basis. As a result, sleeping sickness in livestock, or Nagana, is a particular detriment to the livelihood of these people. It is the main reason behind the absence of draught power, which contributes to eighty percent of the continent's land still being tilled by hand. This not only puts undue stress on the farmers, but an absence of cattle means less manure is also available to act as organic fertilizer. These problems lead to lower yields of crops and fodder plants. Meat and milk, essential components of a healthy, well-balanced diet, are also lacking. Per year, the three million deaths in cattle are estimated to incur monetary losses of 0.6 to 1.2 billion US dollars (Healthy).

Researcher Pere Simarro states it best when he says: "Sleeping sickness, coupled with Nagana, the animal form of African trypanosomiasis, has been a major obstacle to sub-Saharan African rural development and a stumbling block to agricultural production…both human and animal trypanosomiasis are implicated in the underdevelopment of the African continent, and are considered a major obstacle in the establishment of a flourishing agriculture to provide food security and to lead to sustainable economic growth and healthy populations" (2008).

## Impact of Tsetse Fly Research

The protozoa (species *Trypanosoma brucei*) that cause trypanosomiasis are delivered to the host through a vector, the tsetse fly (genus *Glossina*), in much the same way that malaria is transmitted through mosquitoes. The control of the vector population has always been the most significant strategy in stopping the spread of the disease. Prior to the discovery of insecticides, the clearing of vegetation and shooting of wild animals were utilized in the maintenance of the fly population. After the discovery of these organic compounds, massive spraying campaigns were begun in a number of countries. According to Jordan, "many of these operations were successful, and, because of highly selective application of insecticide, caused little contamination of the environment" (1995). This method was eventually replaced with a much more cost-effective one, trapping.

Currently, one of the most effective means of tsetse population control, the "push-pull" philosophy, employs repelling collars on livestock to "push" the flies away and attracting odors to "pull" them into biconical traps, which have been improved over the years for maximum catch (see Figure 2). They are predominantly blue in color because tests have concluded that this shade attracts tsetse from long distances. The inside, however, is composed of black cloth for luring the flies into the hole from a short range.

**Figure 2: Biconical Trap**

Of the three species of tsetse fly, the riverine (sub-genus *Palpalis*) is especially important. It is estimated that nine out of ten cases of HAT start with a bite from a subspecies of *Glossina fuscipes*, a member of the *Palpalis* group (Omolo *et al.* 2009). Unfortunately, the use of artificial baits has provided meager results in the riverine species, proving to be much more effective in attracting the *Morsitans*, or savannah, species of tsetse. The baits are unproductive because no artificial attractants are known; traps must therefore be deployed at densities of 30-40 $km^{-2}$ to have a significant impact, making the method prohibitively expensive (Green 1994).

This project aims to decrease the *Glossina fuscipes fuscipes* population, and therefore lower the incidence of HAT, by attempting to identify attractants that will help to increase the success rate of traps.

# Assessment of the Usefulness of Host Urine Samples as Odor Baits in the Control of the *Glossina fuscipes fuscipes* Population

## Materials and Methodology

The study was conducted between June 16 and July 4, 2009 in two different habitats-Kirindo, a strip of land along the Lake Victoria shore, Mbita Division, Homa Bay district, Western Kenya, and small Chamaunga, an island approximately 3 km from the Icipe Thomas R. Odhiambo Field Station at Mbita. Mwangelwa *et al.* has described in detail the climate and vegetation of the area (1990). The weather is generally sunny all year round with little precipitation and no distinct seasons. Unlike Kirindo, Chamaunga is mostly uninhabited, apart from the intermittent visits by local fishermen and entomologists. In both habitats, the vegetation has been degraded by human activity. Apart from monitor lizards, humans and domestic livestock such as goats and cattle are the main *G. f. fuscipes* hosts in the vicinity; wild mammalian hosts have for the most part been driven away by the aforementioned destruction of habitat.

Seven different sites-three in Kirindo and four on Chamaunga-located near the water's edge were selected for the present study. In Kirindo, a single biconical trap was set at each site. One trap each was baited with goat urine (0.0057g/min) and monitor lizard (*Varanus niloticus*) urine (0.0055g/min) while the third was left unbaited as a control.

*NOTE: Refer to Appendix A for a map and GPS coordinates of the sites from both habitats utilized for this experiment.*

Upon collection, all urine samples were allowed to ferment for forty-eight hours before being placed in the refrigerator and stored at approximately 4°C for a period of time exceeding one year. The samples were then removed from the refrigerator on the first day of the experiment, June 16th, and stored at outside air temperature subsequently throughout the duration of the trial. Each urine sample was placed in a uniform small plastic bottle ($V \sim 135 cm^3$) and attached to the base of the trap approximately 20 cm from ground level.

Randomized Latin square designs incorporating effects of treatments, sites and day were used. The three treatments (control, goat, and lizard) were alternated daily between the three sites for nine nearly consecutive days.

This setup was then repeated on the island of small Chamaunga with a few minor alterations. To ensure that no contamination of odors was taking place, the distance between sites was increased from an average of 43.7 m in Kirindo to 58.4 m in Chamaunga. A fourth treatment, ox urine (0.0062g/min) was added as another means of control; tsetse response to ox urine has been well documented, most notably in Hall *et al.* 1984 and Den Otter 1991. New samples, prepared and handled in the same manner as mentioned above, for each host were utilized and the four treatments (control, goat, lizard, and ox) were alternated daily between the four sites for eight

nearly consecutive days. In order to eliminate sample height on the base as a variable, a piece of masking tape was placed around the base of each trap and each day, the treatment was fixed so that the upper edge of the container was in line with the bottom edge of the tape (see Figure 3).

**Figure 3: Urine Sample and Tape Wound**

**Around the Base of the Biconical Trap**

*NOTE: Refer to Appendix B for the randomized Latin square designs for both trials of Experiment I-Kirindo and Chamaunga.*

As in the first run, the direction each sample was facing on the trap was not maintained on a consistent basis. However, the effect of this is estimated to be minimal as the wind direction in both locations is erratic and highly unpredictable.

Each day, the traps were placed for approximately 210 minutes (900-1230h) and the flies collected, sexed, and counted. The number of stomoxys (*Stomoxys calcitrans*) and tabanids (genus *Tabanus*), other biting flies present in the ecosystem, were recorded in addition to the general weather conditions of the day. Ambient temperature, humidity, and light intensity were not measured.

*NOTE: Refer to Appendix C for the Excel spreadsheet used in the recording of daily data in both habitats.*

**Results**

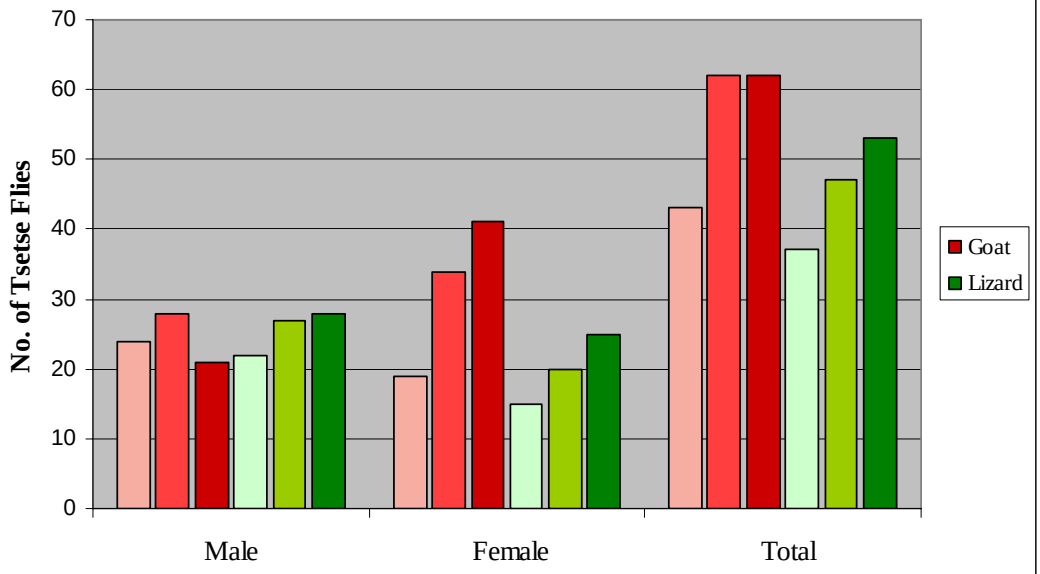
Fly catches were transformed to the logarithmic scale ($\log_{10}$) and analyzed using GenStat computer software (see Table 1).

| Table 1. Log Transformed Mean Catch of Tsetse Caught with Urine Baited Traps | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Kirindo** | | | | **Chamaunga** | | | |
| **Treatment** | **Male** | **Female** | **Total** | **Treatment** | **Male** | **Female** | **Total** |
| Control | 0.889 | 0.873 | 1.174 | Control | 0.747 | 0.880 | 1.101 |
| Goat | 0.945 | 1.028 | 1.276 | Goat | 0.945 | 1.028 | 1.099 |
| Lizard | 0.933 | 0.857 | 1.195 | Lizard | 0.933 | 0.857 | 1.133 |
| | | | | Ox | 0.829 | 0.871 | 1.129 |
| s.e.d | 0.0801 | 0.0806 | 0.0548 | s.e.d | 0.0843 | 0.0823 | 0.0707 |
| s.e.d, standard error difference; *transformed means* not significant | | | | | | | |

Due to the general attractive nature of the monitor lizard, as determined by previous experiments, it was expected that the urine sample of this animal, *Glos f. fuscipes*' primary source of a blood meal, would also procure the most flies in total. This, however, was not the case as the goat sample succeeded in acquiring nineteen more flies than the lizard, 266 as opposed to 247 tsetse flies in total. Surprising was the number of females caught using goat urine, which was 1.36 times that of the control, and the total number of tsetse using the same comparison, which yielded a number of 1.25 times.

Although these numbers are not considered a significant indication of attraction of flies to goat urine, a specific trend is established when block progression is taken into account. A general upward trend was observed in the number of flies caught using both lizard and goat urine at the Kirindo site (see Figure 4). Block progression of the Chamaunga experiment was not as conclusive, due in part to variable weather affecting fly catches. In this instance, a third block would have been necessary in order to establish a pattern, but time constraints made this repetition unfeasible.

**Figure 4: Block Progression-Kirindo**




## Landing Response of *Glossina fuscipes fuscipes* on Electric Targets Incorporating Host Urine Samples as Olfactory Cues

### Materials and Methodology

This study was conducted between July 6 and July 14, 2009 in the same habitat used in the second phase of the first experiment, small Chamaunga; the same four sites were also utilized. At each site, a horizontally oriented 50cm by 1m electric target was flanked by a vertically oriented net of the same size (see Figure 5). Both are composed of a metal frame with copper wires spaced 0.8cm apart across the area on both sides. A piece of black mosquito netting was secured between the wires on the horizontal target only. Both the target and the flanking net were supplied with power from a standard car battery and spark box.

This setup was maintained parallel to the line of the shore in order to optimize the fly catch. The flank was uniformly placed closer to the lake's shore than the target in order to be able to more accurately measure the landing response of tsetse flies. Once again, one trap each was baited with goat, monitor lizard, and ox urine with the fourth site acting as a control. New samples, prepared and handled in the same manner as the previous experiment, were used for each host.

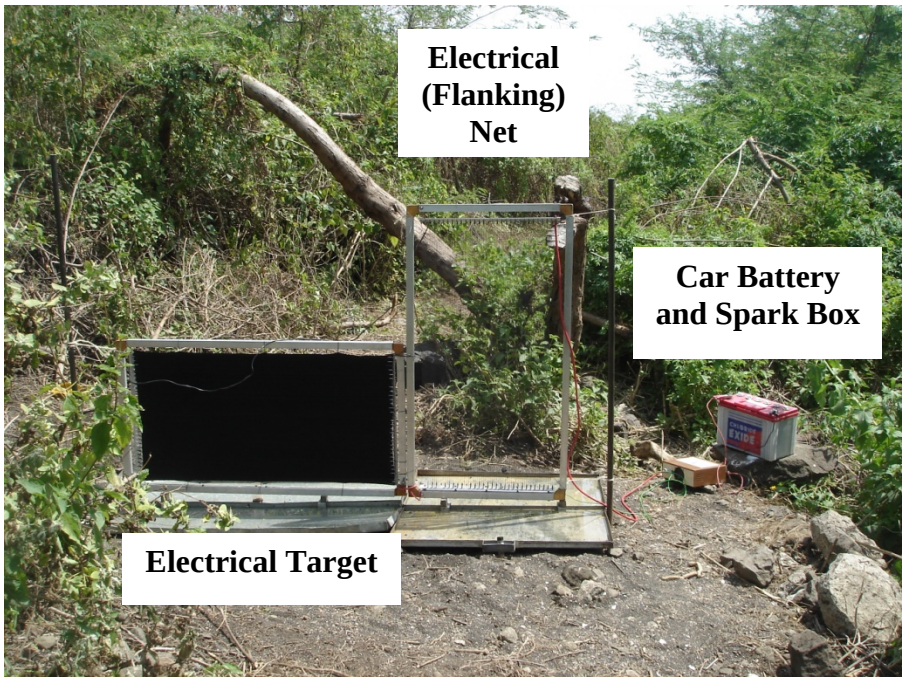



**Figure 5: E-Target/Net Setup**

Randomized Latin square designs were used where the four treatments (control, goat, lizard, and ox) were alternated daily between the four sites for eight nearly consecutive days.

*NOTE: Refer to Appendix D for the randomized Latin square designs for Experiment II.*

Following the conclusion of the experiment on Day 5 (July 10), the monitor lizard sample was diluted approximately ten times when it came in contact with water. In order to ensure that the reduced concentration did not affect the catch, every sample was replaced prior to the start of the experiment the next day.

Each day, the traps were placed for approximately 210 minutes (900-1230h) and the flies retained after electrocution as they fell on metal trays filled with water and placed beneath the traps. The gender, face (upwind or downwind) and type of killing device (target or flank) was recorded for each tsetse. The number of stomoxys and tabanids were also recorded in addition to the general weather conditions of the day. Ambient temperature, humidity, and light intensity were not recorded.

*NOTE: Refer to Appendix E for the Excel spreadsheet used in the recording of daily data in Experiment II.*

**Results**

The E-Target/Net method is more representative of the true behavior patterns of the flies. Because the flanking net is invisible to the tsetse, a comparison between the number of flies on

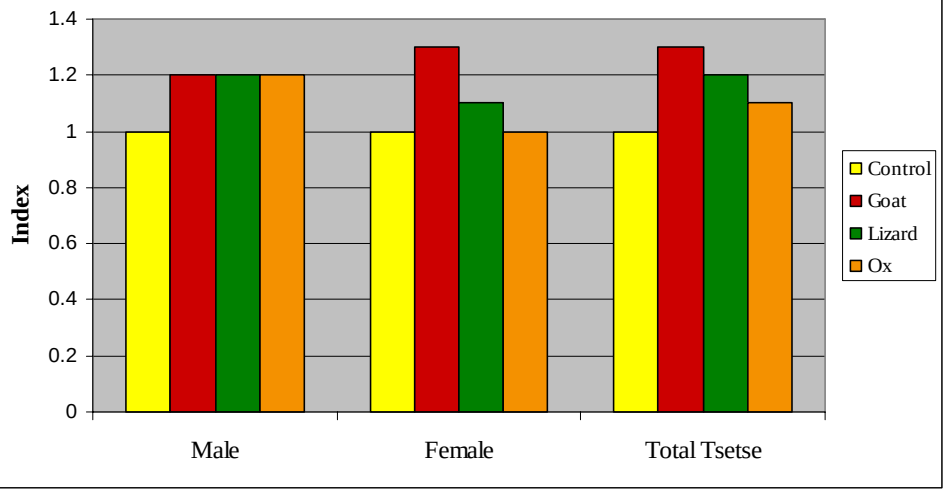
the target and those on the net establishes whether or not the host urine sample succeeded in eliciting a landing response or simply just attraction (see Table 2). If many more flies land on the net rather than on the target, an assumption can be made that the urine sample being tested is not effective. The tsetse flies simply mill about until they eventually unknowingly fall into the confines of the invisible net.

**Table 2: Tsetse Landing Response**

| Treatment | Male | Female | Total |
|---|---|---|---|
| Control | 58.3% | 35.3% | 43.8% |
| Goat | 47.2% | 36.6% | 40.8% |
| Lizard | 57.1% | 38.0% | 45.4% |
| Ox | 57.8% | 23.6% | 36.2% |

Fly catches were once again transformed to the logarithmic scale ($\log_{10}$) and analyzed using GenStat computer software. Catch indices were established by comparing each of the other treatments to the control (see Figure 6).

**Figure 6: Catch indices of Gff in E-target/net baited with host urine samples**




## Extraction and Analysis of Organic Compounds from Sample Goat Urine

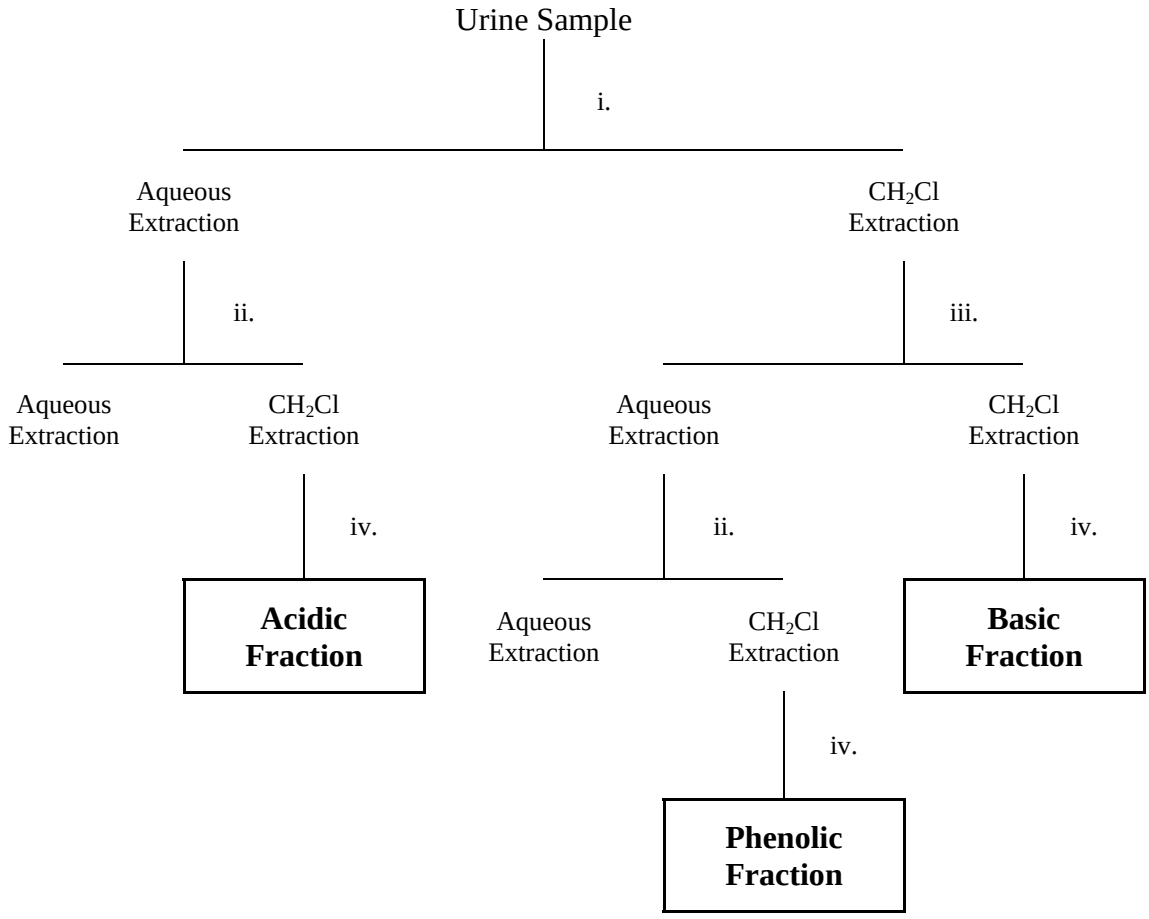
Given the block progression in the first experiment and the catch indices in the second experiment, one final experiment was designed to evaluate the organic components of goat urine, the treatment that showed the most promise in terms of being utilized as a tsetse fly attractant.

## Materials and Methodology

The last experiment was conducted between July 17 and July 24, 2009; extraction of the fractions from the sample took place in the Tsetse Laboratory at the Icipe Mbita Point Field Station. Gas Chromatograph (GC) and Gas Chromatograph coupled to Mass Spectrometer (GCMS) analytical instruments in the Behavioral and Chemical Ecology Lab at the Icipe campus in Nairobi were used to analyze organic extracts from the goat urine. 500mL of goat urine were partitioned to facilitate the extraction of organic compounds in three separate fractions (non-acidic, phenolic, and acidic). The general procedure from Bursell et al, 1988 was adopted with a few minor changes (see Figure 7).

Before beginning the actual extraction, it was necessary to prepare the 5N sulfuric acid and 2M Sodium hydroxide solutions that would be used. 40g of NaOH and 72.5 g of $H_2SO_4$ were each dissolved in 500mL of distilled water.

**Figure 7: Fraction Extraction Procedure**



i). Saturate 500 mL of urine sample with NaCl (approximately 170g when using goat urine) and extract three times with a total volume of 350 mL of $CH_2Cl$ (in increments of 150, 100, and 100 mL).

ii). Acidify with 5N $H_2SO_4$, saturate with NaCl, and extract three times with a total volume of 350 mL of $CH_2Cl$ (in increments of 150, 100, and 100 mL).

iii). Concentrate to 100 mL ,by allowing the solvent to evaporate in the fume hood, and extract three times with a total volume of 100 mL of 2M NaOH (in increments of 50, 25, and 25 mL).

iv). Rinse with distilled water to remove any remaining $H_2SO_4$ and NaOH then dry thoroughly with $MgSO_4$ to remove excess water.

During the extraction process, the organic layer of the basic and acidic fractions was removed using a syringe rinsed with Dichloromethane (DCM) solvent, due to the unavailability of a separating funnel, which was used in the extraction of the phenolic fraction. In between each of the three extractions, the sample was covered with aluminum foil in order to prevent the evaporation of the solvent, which is highly volatile. 5 mL of each fraction was placed into a vial with a Teflon-coated cap and labeled as the unconcentrated sample. The remaining volume was concentrated to approximately 10 mL, of which 5 mL was placed into another vial with a Teflon-coated cap and labeled as the concentrated sample. This was done in order to ensure that peaks would be present on the chromatograms once Gas Chromatography (GC) analysis would be run.

All beakers, graduated cylinders, and syringes were washed with a series of water, distilled water, acetone, and DCM in order to ensure minimum contamination of the samples.

Upon completion of the extraction of all three fractions, the samples were then refrigerated for 36 hours at 4°C before being transported by road to Nairobi and immediately upon arrival stored at -10°C.

40µL of each unconcentrated sample was loaded into a Hewlett Packard model 5790A model gas chromatograph (temperature program: 35°C to 270°C at 10°C/min) and a chromatogram was obtained. Dichloromethane (DCM) was also analyzed so that contaminants in this solvent could be excluded from the samples. During preparation of the samples, the syringe being used was rinsed with the solvent three times before each sample preparation.

In addition, 1µL of an internal standard, methyl salicylate, was added to the three fractions. The standard was chosen due to its greatly varying chemical structure. This ensured that its peak would be well separated from the rest of the chromatogram. Utilizing knowledge of the volume and concentration of the standard as well as the area of its curve on the graph, quantification of the organic compounds present in the urine sample were achieved.

This was done in order to ascertain the appropriate level of compounds in the concentration of sample used. Once the desired concentration was discovered, each of the samples was prepared again in the exact same manner and run with an Agilent model 7890A GCMS system (temperature program: 35°C to 280°C at 5°C/min). The chromatograms were analyzed using the 5795C Mass Spec Data Analysis computer program and mass spectral data of potential attractants and other compounds of interest were obtained.

**Results**

Several compounds of interest were discovered in the phenolic and acidic fractions, but not in the non-acidic fraction (see Figures 8 and 9).

*NOTE: Refer to Appendix F for the mass spectroscopies of the interesting compounds in found in the phenolic and acidic fractions. Refer to Appendix G for the chromatogram percent area reports.*

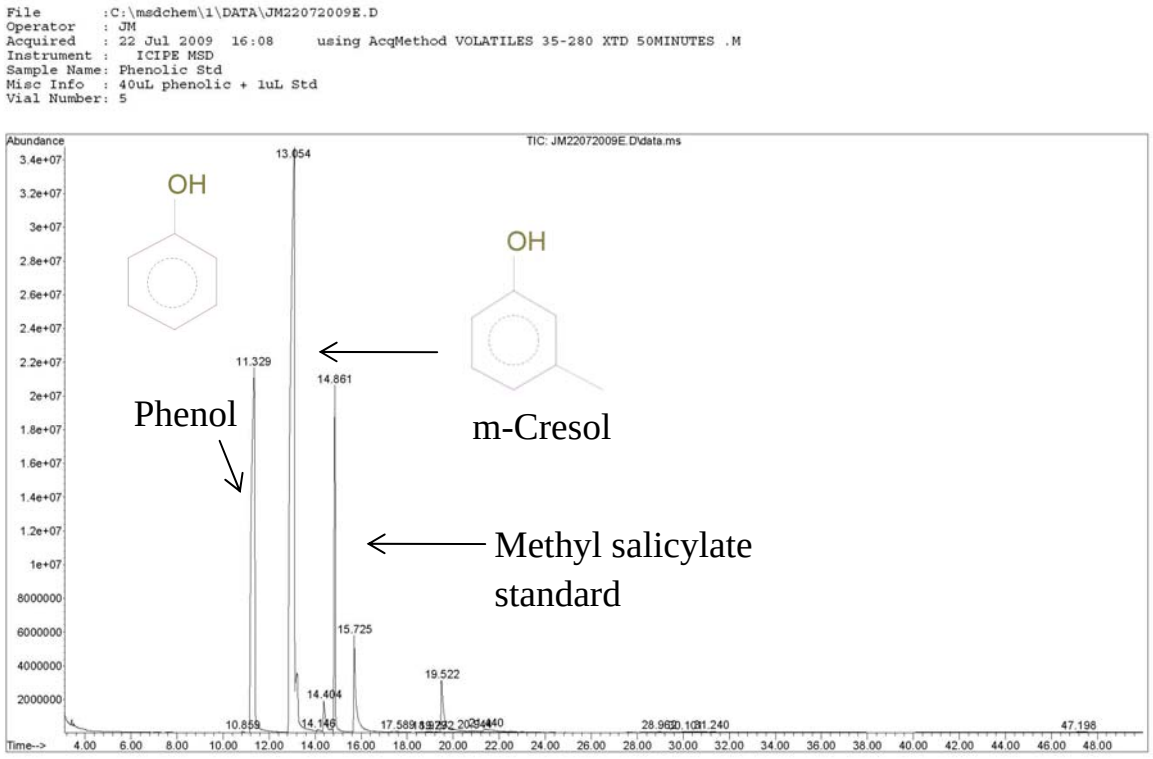



**Figure 8: Gas Chromatogram of Phenolic Fraction**

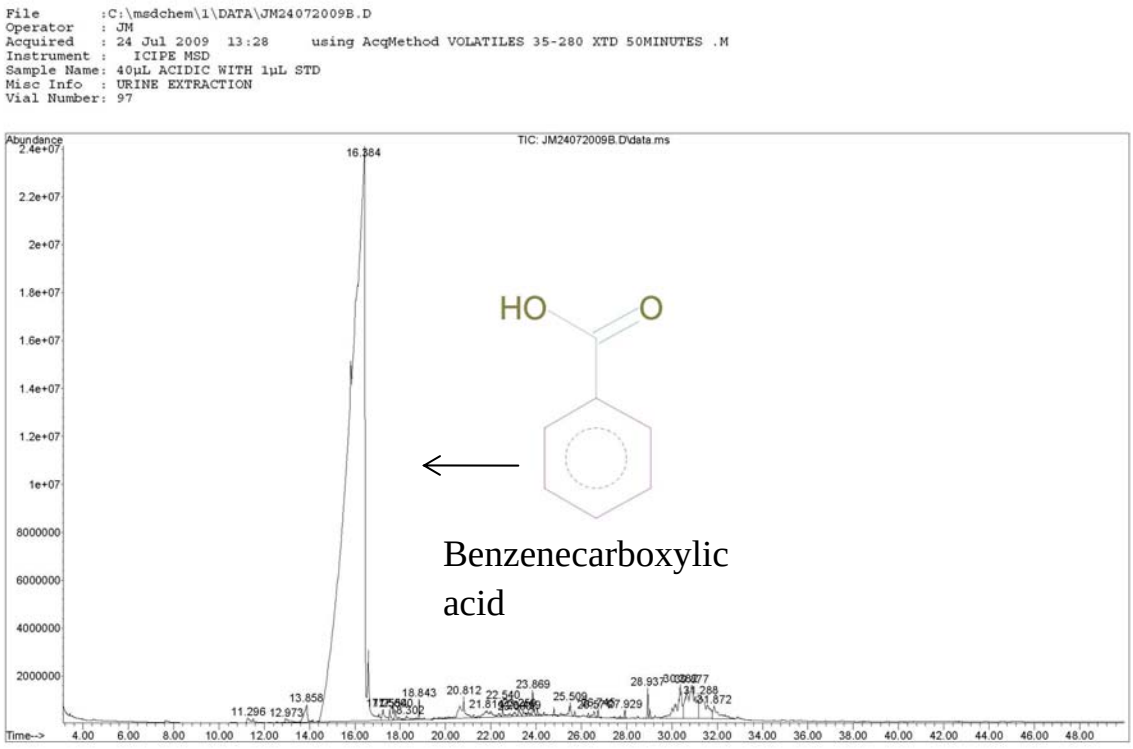



**Figure 9: Gas Chromatogram of Acidic Fraction**

Using the percent area and volume of the methyl salicylate standard, the volume of these organic compounds in the solution can be calculated. A continuation of this line of research would result in placing these compounds underneath a biconical trap and mimicking the first experiment. Therefore, the volume in urine sample becomes important in order to reproduce the experiment using naturally-occurring levels of organic compounds.

## Discussion and Recommendations

Even though the goat, monitor lizard and ox are *Glossina fuscipes fuscipes* hosts, their urine samples would not be effective in maintaining control of the fly population. Although the data was not statistically significant, the amount of flies caught using goat urine was elevated enough that this treatment could potentially be utilized for more accurate sampling of the fly population.

Further experimentation could be conducted into the organic compounds discovered in the last experiment. The phenolic nature of the first two coincides with prior research that tsetse flies are attracted to this class of substances when found in the urine of other animals.

Finally, an experiment that utilizes fermentation of the urine samples as an independent variable might lead to interesting results, based on the block progression data obtained from the first experiment.

# Conclusion

## Asante Sana

It didn't take me long at all to realize the vast differences between the "inside" and "outside" world. Within the gated Icipe campus, under the ever-watchful eyes of the hired security guards, I oftentimes forgot that I was even in Kenya. The paved roads, running water, electricity, and green foliage, a result of the only irrigation system for miles around, were not a huge departure from life back home. Only during my weekly laundry sessions-I hand washed my clothes due to the absence of machines-and the occasional blackout was I jolted back into reality. But inside, these moments were still extremely rare. The second I stepped outside those gates on my way to the research sites each morning, I felt as if I had set foot in a different world altogether. Now this was more like the stereotypical scene I had naively envisioned before setting out on my journey this summer, but not quite… True, I was experiencing one of the most destitute areas of Kenya. Most of the houses in this region are constructed of mud walls and metal sheet roofs; cattle in the middle of the road is a common occurrence and the nearest hospital is three hours away.

But before I came to Kenya, I thought that this is where it stopped. I mean what else could possibly exist in an area characterized by its high insect population and low annual rainfall? But I was wrong. Quite a bit lay hidden beneath the surface. Before I arrived, I was privy to the mindset that this experience needed to be "fun", that if I didn't experience the touristy side of Kenya, I would be missing out. And while some of my fondest memories come from being given the privilege to meet Mama Sarah, President Obama's paternal grandmother, or witnessing the majesty of giraffes at Maasai Mara, these snapshots in time are not what define my experience. Chatting about the state of the world while sipping afternoon tea or being invited into a colleague's home for dinner-those are the simple everyday happenings that made my time in Kenya truly rewarding. It was in this time that I developed a deep sense of respect for the mindset with which everything is approached in this East African country. Time seems to stand still because it carries little meaning, and while this was absolutely frustrating at first, I grew to appreciate everything else that much more as a result. Now that is what amazed me most-that people are able to be so completely content with what little they do have compared to all of the luxuries I have been used to my entire life. It was a humbling experience to say the least.

The most poignant moment throughout the sixty days came when I was walking back from the fields for lunch. I stopped by the school because I had heard noise coming from the clearing behind the main building; the students were putting on a performance for their teachers, administrators, and parents. I was immediately beckoned towards a chair, just in time to watch a young girl walk up to the microphone all by herself. She recited the following adapted version of *The Boy* by Tony Bradman:

*I am the child in the playground,*
*The child who stands by the wall,*

*The child that nobody likes much,*
*And some don't like at all.*
*I am the child with a problem,*
*The child at the back of the class,*
*The child who finds it hard to read,*
*And tests too hard to pass.*
*I am the child no one plays with,*
*The child that walks home alone,*
*The child that some wish didn't exist,*
*And who wishes his heart was stone.*
*I am the child with no future,*
*The child with a difficult past,*
*The child who ought to be first in the queue,*
*And somehow is always…. the last.*

My eyes literally started watering as she continued with her performance. Only someone who has had to bear such suffering would've been able to deliver the piece with any sort of convincingness. It is then perhaps the most troubling thing in the world to say that her performance was flawless.

Even though my life has now been consumed with studies and other such regular activities again, I will never forget all the priceless lessons that Kenya so selflessly offered to me. They will without a doubt be the most useful as I continue down the road towards my goal of making the world a better place to live in, for everyone. I highly doubt I will ever truly be able to express in words how much this means to be. Thank you. Or as one would say in Swahili, asante sana.

# References


4Hs Paradigm, The: Environmental, Human, Plant, and Animal Health. Icipe: African Insect Science for Food and Health.

African Trypanosomiasis (Sleeping Sickness). 26 June 2009. World Health Organization. Aug. 2006 <http://www.who.int/mediacentre/factsheets/fs259/en/ August 2006>.

Building Capacity: Through Research and Strategic Partnerships. Icipe: African Insect Science for Food and Health.

Bursell E, Gough AJE, Beevor PS, Cork A, Hall DR, Vale GA (1988). Identification of Components of Cattle Urine Attractive to Tsetse Flies, *Glossina* spp. (Diptera: Glossinidae). Bull Entomol Res 78: 281-291.

Den Otter CJ (1991). Olfactory Responses of Tsetse Flies to Phenols from Buffalo Urine. Phys Entomol 16: 401-410.

Disease, The: Human African Trypanosomiasis. 11 July 2009. World Health Organization. 2009 <http://www.who.int/trypanosomiasis_african/disease/en/index.html>.

Green CH (1994). Bait Methods for Tsetse Fly Control. Adv Parasitol 34: 229-291.

Hall DR, Beevor PS, Cork A, Brenda FN, Vale GA (1984). A Potent Olfactory Stimulant and Attractant for Tsetse Isolated from Cattle Odours. Insect Sci. Applic. 5: 335-339.

Healthy Livestock, Healthy People: Tackling Tsetse and Ticks in Africa. Icipe: African Insect Science for Food and Health.

Jordan AM. Control of Tsetse Flies (Diptera: Glossinidae) With the Aid of Attractants (1995). Journal of the American Mosquito Control Association. 11 (2): 249-255.

Meeting the Needs of a Changing World: Icipe's Vision and Strategy 2007-2012. Icipe Science Press

Mwangelwa MI, Dransfield RD, Oteino LH, Mbata KJ (1990). Distribution and Diel Activity Patterns of *Glossina fuscipes fuscipes* Newstead on Rusinga Island and Mainland in Mbita, Kenya. Insect Sci Appl 11: 315-321.

Simarro PP, Jannin J, Cattand P. (2008) Eliminating Human African Trypanosomiasis: Where do We Stand and What Comes Next. PLoS Med 5: e55. doi:10.1371/journal.pmed.0050055.

Omolo MO, Hassanali A, Mpiana S, Esterhuizen J, Lindh J, et al. (2009) Prospects for Devoloping Odour Baits to Control *Glossina fuscipes* spp., the Major Vector of Human African Trypanosomiasis. PLoS Negl Trop Dis 3(5): e435. doi:10.1371/journal.pntd.0000435.

One Coordinated HIV Prevention and Treatment Program for Suba District, Kenya. 3 July 2009. Family AIDS Care and Education Services-Kenya, AIDS Research Institute, University of California-San Francisco. 2009 <http://www.faces-kenya.org/publications/suba-poster.pdf>.

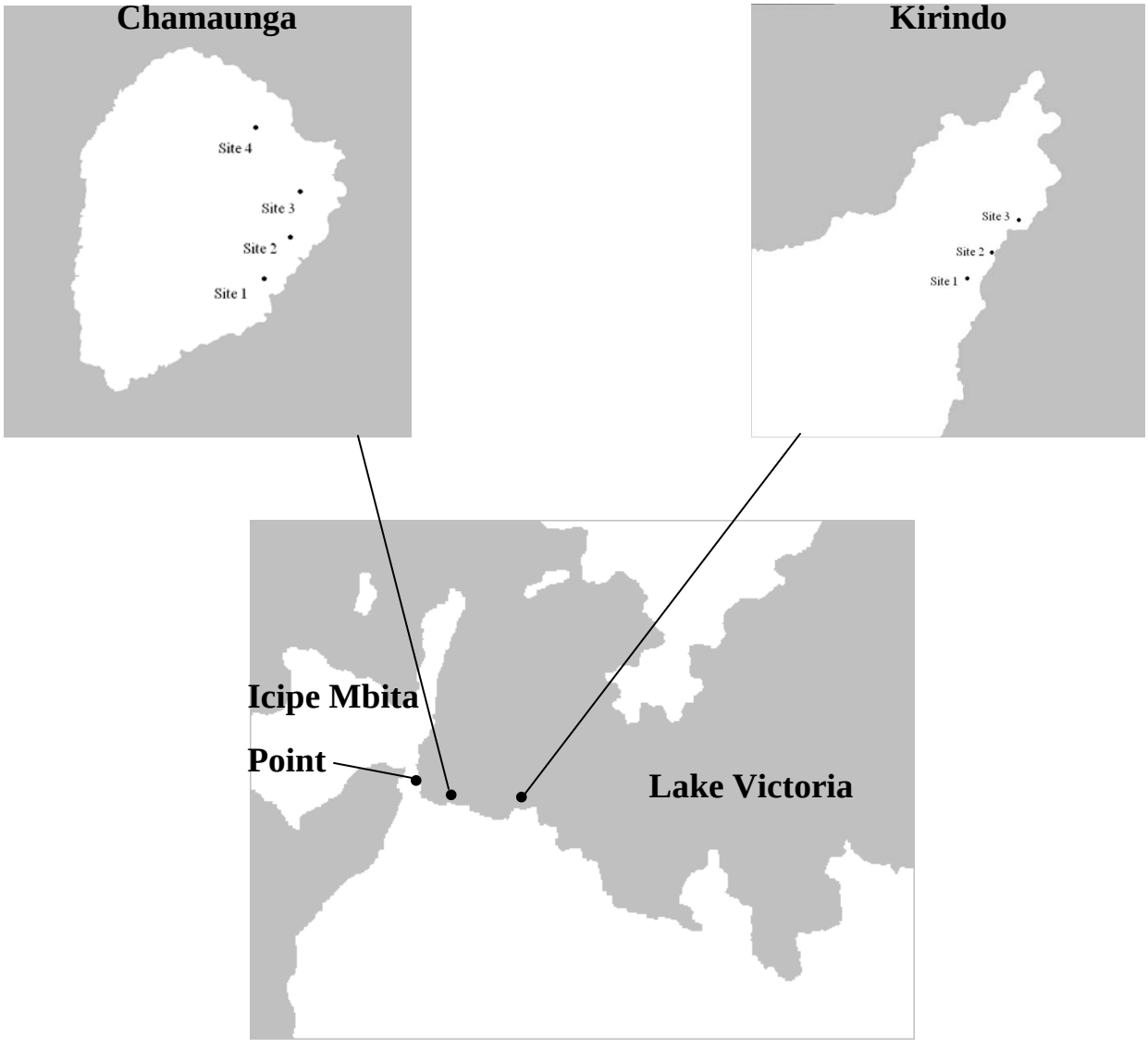
APPENDIX A: SITE MAP AND GPS COORDINATES

## Kirindo

| | | |
|---|---|---|
| Site 1 | S 00° 26.216' | E 34° 15.301' |
| Site 2 | S 00° 26.201' | E 34° 15.316' |
| Site 3 | S 00° 26.181' | E 34° 15.333' |

## Chamaunga

| | | |
|---|---|---|
| Site 1 | S 00° 25.918' | E 34° 13.673' |
| Site 2 | S 00° 25.895' | E 34° 13.687' |
| Site 3 | S 00° 25.870' | E 34° 13.692' |
| Site 4 | S 00° 25.835' | E 34° 13.668' |



APPENDIX B: EXPERIMENT I LATIN SQUARE BLOCKS

Key for charts: C: Control G: Goat L: Lizard O: Ox

Note that all dates are in the European Style Date Format

## Kirindo

| **Block I** | | | | |
|---|---|---|---|---|
| Date | Day | Site 1 | Site 2 | Site 3 |
| 16.06.09 | 1 | L | C | G |
| 17.06.09 | 2 | C | G | L |
| 18.06.09 | 3 | G | L | C |

| **Block II** | | | | |
|---|---|---|---|---|
| Date | Day | Site 1 | Site 2 | Site 3 |
| 19.06.09 | 1 | C | L | G |
| 20.06.09 | 2 | L | G | C |
| 22.06.09 | 3 | G | C | L |

| **Block III** | | | | |
|---|---|---|---|---|
| Date | Day | Site 1 | Site 2 | Site 3 |
| 23.06.09 | 1 | L | G | C |
| 24.06.09 | 2 | G | C | L |
| 25.06.09 | 3 | C | L | G |

## Chamaunga

| **Block I** | | | | | |
|---|---|---|---|---|---|
| Date | Day | Site 1 | Site 2 | Site 3 | Site 4 |
| 27.06.09 | 1 | L | C | O | G |
| 28.06.09 | 2 | G | L | C | O |
| 29.06.09 | 3 | C | O | G | L |
| 30.06.09 | 4 | O | G | L | C |

| **Block II** | | | | | |
|---|---|---|---|---|---|
| Date | Day | Site 1 | Site 2 | Site 3 | Site 4 |
| 01.07.09 | 1 | G | O | C | L |
| 02.07.09 | 2 | C | L | G | O |
| 03.07.09 | 3 | O | C | L | G |
| 04.07.09 | 4 | L | G | O | C |

APPENDIX C: EXPERIMENT I DATA SPREADSHEET

## Kirindo

| Date | Day | Site | Treatment | Code | Device | Male | Female | Total | Sto. | Tab. | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 16.06 | 1 | 1 | Lizard | L | Trap | 7 | 4 | 11 | 0 | 0 | |
| 16.06 | 1 | 2 | Control | C | Trap | 8 | 1 | 9 | 0 | 0 | |
| 16.06 | 1 | 3 | Goat | G | Trap | 11 | 7 | 18 | 0 | 1 | |
| 17.06 | 2 | 1 | Control | C | Trap | 10 | 13 | 23 | 2 | 0 | |
| 17.06 | 2 | 2 | Goat | G | Trap | 6 | 3 | 9 | 0 | 1 | |
| 17.06 | 2 | 3 | Lizard | L | Trap | 13 | 5 | 18 | 2 | 0 | |
| 18.06 | 3 | 1 | Goat | G | Trap | 7 | 9 | 16 | 0 | 0 | |
| 18.06 | 3 | 2 | Lizard | L | Trap | 2 | 6 | 8 | 0 | 0 | |
| 18.06 | 3 | 3 | Control | C | Trap | 9 | 4 | 13 | 0 | 0 | |
| 19.06 | 4 | 1 | Control | C | Trap | 9 | 10 | 19 | 1 | 0 | |
| 19.06 | 4 | 2 | Lizard | L | Trap | 11 | 6 | 17 | 0 | 0 | |
| 19.06 | 4 | 3 | Goat | G | Trap | 9 | 10 | 19 | 1 | 0 | |
| 20.06 | 5 | 1 | Lizard | L | Trap | 4 | 11 | 15 | 2 | 0 | |
| 20.06 | 5 | 2 | Goat | G | Trap | 10 | 12 | 22 | 0 | 1 | |
| 20.06 | 5 | 3 | Control | C | Trap | 11 | 9 | 20 | 2 | 0 | |
| 22.06 | 6 | 1 | Goat | G | Trap | 9 | 12 | 21 | 2 | 1 | |
| 22.06 | 6 | 2 | Control | C | Trap | 4 | 5 | 9 | 1 | 0 | |
| 22.06 | 6 | 3 | Lizard | L | Trap | 12 | 3 | 15 | 0 | 0 | |
| 23.06 | 7 | 1 | Lizard | L | Trap | 14 | 7 | 21 | 0 | 0 | |
| 23.06 | 7 | 2 | Goat | G | Trap | 11 | 17 | 28 | 0 | 0 | |
| 23.06 | 7 | 3 | Control | C | Trap | 6 | 11 | 17 | 0 | 0 | |
| 24.06 | 8 | 1 | Goat | G | Trap | 5 | 13 | 18 | 0 | 0 | |
| 24.06 | 8 | 2 | Control | C | Trap | 3 | 4 | 7 | 0 | 0 | |
| 24.06 | 8 | 3 | Lizard | L | Trap | 8 | 12 | 20 | 0 | 0 | |
| 25.06 | 9 | 1 | Control | C | Trap | 5 | 12 | 17 | 0 | 0 | |
| 25.06 | 9 | 2 | Lizard | L | Trap | 6 | 6 | 12 | 0 | 0 | |
| 25.06 | 9 | 3 | Goat | G | Trap | 5 | 11 | 16 | 12 | 1 | * |

## Chamaunga

| Date | Day | Site | Treatment | Code | Device | Male | Female | Total | Sto. | Tab. | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 27.06 | 1 | 1 | Lizard | L | Trap | 4 | 12 | 16 | 0 | 0 | |
| 27.06 | 1 | 2 | Control | C | Trap | 2 | 19 | 21 | 0 | 0 | |
| 27.06 | 1 | 3 | Ox | O | Trap | 12 | 12 | 24 | 0 | 1 | |
| 27.06 | 1 | 4 | Goat | G | Trap | 5 | 5 | 10 | 0 | 0 | |
| 28.06 | 2 | 1 | Goat | G | Trap | 12 | 3 | 15 | 0 | 0 | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 28.06 | 2 | 2 | Lizard | L | Trap | 13 | 5 | 18 | 0 | 0 | |
| 28.06 | 2 | 3 | Control | C | Trap | 13 | 15 | 28 | 0 | 0 | |
| 28.06 | 2 | 4 | Ox | O | Trap | 4 | 2 | 6 | 0 | 0 | |
| 29.06 | 3 | 1 | Control | C | Trap | 4 | 5 | 9 | 0 | 0 | ** |
| 29.06 | 3 | 2 | Ox | O | Trap | 3 | 2 | 5 | 0 | 0 | ** |
| 29.06 | 3 | 3 | Goat | G | Trap | 2 | 4 | 6 | 2 | 0 | ** |
| 29.06 | 3 | 4 | Lizard | L | Trap | 5 | 1 | 6 | 0 | 0 | ** |
| 30.06 | 4 | 1 | Ox | O | Trap | 10 | 16 | 26 | 0 | 0 | |
| 30.06 | 4 | 2 | Goat | G | Trap | 5 | 5 | 10 | 1 | 0 | |
| 30.06 | 4 | 3 | Lizard | L | Trap | 12 | 14 | 26 | 0 | 0 | |
| 30.06 | 4 | 4 | Control | C | Trap | 3 | 5 | 8 | 0 | 0 | |
| 01.07 | 5 | 1 | Goat | G | Trap | 4 | 8 | 12 | 0 | 0 | |
| 01.07 | 5 | 2 | Ox | O | Trap | 4 | 12 | 16 | 0 | 0 | |
| 01.07 | 5 | 3 | Control | C | Trap | 6 | 8 | 14 | 0 | 0 | |
| 01.07 | 5 | 4 | Lizard | L | Trap | 6 | 5 | 11 | 0 | 0 | |
| 02.07 | 6 | 1 | Control | C | Trap | 8 | 6 | 14 | 0 | 0 | |
| 02.07 | 6 | 2 | Lizard | L | Trap | 7 | 8 | 15 | 0 | 0 | |
| 02.07 | 6 | 3 | Goat | G | Trap | 10 | 12 | 22 | 2 | 0 | |
| 02.07 | 6 | 4 | Ox | O | Trap | 4 | 5 | 9 | 0 | 0 | |
| 03.07 | 7 | 1 | Ox | O | Trap | 9 | 5 | 14 | 0 | 0 | |
| 03.07 | 7 | 2 | Control | C | Trap | 5 | 4 | 9 | 0 | 0 | |
| 03.07 | 7 | 3 | Lizard | L | Trap | 4 | 4 | 8 | 0 | 0 | |
| 03.07 | 7 | 4 | Goat | G | Trap | 6 | 2 | 8 | 3 | 1 | |
| 04.07 | 8 | 1 | Lizard | L | Trap | 6 | 4 | 10 | 0 | 0 | |
| 04.07 | 8 | 2 | Goat | G | Trap | 4 | 12 | 16 | 0 | 0 | |
| 04.07 | 8 | 3 | Ox | O | Trap | 5 | 9 | 14 | 0 | 0 | |
| 04.07 | 8 | 4 | Control | C | Trap | 2 | 2 | 4 | 0 | 0 | |

* Unusually high number of stomoxys is in all likelihood due to presence of grazing cattle the previous afternoon, not the effect of the treatment itself

** Low catch most likely a result of cold, rainy weather

APPENDIX D: EXPERIMENT II LATIN SQUARE BLOCKS

Key for charts: C: Control G: Goat L: Lizard O: Ox

Note that all dates are in the European Style Date Format

| **Block I** | | | | | |
|---|---|---|---|---|---|
| Date | Day | Site 1 | Site 2 | Site 3 | Site 4 |
| 06.07.09 | 1 | O | L | C | G |
| 07.07.09 | 2 | C | G | O | L |
| 08.07.09 | 3 | L | O | G | C |
| 09.07.09 | 4 | G | C | L | O |

| **Block II** | | | | | |
|---|---|---|---|---|---|
| Date | Day | Site 1 | Site 2 | Site 3 | Site 4 |
| 10.07.09 | 1 | C | O | L | G |
| 11.07.09 | 2 | G | L | O | C |
| 13.07.09 | 3 | O | G | C | L |
| 14.07.09 | 4 | L | C | G | O |

APPENDIX E: EXPERIMENT II DATA SPREADSHEET

| Date | Day | Site | Treatment | Code | Device | Direction | Male | Female | Total | Sto. | Tab. | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 06.07 | 1 | 1 | Ox | O | Target | Upwind | 4 | 2 | 6 | 1 | 0 | |
| 06.07 | 1 | 1 | Ox | O | Target | Downwind | 3 | 1 | 4 | 0 | 0 | |
| 06.07 | 1 | 1 | Ox | O | Flank | Upwind | 0 | 3 | 3 | 0 | 0 | |
| 06.07 | 1 | 1 | Ox | O | Flank | Downwind | 1 | 5 | 6 | 0 | 0 | |
| 06.07 | 1 | 2 | Lizard | L | Target | Upwind | 2 | 2 | 4 | 0 | 0 | |
| 06.07 | 1 | 2 | Lizard | L | Target | Downwind | 3 | 2 | 5 | 0 | 0 | |
| 06.07 | 1 | 2 | Lizard | L | Flank | Upwind | 3 | 3 | 6 | 0 | 0 | |
| 06.07 | 1 | 2 | Lizard | L | Flank | Downwind | 6 | 8 | 14 | 0 | 0 | |
| 06.07 | 1 | 3 | Control | C | Target | Upwind | 3 | 1 | 4 | 0 | 0 | |
| 06.07 | 1 | 3 | Control | C | Target | Downwind | 6 | 1 | 7 | 0 | 0 | |
| 06.07 | 1 | 3 | Control | C | Flank | Upwind | 8 | 9 | 17 | 0 | 1 | |
| 06.07 | 1 | 3 | Control | C | Flank | Downwind | 2 | 5 | 7 | 0 | 0 | |
| 06.07 | 1 | 4 | Goat | G | Target | Upwind | 3 | 1 | 4 | 1 | 0 | |
| 06.07 | 1 | 4 | Goat | G | Target | Downwind | 2 | 4 | 6 | 0 | 0 | |
| 06.07 | 1 | 4 | Goat | G | Flank | Upwind | 4 | 4 | 8 | 1 | 0 | |
| 06.07 | 1 | 4 | Goat | G | Flank | Downwind | 3 | 1 | 4 | 1 | 0 | |
| 07.07 | 2 | 1 | Control | C | Target | Upwind | 4 | 8 | 12 | 0 | 0 | |
| 07.07 | 2 | 1 | Control | C | Target | Downwind | 6 | 4 | 10 | 1 | 0 | |
| 07.07 | 2 | 1 | Control | C | Flank | Upwind | 1 | 1 | 2 | 0 | 0 | |
| 07.07 | 2 | 1 | Control | C | Flank | Downwind | 3 | 9 | 12 | 0 | 1 | |
| 07.07 | 2 | 2 | Goat | G | Target | Upwind | 2 | 4 | 6 | 0 | 0 | |
| 07.07 | 2 | 2 | Goat | G | Target | Downwind | 0 | 3 | 3 | 0 | 0 | |
| 07.07 | 2 | 2 | Goat | G | Flank | Upwind | 3 | 10 | 13 | 0 | 0 | |
| 07.07 | 2 | 2 | Goat | G | Flank | Downwind | 0 | 6 | 6 | 0 | 0 | |
| 07.07 | 2 | 3 | Ox | O | Target | Upwind | 1 | 2 | 3 | 0 | 0 | |
| 07.07 | 2 | 3 | Ox | O | Target | Downwind | 6 | 1 | 7 | 1 | 0 | |
| 07.07 | 2 | 3 | Ox | O | Flank | Upwind | 3 | 7 | 10 | 0 | 1 | |
| 07.07 | 2 | 3 | Ox | O | Flank | Downwind | 4 | 3 | 7 | 0 | 0 | |
| 07.07 | 2 | 4 | Lizard | L | Target | Upwind | 0 | 2 | 2 | 1 | 0 | |
| 07.07 | 2 | 4 | Lizard | L | Target | Downwind | 1 | 4 | 5 | 1 | 0 | |
| 07.07 | 2 | 4 | Lizard | L | Flank | Upwind | 2 | 2 | 4 | 1 | 1 | |
| 07.07 | 2 | 4 | Lizard | L | Flank | Downwind | 1 | 4 | 5 | 0 | 0 | |
| 08.07 | 3 | 1 | Lizard | L | Target | Upwind | 3 | 6 | 9 | 0 | 0 | |
| 08.07 | 3 | 1 | Lizard | L | Target | Downwind | 4 | 4 | 8 | 0 | 1 | |
| 08.07 | 3 | 1 | Lizard | L | Flank | Upwind | 5 | 6 | 11 | 2 | 1 | |
| 08.07 | 3 | 1 | Lizard | L | Flank | Downwind | 1 | 12 | 13 | 0 | 1 | |
| 08.07 | 3 | 2 | Ox | O | Target | Upwind | 1 | 3 | 4 | 0 | 0 | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 08.07 | 3 | 2 | Ox | O | Target | Downwind | 1 | 3 | 4 | 0 | 0 | |
| 08.07 | 3 | 2 | Ox | O | Flank | Upwind | 1 | 7 | 8 | 0 | 0 | |
| 08.07 | 3 | 2 | Ox | O | Flank | Downwind | 3 | 13 | 16 | 1 | 1 | |
| 08.07 | 3 | 3 | Goat | G | Target | Upwind | 5 | 2 | 7 | 0 | 0 | |
| 08.07 | 3 | 3 | Goat | G | Target | Downwind | 5 | 1 | 6 | 0 | 0 | |
| 08.07 | 3 | 3 | Goat | G | Flank | Upwind | 5 | 6 | 11 | 0 | 0 | |
| 08.07 | 3 | 3 | Goat | G | Flank | Downwind | 2 | 8 | 10 | 0 | 2 | |
| 08.07 | 3 | 4 | Control | C | Target | Upwind | 1 | 0 | 1 | 0 | 2 | |
| 08.07 | 3 | 4 | Control | C | Target | Downwind | 2 | 3 | 5 | 4 | 0 | |
| 08.07 | 3 | 4 | Control | C | Flank | Upwind | 0 | 4 | 4 | 2 | 1 | |
| 08.07 | 3 | 4 | Control | C | Flank | Downwind | 1 | 2 | 3 | 1 | 0 | |
| 09.07 | 4 | 1 | Goat | G | Target | Upwind | 5 | 5 | 10 | 2 | 0 | |
| 09.07 | 4 | 1 | Goat | G | Target | Downwind | 1 | 3 | 4 | 0 | 1 | |
| 09.07 | 4 | 1 | Goat | G | Flank | Upwind | 2 | 3 | 5 | 1 | 2 | |
| 09.07 | 4 | 1 | Goat | G | Flank | Downwind | 2 | 5 | 7 | 0 | 2 | |
| 09.07 | 4 | 2 | Control | C | Target | Upwind | 1 | 4 | 5 | 0 | 0 | |
| 09.07 | 4 | 2 | Control | C | Target | Downwind | 3 | 4 | 7 | 0 | 1 | |
| 09.07 | 4 | 2 | Control | C | Flank | Upwind | 0 | 0 | 0 | 0 | 0 | |
| 09.07 | 4 | 2 | Control | C | Flank | Downwind | 1 | 6 | 7 | 0 | 1 | |
| 09.07 | 4 | 3 | Lizard | L | Target | Upwind | 1 | 0 | 1 | 0 | 0 | |
| 09.07 | 4 | 3 | Lizard | L | Target | Downwind | 1 | 4 | 5 | 0 | 0 | |
| 09.07 | 4 | 3 | Lizard | L | Flank | Upwind | 1 | 7 | 8 | 0 | 0 | |
| 09.07 | 4 | 3 | Lizard | L | Flank | Downwind | 3 | 3 | 6 | 3 | 1 | |
| 09.07 | 4 | 4 | Ox | O | Target | Upwind | 0 | 0 | 0 | 1 | 0 | |
| 09.07 | 4 | 4 | Ox | O | Target | Downwind | 0 | 1 | 1 | 4 | 0 | |
| 09.07 | 4 | 4 | Ox | O | Flank | Upwind | 1 | 3 | 4 | 3 | 1 | |
| 09.07 | 4 | 4 | Ox | O | Flank | Downwind | 1 | 4 | 5 | 1 | 1 | |
| 10.07 | 5 | 1 | Control | C | Target | Upwind | 2 | 1 | 3 | 2 | 0 | |
| 10.07 | 5 | 1 | Control | C | Target | Downwind | 3 | 4 | 7 | 0 | 0 | |
| 10.07 | 5 | 1 | Control | C | Flank | Upwind | 1 | 4 | 5 | 1 | 0 | |
| 10.07 | 5 | 1 | Control | C | Flank | Downwind | 1 | 5 | 6 | 1 | 6 | |
| 10.07 | 5 | 2 | Ox | O | Target | Upwind | 2 | 3 | 5 | 0 | 0 | |
| 10.07 | 5 | 2 | Ox | O | Target | Downwind | 0 | 0 | 0 | 0 | 0 | |
| 10.07 | 5 | 2 | Ox | O | Flank | Upwind | 0 | 1 | 1 | 0 | 0 | |
| 10.07 | 5 | 2 | Ox | O | Flank | Downwind | 3 | 6 | 9 | 2 | 1 | |
| 10.07 | 5 | 3 | Lizard | L | Target | Upwind | 4 | 0 | 4 | 0 | 0 | * |
| 10.07 | 5 | 3 | Lizard | L | Target | Downwind | 4 | 2 | 6 | 0 | 0 | |
| 10.07 | 5 | 3 | Lizard | L | Flank | Upwind | 0 | 1 | 1 | 0 | 0 | |
| 10.07 | 5 | 3 | Lizard | L | Flank | Downwind | 2 | 1 | 3 | 0 | 1 | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10.07 | 5 | 4 | Goat | G | Target | Upwind | 0 | 2 | 2 | 0 | 0 | |
| 10.07 | 5 | 4 | Goat | G | Target | Downwind | 0 | 5 | 5 | 2 | 0 | |
| 10.07 | 5 | 4 | Goat | G | Flank | Upwind | 0 | 3 | 3 | 1 | 1 | |
| 10.07 | 5 | 4 | Goat | G | Flank | Downwind | 0 | 2 | 2 | 0 | 1 | |
| 11.07 | 6 | 1 | Goat | G | Target | Upwind | 0 | 2 | 2 | 0 | 0 | ** |
| 11.07 | 6 | 1 | Goat | G | Target | Downwind | 3 | 3 | 6 | 1 | 2 | |
| 11.07 | 6 | 1 | Goat | G | Flank | Upwind | 2 | 4 | 6 | 1 | 0 | |
| 11.07 | 6 | 1 | Goat | G | Flank | Downwind | 0 | 6 | 6 | 0 | 3 | |
| 11.07 | 6 | 2 | Lizard | L | Target | Upwind | 0 | 1 | 1 | 0 | 0 | ** |
| 11.07 | 6 | 2 | Lizard | L | Target | Downwind | 2 | 3 | 5 | 0 | 0 | |
| 11.07 | 6 | 2 | Lizard | L | Flank | Upwind | 0 | 3 | 3 | 0 | 0 | |
| 11.07 | 6 | 2 | Lizard | L | Flank | Downwind | 1 | 6 | 7 | 0 | 1 | |
| 11.07 | 6 | 3 | Ox | O | Target | Upwind | 2 | 2 | 4 | 2 | 0 | ** |
| 11.07 | 6 | 3 | Ox | O | Target | Downwind | 5 | 1 | 6 | 0 | 0 | |
| 11.07 | 6 | 3 | Ox | O | Flank | Upwind | 3 | 8 | 11 | 0 | 2 | |
| 11.07 | 6 | 3 | Ox | O | Flank | Downwind | 2 | 1 | 3 | 1 | 0 | |
| 11.07 | 6 | 4 | Control | C | Target | Upwind | 0 | 0 | 0 | 0 | 0 | ** |
| 11.07 | 6 | 4 | Control | C | Target | Downwind | 0 | 0 | 0 | 2 | 1 | |
| 11.07 | 6 | 4 | Control | C | Flank | Upwind | 1 | 4 | 5 | 1 | 0 | |
| 11.07 | 6 | 4 | Control | C | Flank | Downwind | 0 | 2 | 2 | 0 | 0 | |
| 13.07 | 7 | 1 | Ox | O | Target | Upwind | 5 | 0 | 5 | 0 | 0 | |
| 13.07 | 7 | 1 | Ox | O | Target | Downwind | 4 | 6 | 10 | 4 | 0 | |
| 13.07 | 7 | 1 | Ox | O | Flank | Upwind | 1 | 7 | 8 | 0 | 1 | |
| 13.07 | 7 | 1 | Ox | O | Flank | Downwind | 2 | 9 | 11 | 0 | 2 | |
| 13.07 | 7 | 2 | Goat | G | Target | Upwind | 1 | 1 | 2 | 0 | 0 | |
| 13.07 | 7 | 2 | Goat | G | Target | Downwind | 1 | 4 | 5 | 0 | 0 | |
| 13.07 | 7 | 2 | Goat | G | Flank | Upwind | 1 | 4 | 5 | 0 | 0 | |
| 13.07 | 7 | 2 | Goat | G | Flank | Downwind | 1 | 3 | 4 | 0 | 1 | |
| 13.07 | 7 | 3 | Control | C | Target | Upwind | 1 | 0 | 1 | 1 | 0 | |
| 13.07 | 7 | 3 | Control | C | Target | Downwind | 2 | 2 | 4 | 1 | 0 | |
| 13.07 | 7 | 3 | Control | C | Flank | Upwind | 4 | 6 | 10 | 1 | 0 | |
| 13.07 | 7 | 3 | Control | C | Flank | Downwind | 2 | 2 | 4 | 0 | 0 | |
| 13.07 | 7 | 4 | Lizard | L | Target | Upwind | 1 | 1 | 2 | 0 | 0 | |
| 13.07 | 7 | 4 | Lizard | L | Target | Downwind | 1 | 1 | 2 | 0 | 0 | |
| 13.07 | 7 | 4 | Lizard | L | Flank | Upwind | 0 | 2 | 2 | 1 | 1 | |
| 13.07 | 7 | 4 | Lizard | L | Flank | Downwind | 1 | 1 | 2 | 0 | 0 | |
| 14.07 | 8 | 1 | Lizard | L | Target | Upwind | 4 | 1 | 5 | 3 | 0 | |
| 14.07 | 8 | 1 | Lizard | L | Target | Downwind | 5 | 5 | 10 | 0 | 0 | |
| 14.07 | 8 | 1 | Lizard | L | Flank | Upwind | 1 | 7 | 8 | 0 | 0 | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 14.07 | 8 | 1 | Lizard | L | Flank | Downwind | 0 | 6 | 6 | 1 | 0 | |
| 14.07 | 8 | 2 | Control | C | Target | Upwind | 0 | 3 | 3 | 0 | 0 | |
| 14.07 | 8 | 2 | Control | C | Target | Downwind | 1 | 1 | 2 | 0 | 0 | |
| 14.07 | 8 | 2 | Control | C | Flank | Upwind | 0 | 4 | 4 | 0 | 2 | |
| 14.07 | 8 | 2 | Control | C | Flank | Downwind | 0 | 3 | 3 | 0 | 0 | |
| 14.07 | 8 | 3 | Goat | G | Target | Upwind | 4 | 0 | 4 | 0 | 0 | |
| 14.07 | 8 | 3 | Goat | G | Target | Downwind | 2 | 1 | 3 | 1 | 0 | |
| 14.07 | 8 | 3 | Goat | G | Flank | Upwind | 4 | 3 | 7 | 1 | 0 | |
| 14.07 | 8 | 3 | Goat | G | Flank | Downwind | 9 | 3 | 12 | 1 | 0 | |
| 14.07 | 8 | 4 | Ox | O | Target | Upwind | 3 | 1 | 4 | 4 | 0 | |
| 14.07 | 8 | 4 | Ox | O | Target | Downwind | 0 | 0 | 0 | 2 | 0 | |
| 14.07 | 8 | 4 | Ox | O | Flank | Upwind | 1 | 7 | 8 | 3 | 3 | |
| 14.07 | 8 | 4 | Ox | O | Flank | Downwind | 1 | 0 | 1 | 7 | 0 | |

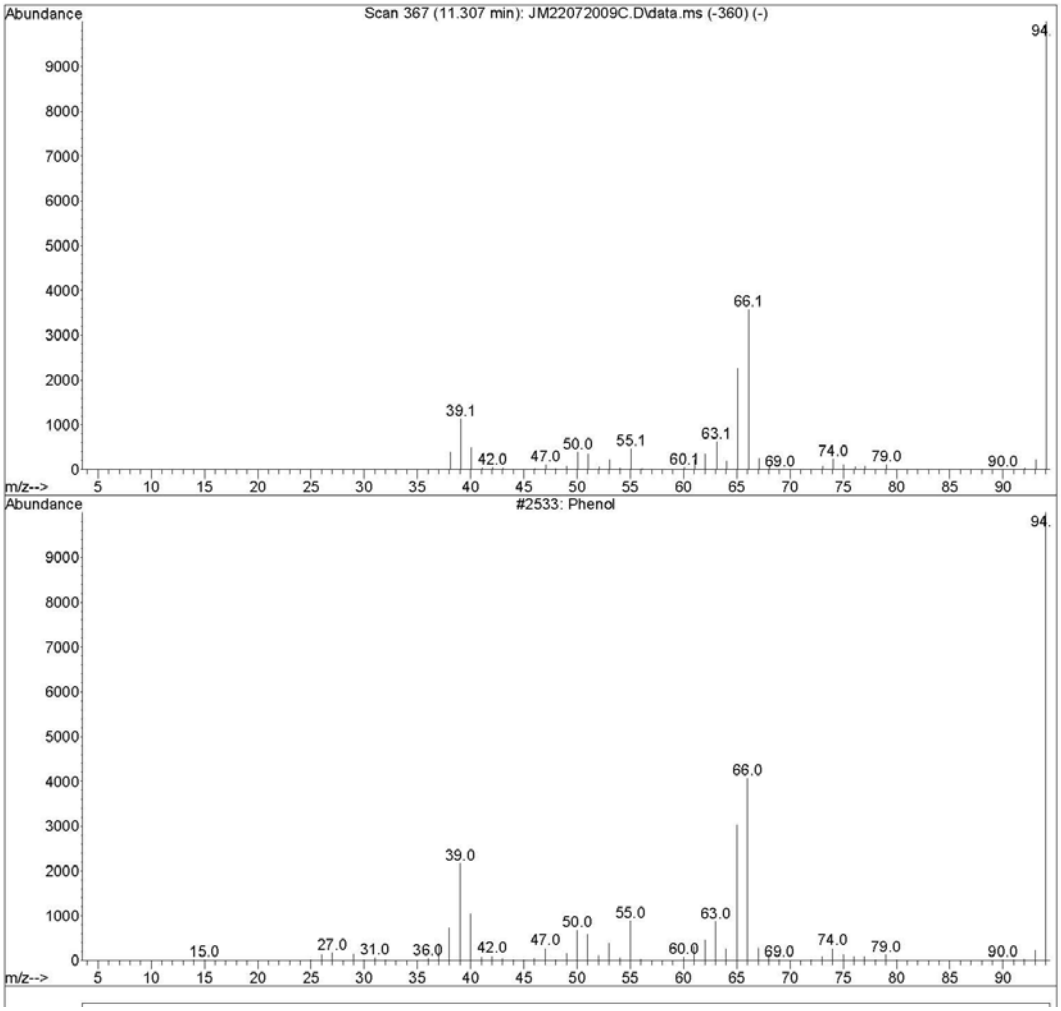
* Urine sample accidentally diluted 10x with water

** Fresh urine samples were used for all treatments the next day

APPENDIX F: EXPERIMENT III MASS SPECTROSCOPIES OF INTEREST

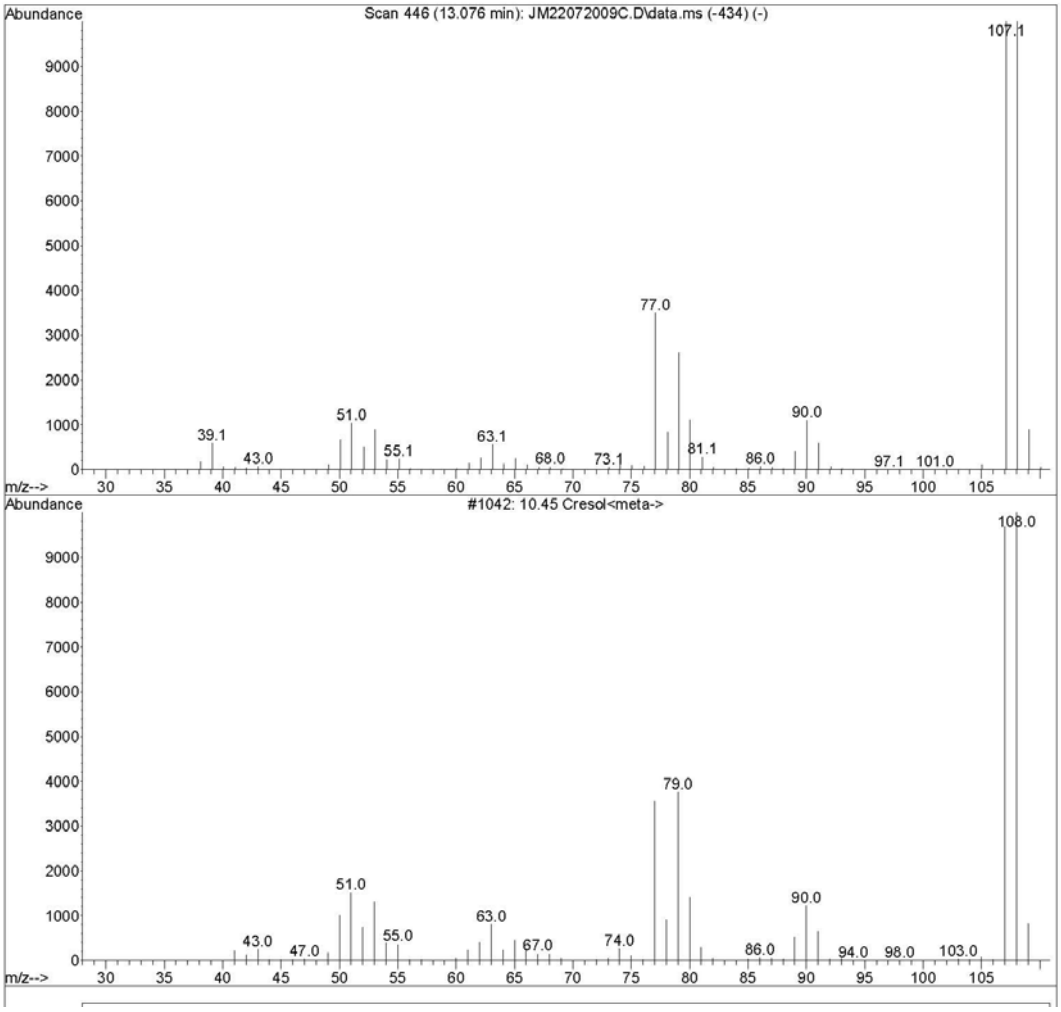
## Phenol

Library Searched : C:\Database\NIST05a.L
Quality : 91
ID : Phenol



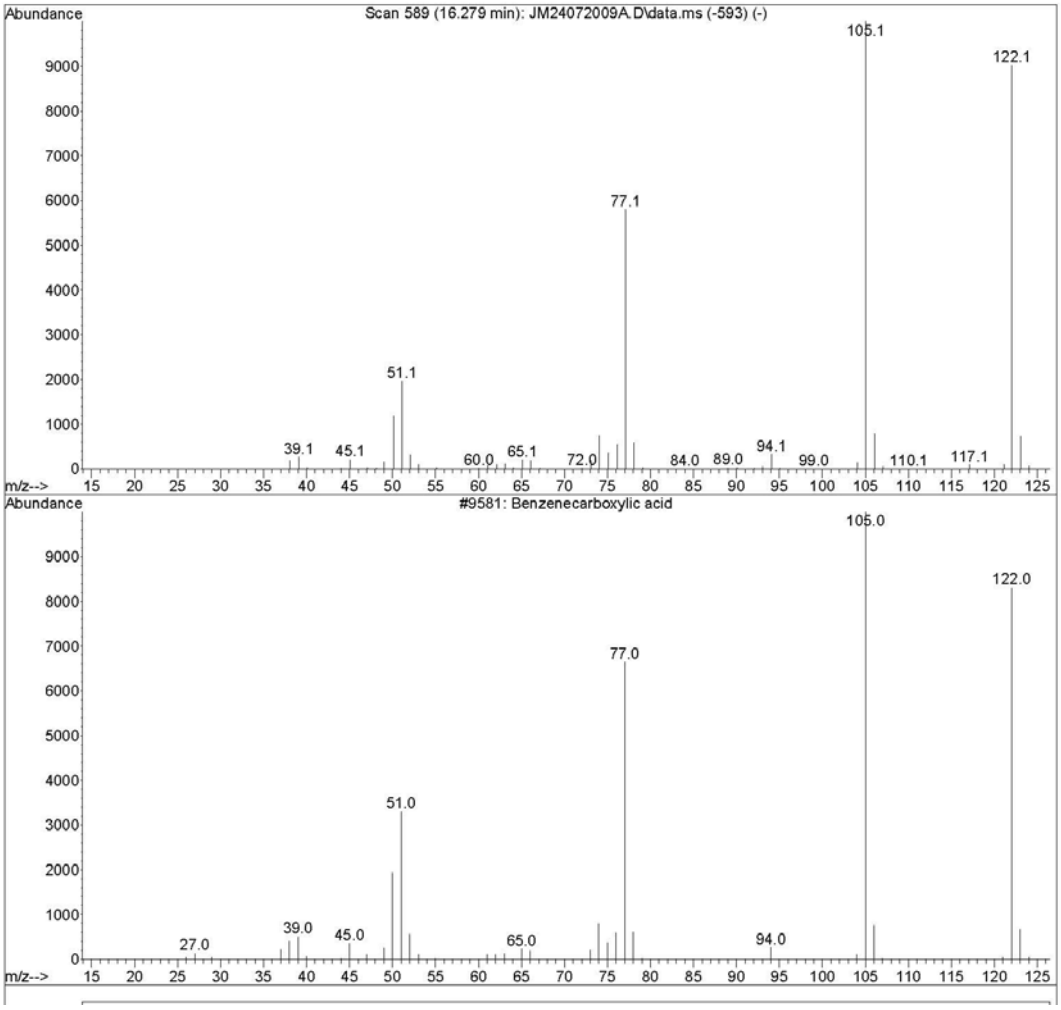

## m-Cresol

Library Searched : C:\Database\Adams2.L
Quality : 97
ID : 10.45 Cresol<meta->




Benzenecarboxylic acid

Library Searched : C:\Database\NIST05a.L
Quality : 94
ID : Benzenecarboxylic acid



APPENDIX G: EXPERIMENT III CHROMATOGRAM PERCENT AREA REPORTS

## Phenolic

Data Path : C:\msdchem\1\DATA\

Data File : JM22072009E.D

Acq On : 22 Jul 2009 16:08

Operator : JM

Sample : Phenolic Std

Misc : 40uL phenolic + 1uL Std

ALS Vial : 5 Sample Multiplier: 1

Integration Parameters: autoint1.e

Integrator: ChemStation

Method : C:\msdchem\1\METHODS\default-new-drs-2.m

Title :

Signal : TIC: JM22072009E.D\data.ms

| peak # | R.T. first min | max scan | last scan | PK | peak scan | corr. TY | corr. height | area | % max. | % total |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 7.113 | 155 | 180 | 196 | BV 2 | 519 | -72668 | - | 0.00% | -0.001% |
| 2 | 7.654 | 196 | 204 | 261 | PV 2 | 22006 | 3085266 | | 0.07% | 0.035% |
| 3 | 9.362 | 277 | 280 | 297 | VV 2 | 6703 | 676239 | | 0.02% | 0.008% |
| 4 | 9.851 | 297 | 302 | 325 | VV 3 | 16999 | 297547 | | 0.03% | 0.015% |
| 5 | 10.469 | 325 | 330 | 342 | VV 3 | 6411 | 468798 | | 0.01% | 0.005% |
| 6 | 10.859 | 342 | 347 | 359 | PV | 68524 | 6059807 | | 0.14% | 0.069% |
| 7 | 11.329 | 359 | 368 | 424 | VV | 20310075 | 2279241832 | | 51.33% | 26.040% |

**Phenol**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 8 | 13.054 | 424 | 445 | 491 | VV 3 | 31906134 | 4440400644 | 100.00% | 50.731% |
| | **m-Cresol** | | | | | | | | |
| 9 | 14.146 | 491 | 494 | 501 | VV 2 | 178892 | 19710445 | 0.44% | 0.225% |
| 10 | 14.404 | 501 | 505 | 521 | VV | 1509088 | 141776913 | 3.19% | 1.620% |
| 11 | 14.861 | 521 | 526 | 550 | VV | 19174058 | 735903008 | 16.57% | 8.408% |
| 12 | 15.518 | 550 | 555 | 561 | VV 3 | 75946 | 9561844 | 0.22% | 0.109% |
| 13 | 15.725 | 561 | 564 | 626 | VV | 4629429 | 425764490 | 9.59% | 4.864% |
| 14 | 17.589 | 626 | 648 | 678 | VV 6 | 87307 | 27030766 | 0.61% | 0.309% |
| 15 | 18.502 | 678 | 688 | 694 | VV 7 | 23725 | 4600377 | 0.10% | 0.053% |
| 16 | 18.979 | 694 | 710 | 718 | VV 8 | 64220 | 15295576 | 0.34% | 0.175% |
| 17 | 19.272 | 718 | 723 | 729 | VV 2 | 63534 | 8032981 | 0.18% | 0.092% |
| 18 | 19.524 | 729 | 734 | 787 | VV | 2525232 | 330391457 | 7.44% | 3.775% |
| 19 | 20.945 | 787 | 797 | 809 | VV | 116423 | 30377744 | 0.68% | 0.347% |
| 20 | 21.439 | 809 | 819 | 864 | VV 3 | 215521 | 88566473 | 1.99% | 1.012% |
| 21 | 22.511 | 864 | 867 | 886 | VV 5 | 88371 | 16115444 | 0.36% | 0.184% |
| 22 | 23.059 | 886 | 892 | 939 | VV 4 | 54923 | 19132781 | 0.43% | 0.219% |
| 23 | 24.207 | 939 | 943 | 978 | VV 7 | 28157 | 6920583 | 0.16% | 0.079% |
| 24 | 25.265 | 978 | 990 | 996 | VV 7 | 7911 | 1366990 | 0.03% | 0.016% |
| 25 | 26.056 | 1013 | 1026 | 1031 | PV 7 | 3915 | 325395 | 0.01% | 0.004% |
| 26 | 26.911 | 1031 | 1064 | 1087 | VV 4 | 11105 | 5274600 | 0.12% | 0.060% |
| 27 | 27.734 | 1087 | 1100 | 1112 | VV 7 | 21227 | 4117372 | 0.09% | 0.047% |
| 28 | 28.965 | 1112 | 1155 | 1164 | VV 2 | 54936 | 15208646 | 0.34% | 0.174% |
| 29 | 30.100 | 1164 | 1206 | 1222 | VV 2 | 47366 | 28762626 | 0.65% | 0.329% |
| 30 | 30.610 | 1222 | 1229 | 1251 | VV 2 | 46166 | 15370236 | 0.35% | 0.176% |
| 31 | 31.240 | 1251 | 1257 | 1327 | VV | 54845 | 26230398 | 0.59% | 0.300% |
| 32 | 32.882 | 1327 | 1330 | 1395 | VV | 18745 | 8681221 | 0.20% | 0.099% |
| 33 | 34.857 | 1413 | 1418 | 1445 | VV | 5867 | 765368 | 0.02% | 0.009% |
| 34 | 40.796 | 1632 | 1684 | 1694 | PV | 9632 | 3084480 | 0.07% | 0.035% |
| 35 | 42.323 | 1694 | 1752 | 1774 | VV | 9021 | 9278301 | 0.21% | 0.106% |
| 36 | 44.564 | 1774 | 1852 | 1872 | VV | 7183 | 7458136 | 0.17% | 0.085% |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 37 | 45.149 | 1872 | 1878 | 1923 | VB | 4893 | 1514049 | 0.03% | 0.017% |
| 38 | 47.218 | 1935 | 1970 | 2066 | BV 3 | 37757 | 14830213 | 0.33% | 0.169% |
| 39 | 49.539 | 2066 | 2074 | 2085 | VBA3 | 2211 | 275873 | 0.01% | 0.003% |

**Sum of corrected areas: 8752882251**

## Acidic

Data Path : C:\msdchem\1\DATA\

Data File : JM24072009B.D

Acq On : 24 Jul 2009 13:28

Operator : JM

Sample : 40µL ACIDIC WITH 1µL STD

Misc : URINE EXTRACTION

ALS Vial : 97 Sample Multiplier: 1

Integration Parameters: autoint1.e

Integrator: ChemStation

Method : C:\msdchem\1\METHODS\default-new-drs-2.m

Title :

Signal : TIC: JM24072009B.D\data.ms

| peak # | R.T. first min | max scan | last scan | PK scan | peak TY | corr. height | corr. area | % of % max. | total |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 4.483 | 43 | 62 | 94 | BV 2 | 26773 - | 266053 | -0.00% | -0.002% |
| 2 | 5.271 | 94 | 98 | 197 | PV | 9881 | 1279224 | 0.01% | 0.008% |
| 3 | 7.675 | 197 | 205 | 272 | PV 2 | 68822 | 7214304 | 0.06% | 0.043% |
| 4 | 9.288 | 272 | 277 | 295 | PV 5 | 5121 | 707768 | 0.01% | 0.004% |
| 5 | 9.986 | 295 | 308 | 326 | PV 4 | 12040 | 2331930 | 0.02% | 0.014% |
| 6 | 10.715 | 326 | 341 | 358 | VV 3 | 28634 | 4645326 | 0.04% | 0.027% |
| 7 | 11.296 | 358 | 367 | 403 | PV 2 | 201347 | 35681269 | 0.30% | 0.210% |
| 8 | 12.203 | 403 | 407 | 418 | VV 2 | 26294 | 4502054 | 0.04% | 0.027% |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 9 | 12.576 | 418 | 424 | 429 | VV 2 | 59238 | 4188743 | 0.03% | 0.025% |
| 10 | 12.973 | 429 | 441 | 464 | VV 2 | 168059 | 34058927 | 0.28% | 0.201% |
| 11 | 13.858 | 464 | 481 | 498 | VV 2 | 678521 | 94783054 | 0.79% | 0.559% |
| 12 | 16.384 | 498 | 594 | 628 | VV | 22929097 1 | 1982758839 | 100.00% | 70.646% |
| | **Benzenecarboxlyic Acid** | | | | | | | | |
| 13 | 17.255 | 628 | 633 | 643 | VV 3 | 559959 | 63673758 | 0.53% | 0.375% |
| 14 | 17.554 | 643 | 646 | 653 | VV | 521387 | 38678252 | 0.32% | 0.228% |
| 15 | 17.800 | 653 | 657 | 675 | VV 3 | 500847 | 71236875 | 0.59% | 0.420% |
| 16 | 18.302 | 675 | 679 | 695 | VV 7 | 269122 | 53853706 | 0.45% | 0.318% |
| 17 | 18.843 | 695 | 703 | 711 | VV | 818747 | 56528700 | 0.47% | 0.333% |
| 18 | 19.062 | 711 | 713 | 729 | VV 7 | 222470 | 41000729 | 0.34% | 0.242% |
| 19 | 19.746 | 729 | 744 | 758 | VV 7 | 241919 | 84679187 | 0.71% | 0.499% |
| 20 | 20.649 | 758 | 784 | 815 | VV 5 | 718913 | 294177743 | 2.46% | 1.734% |
| 21 | 21.814 | 815 | 836 | 856 | VV 5 | 533424 | 211150873 | 1.76% | 1.245% |
| 22 | 22.540 | 856 | 869 | 887 | VV 3 | 805581 | 159379189 | 1.33% | 0.940% |
| 23 | 23.060 | 887 | 892 | 896 | VV 4 | 480907 | 50898597 | 0.42% | 0.300% |
| 24 | 23.259 | 896 | 901 | 906 | VV 2 | 581513 | 58671257 | 0.49% | 0.346% |
| 25 | 23.460 | 906 | 910 | 915 | VV 3 | 503045 | 49610315 | 0.41% | 0.292% |
| 26 | 23.869 | 915 | 928 | 946 | VV 3 | 1130204 | 196137284 | 1.64% | 1.156% |
| 27 | 24.356 | 946 | 950 | 967 | VV 10 | 470590 | 106802725 | 0.89% | 0.630% |
| 28 | 24.802 | 967 | 970 | 976 | VV | 649613 | 48051828 | 0.40% | 0.283% |
| 29 | 25.117 | 976 | 984 | 991 | VV 2 | 438185 | 75836569 | 0.63% | 0.447% |
| 30 | 25.509 | 991 | 1001 | 1020 | VV 4 | 785528 | 159939075 | 1.33% | 0.943% |
| 31 | 26.087 | 1020 | 1027 | 1035 | VV 7 | 349183 | 63869893 | 0.53% | 0.377% |
| 32 | 26.571 | 1035 | 1049 | 1054 | VV 4 | 524134 | 90238345 | 0.75% | 0.532% |
| 33 | 26.745 | 1054 | 1056 | 1080 | VV | 540802 | 99352598 | 0.83% | 0.586% |
| 34 | 27.458 | 1080 | 1088 | 1095 | VV 5 | 303905 | 52299164 | 0.44% | 0.308% |
| 35 | 27.929 | 1095 | 1109 | 1117 | VV 3 | 546169 | 80704818 | 0.67% | 0.476% |
| 36 | 28.510 | 1117 | 1135 | 1149 | VV 6 | 285567 | 106957281 | 0.89% | 0.631% |
| 37 | 28.941 | 1149 | 1154 | 1164 | VV | 1320934 | 82925075 | 0.69% | 0.489% |
| 38 | 30.157 | 1164 | 1209 | 1212 | VV 3 | 800829 | 254339094 | 2.12% | 1.499% |
| 39 | 30.379 | 1212 | 1219 | 1224 | VV | 1543297 | 157416232 | 1.31% | 0.928% |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 40 | 30.665 | 1224 | 1231 | 1235 | VV | 1465033 | 164382880 | 1.37% | 0.969% |
| 41 | 30.878 | 1235 | 1241 | 1252 | VV | 1609079 | 256738681 | 2.14% | 1.514% |
| 42 | 31.289 | 1252 | 1259 | 1281 | VV | 3 1138400 | 335794960 | 2.80% | 1.980% |
| 43 | 31.871 | 1281 | 1285 | 1327 | VV | 737204 | 236896353 | 1.98% | 1.397% |
| 44 | 32.908 | 1327 | 1331 | 1398 | VV 3 | 305761 | 167897334 | 1.40% | 0.990% |
| 45 | 34.499 | 1398 | 1402 | 1415 | VV 2 | 155231 | 31992701 | 0.27% | 0.189% |
| 46 | 34.896 | 1415 | 1420 | 1432 | VV 2 | 182251 | 35985545 | 0.30% | 0.212% |
| 47 | 35.285 | 1432 | 1438 | 1447 | VV 2 | 157197 | 29577240 | 0.25% | 0.174% |
| 48 | 35.657 | 1447 | 1454 | 1477 | VV 2 | 156701 | 55509861 | 0.46% | 0.327% |
| 49 | 36.268 | 1477 | 1481 | 1501 | VV 2 | 132786 | 41048969 | 0.34% | 0.242% |
| 50 | 36.789 | 1501 | 1505 | 1516 | VV 5 | 133832 | 25298963 | 0.21% | 0.149% |
| 51 | 37.184 | 1516 | 1522 | 1531 | VV 5 | 123696 | 24443561 | 0.20% | 0.144% |
| 52 | 37.508 | 1531 | 1537 | 1571 | VV 4 | 147143 | 66430915 | 0.55% | 0.392% |
| 53 | 38.609 | 1571 | 1586 | 1605 | VV 4 | 121302 | 52642166 | 0.44% | 0.310% |
| 54 | 39.144 | 1605 | 1610 | 1621 | VV 4 | 115921 | 25365340 | 0.21% | 0.150% |
| 55 | 39.549 | 1621 | 1628 | 1644 | VV 4 | 120645 | 35124882 | 0.29% | 0.207% |
| 56 | 40.136 | 1644 | 1654 | 1665 | VV 4 | 120951 | 33451949 | 0.28% | 0.197% |
| 57 | 40.817 | 1665 | 1685 | 1703 | VV 8 | 139449 | 63526015 | 0.53% | 0.375% |
| 58 | 41.395 | 1703 | 1710 | 1731 | VV 8 | 129316 | 47915456 | 0.40% | 0.282% |
| 59 | 42.247 | 1731 | 1748 | 1766 | VV 8 | 128976 | 59049179 | 0.49% | 0.348% |
| 60 | 43.880 | 1811 | 1821 | 1843 | VV 8 | 128222 | 53972605 | 0.45% | 0.318% |
| 61 | 44.609 | 1843 | 1854 | 1864 | VV 8 | 136947 | 36244816 | 0.30% | 0.214% |
| 62 | 45.084 | 1864 | 1875 | 1880 | VV 8 | 136047 | 29292662 | 0.24% | 0.173% |
| 63 | 45.293 | 1880 | 1884 | 1900 | VV 8 | 135402 | 35649766 | 0.30% | 0.210% |
| 64 | 49.595 | 2067 | 2076 | 2089 | VBA8 | 132221 | 37114762 | 0.31% | 0.219% |

**Sum of corrected areas: 16961642107**