chats

Children's Health after the Storms (CHATS)

September 2013

Children's Health after the Storms

Final Report

Prepared for

Centers for Disease Control and Prevention
National Center for Environmental Health
Division of Environmental Hazards and Health Effects
ATTN: Mr. Gary Teague
4770 Buford Highway
Room 06105, Mailstop F-60
Atlanta, GA 30341
E-mail: gteague@cdc.gov

Prepared by

RTI International
Research Triangle Park, NC 27709

RTI Project Number 0212734

RTI International is a trade name of Research Triangle Institute.

Children's Health after the Storms

Final Report

September 2013

Centers for Disease Control and Prevention
National Center for Environmental Health
Division of Environmental Hazards and Health Effects

Mr. Gary Teague
Project Officer
4770 Buford Highway
Room 06105, Mailstop F-60
Atlanta, GA 30341
E-mail: gteague@cdc.gov
Fax: (770) 488-3460

Contents

Executive Summary **1**

1. Background **13**

1.1 Purpose of the CHATS Study ... 13

1.2 CHATS Study Objectives ... 14

1.3 Purpose of the Feasibility Study ... 14

1.4 Study Design ... 15

2. Study Methods **17**

2.1 Sample Design ... 17

2.1.1 Exposed Sample ... 17

2.1.2 Unexposed Sample ... 24

2.2 Tracing Sample ... 29

2.2.1 Preliminary Review of Sample File ... 30

2.2.2 Batch Tracing ... 30

2.2.3 Interactive Tracing ... 30

2.2.4 Field Tracing ... 31

2.2.5 Tracing Unit Systems Security ... 31

2.2.6 50-HouseholdTest of Tracing Protocol for Four States ... 32

2.3 Screening and Interviewing Instruments ... 32

2.3.1 Screening Instrument ... 33

2.3.2 Informed Consent ... 33

2.3.3 Administering the Interview ... 34

2.3.4 Baseline Assessment ... 34

2.3.5 Follow-up Assessment ... 36

2.3.6 Central Site Assessment ... 37

2.4 Staff Recruitment and Training ... 38

2.4.1 Field Interviewers ... 38

2.4.2 Nurse Training ... 41

2.4.3 Abstractor Training ... 42

2.5 Subject Recruitment 42
2.5.1 Screening 42
2.5.2 Baseline Assessment 43
2.5.3 Follow-up Assessment 44
2.6 Environmental Assessment 46
2.6.1 Environmental Sample Box Preparation and Shipping to Field Interviewers 46
2.6.2 Week-Long Sample Collections at the Household—Exposure Measuring Devices 47
2.6.3 Other Sample Collections at the Household 52
2.6.4 Additional Environmental Sample Collections (Off-Site) 53
2.6.5 Sample Handling and Shipping to RTI 54
2.7 Health Assessment 55
2.8 Laboratory Analysis and Data Handling 57
2.8.1 Laboratory Protocols 57
2.8.2 Sample Shipping and Handling 65
2.8.3 Data Submission and Processing 67
2.9 Outreach 70
2.9.1 Establishment of a CHATS Community Advisory Panel (CAP) 71
2.9.2 CHATS Partnership Development and Public Forums 71
2.9.3 Design and Implementation of a Local Media/Advertising Campaign 73

3. Findings 75

3.1 Public Outreach Results 75
3.1.1 Media Campaign Results 79
NEW ORLEANS, LA 80
3.2 Study Participant Locating Results 81
3.2.1 Locating Outcomes 81
3.3 Study Participant Recruitment Results 84
3.3.1 Screening 84
3.3.2 Baseline Assessment 87
3.3.3 Follow-up Assessment 88

3.4 Feasibility of Enrolling and Retaining Participants 89
3.4.1 Enrolling Participants for Baseline 89
3.4.2 Retaining Participants for Follow-up 93
3.5 Locating Medical Records 94
3.6 Health Assessment Outcomes 96
3.7 Evaluate Operational Issues 102
3.7.1 Health Assessment Compliance 102
3.7.2 Environmental Assessment Compliance 103
3.8 Evaluate Selection Bias 108
3.8.1 Exposed Sample 108
3.8.2 Unexposed Sample 108
3.8.3 Participation Rate by Various Characteristics 109
3.9 Evaluate Information Bias 109
3.10 Evaluate Quality of Data 111
3.10.1 Quality of Interview Data 111
3.10.2 Quality Assurance (QA) Activities of Laboratory Data 114
3.10.3 Record Quality Indicators 154
3.11 Laboratory Findings 161
3.12 Special Studies 179
3.12.1 Air Exchange Rate Calculation 179
3.12.2 Exposure Misclassification Substudy 180

4. Recommendations 201

4.1 Study Design 201
4.1.1 Stratification on Trailer Site 201
4.1.2 Longitudinal Design 201
4.1.3 Collection of Self-reported Historical Information 201
4.2 Sample Design 202
4.2.1 Frame Quality 202
4.2.2 Use of GIS 202
4.2.3 Unexposed Sample 202
4.2.4 Eligibility Rate 203

4.3 Subject Recruitment Recommendations 203
4.3.1 Staffing: Field Interviewer Experience 203
4.3.2 Training 204
4.3.3 Screening Procedures 204
4.3.4 Participant Burden 205
4.4 Environmental Assessment 206
4.5 Health Assessment 208
4.6 Laboratory Analysis 208
4.7 Outreach 210
4.7.1 Public Forums 210

List of Appendices

Appendix A: List of Analytes, Media, and Source of Samples A-1
Appendix B: Protocols for Laboratory Analyses Conducted in the CHATS Feasibility Study B-1

List of Tables

Table		Page
ES-1	Targets and Observed Tracing and Recruitment Numbers, by Exposure Status	3
ES-2	Summary of Feasibility Study Criteria for Objectives 1 and 2	4
2-1a	Feasibility Study Counties/Parishes for Group Temporary Housing Unit Residents (Exposed) with Sample Allocation	21
2-1b	Feasibility Study Counties/Parishes for Private Temporary Housing Unit Residents (Exposed) with Sample Allocation	22
2-1c	Probability of Selection and Design Weight for the Exposed Children's Address by County/State and Temporary Housing Unit Type	24
2-1d	Probability of Selection and Design Weight for the Unexposed Children's Address by Census Block Group	28
2-4a	Field Interviewer Recruitment by Location and Training Session	40
2-4b	Registered Nurse Recruitment by Location and Training Session	41
2-6a	Participant Box Usage Frequency and Inclusion of QC Samples by Box Type	46
2-6b	Types of Environmental Samplers	47
2-8a	Summary of Procedures (Research Operating Protocols [ROPs] and Standard Operating Procedures [SOPs]) Used for Analysis of CHATS Samples	58
2-8b	Frequencies and Percentages of Participant Box Return to RTI over Weekends or Holidays	66
2-8c	Baseline Samples Adversely Affected by Weather, Delays in Delivery, or Other Reasons	67
3-1a	Snapshot of Forum Attendance	75
3-1b	CHATS Public Service Advertisements	80
3-1c	CHATS Billboard and Community Bulletin Board Placements	81
3-2a	Outcomes of Batch Tracing – Summary Findings Based on Two Sources	82
3-2b	Locating Outcomes by Exposed and Unexposed Cohort	83
3-2c	Outcomes of Batch Tracing for Test Cases (Alabama and Texas)	84
3-3a	Summary of Screening Outcomes	84
3-3b	Reasons for Screening Nonresponse among Exposed and Unexposed	84
3-3c	Summary of Baseline Assessment Outcomes by Session	88
3-3d	Summary of Follow-up Assessment Outcomes by Session	89
3-4a	Baseline Session 1 Outcomes by Region	91
3-4b	Baseline Session 1 Outcomes among Exposed Participants	91

3-4c Baseline Session 1 Outcomes among Unexposed Participants 91
3-4d Summary of Baseline Session 1 Appointments by Geographic Region 92
3-5a Medical Records Abstraction Consent Summary from Baseline Visits by State and Exposure Status 95
3-5b Medical Records Accession and Completion Status for Baseline by State 95
3-5c Medical Records Accession and Completion Status for Follow-up by State 95
3-6a History of Asthma or Other Signature Health Outcomes (SHO) Reported by CHATS Participants at Baseline 96
3-6b History of Asthma or Other Signature Health Outcomes (SHO) Reported by CHATS Participants at Follow-up 96
3-6c Presence of Facial Symptoms at Baseline and Follow-up 97
3-6d Presence of Dermal Symptoms at Baseline and Follow-up 97
3-6e Descriptive Statistics of Exhaled Nitric Oxide Measurements by Reported Health Status at Baseline (ppb) 98
3-6f Descriptive Statistics of Exhaled Nitric Oxide Measurements by Reported Health Status at Follow-up (ppb) 98
3-6g Spirometry Quality Grades at Baseline and at Follow-up 99
3-6h Means of Spirometry Measurements by Health Status at Baseline 100
3-6i Means of Spirometry Measurements by Health Status at Follow-up 100
3-6j Number Attaining 80% of Predicted Value for Various Spirometry Measurements by Health Status at Baseline 101
3-6k Number Attaining 80% of Predicted Value for Various Spirometry Measurements by Health Status at Follow-up 101
3-6l Mean Scores for the ACT™ 101
3-6m Mean Scores for the C-ACT™ 101
3-6n Mean Scores for the PedsQL™ 102
3-6o Mean Scores for the PedsQL™Asthma 102
3-7a Biospecimen Refusal at Baseline 103
3-7b Overall Percentage and Number of Environmental Samples that were Successfully Deployed, Retrieved, and Shipped (RQI = 0 or 1) in Each Sampling Platform during Two Study Phases (Number Valid/Number Total Planned Shown in Parentheses) 104
3-7c Percentage and Number of Samples Determined to be Invalid (RQI = 2) during Deployment of Each Sampling Platform during the Two Study Phases (Number Invalid/Number Total Planned Shown in Parentheses) 104
3-7d Percent and Number of Samples Determined to be Invalid (RQI = 2) during Retrieval of Each Sampling Platform during the Two Study Phases (Number Invalid/Number Total Planned Shown in Parentheses) 105
3-7e Various Issues that Prevented Successful Environmental Sample Collection 106

3-7g Average Wearing Compliance (% Waking Time) of Personal Exposure Monitor by Study Phase, State, and Health Group 107
3-9a Comparison of Asthma Diagnosis as Reported by the Participant and by the Medical Records 110
3-9b Verification of Participant Reported Asthma Diagnosis by Medical Records 110
3-9c Comparison of Multiple THU Residence as Reported on the FEMA Applicant List and Self-reported by the Participant 111
3-10a Phthalates and Metabolites 116
3-10b Regression Data for RTI versus LSU Interim Hospital Phthalate Monoester Analyses of QA Samples 117
3-10c Distribution of Phthalate Monoester Concentrations from RTI and LSU Interim Hospital Compared to NHANES Data 118
310d Median Values for Di(2-ethylhexyl) Phthalate (DEHP) Metabolites (ng/mL) 120
3-10e Correlations between Di(2-ethylhexyl) Phthalate (DEHP) Metabolites (r) by Laboratory 120
3-10f VOCs and Metabolites 122
3-10g Statistical Comparison of VOC Metabolite Data from LSU Interim Hospital, RTI Analytes with Significant Measurable Quantities from Both Laboratories 123
3-10h Comparison of Centers for Disease Control and Prevention1 and CHATS Quality Assurance Sample Distributions by Laboratory 126
3-10i Assessment of Dust Samples Stored under Several Conditions 135
3-10j Descriptive Statistics for Percent Difference between Duplicate Carbonyl Badges 136
3-10k Descriptive Statistics for Percent Difference between Duplicate Hydrogen Sulfide Sorbent Tubes 136
3-10l Descriptive Statistics for Percent Difference between NO2 Badges 136
3-10m Descriptive Statistics for Percent Difference between VOC Badges 137
3-10n Descriptive Statistics for Carbonyl Badge Field Blanks 138
3-10o Descriptive Statistics for NO2 Badge Blanks 138
3-10p Descriptive Statistics for VOC Badge Blanks 138
3-10q Dust Phthalates Precision (Calculated as Relative Standard Deviation Eq 3-1; n = 9 pairs) 145
3-10r Quality Control Data for the Standard Curves for Each Analyte and the Limits of Detection 147
3-10s Summary of Quality Control Measures for Integrated PM Mass on MicroPEM™ Filters 148
3-10t A Summary of Coefficient of Variance (%) of Replicate Analysis of PM Mass on MicroPEM™ Filters 149

3-10u Coefficient of Variance (%) of Quality Assurance Measures for Optical Density of Each Light Source Wavelength Assessed for MicroPEM™ Filters 149
3-10v Descriptive Statistics for PM10 Mass and ETS of MicroPEM Filter Field Blanks 149
3-10w Descriptive Statistics for Averaged Real-Time PM10 Exposures Measured by Personal MicroPEMs 152
3-10x Descriptive Statistics for Averaged Real-Time Indoor Temperature and Relative Humidity from HOBO 153
3-11a Distribution of Laboratory Measurements for Environmental Samples: Carbonyls in Air 163
3-11b Distribution of Laboratory Measurements for Environmental Samples: Phthalates in Dust 165
3-11c Distribution of Laboratory Measurements for Environmental Samples: Environmental Tobacco Smoke in PM10 166
3-11d Distribution of Laboratory Measurements for Environmental Samples: Air PM10 166
3-11e Distribution of Laboratory Measurements for Environmental Samples: NO2 in Air 167
3-11f Distribution of Laboratory Measurements for Environmental Samples: H2S 167
3-11g Distribution of Laboratory Measurements for Environmental Samples: VOC in Air 168
3-11h Distribution of Laboratory Measurements for Analyses of Biospecimens: Complete Blood Count (CBC) Results 172
3-11i Distribution of Laboratory Measurements for Analyses of Biospecimens: Cotinine in Urine Results 173
3-11j Distribution of Laboratory Measurements for Analyses of Biospecimens: Clinical Urine Results 173
3-11k Distribution of Laboratory Measurements for Analyses of Biospecimens: Immunoglobulin E (IgE) Results 173
3-11l Distribution of Laboratory Measurements for Analyses of Biospecimens: Urinary VOC and Phthalate Metabolites 174
3-11m Distribution of Microbiologic Measurements for Analyses of MicroPEM Filters 177
3-11n Distribution of Microbiologic Measurements for Analyses of Vacuum Dust 178
3-12a Number of Substudy Participants with Complete Residence Information Required for the Air Exchange Rate Modeling and the Distribution of Modeled Daily Air Exchange Rate (h-1) by Study Phase and Season 179
3-12b Pearson (top panel) and Spearman (bottom panel) Correlation Coefficients between the Four PM10 Sampling Platforms during Baseline 182
3-12c Pearson (top panel) and Spearman (bottom panel) Correlation Coefficients between the Four PM10 Sampling Platforms during Follow-up 183

3-12d Pearson (top panel) and Spearman (bottom panel) Correlation Coefficients of Benzene Concentrations from Three Sampling Platforms during Baseline 185
3-12e Pearson (top panel) and Spearman (bottom panel) Correlation Coefficients of Benzene Concentrations from Three Sampling Platforms during Follow-up 186
3-12f Pearson (top panel) and Spearman (bottom panel) Correlation Coefficients of Formaldehyde Concentrations from Three Sampling Platforms during Baseline 187
3-12g Pearson (top panel) and Spearman (bottom panel) Correlation Coefficients of Formaldehyde Concentrations from Three Sampling Platforms during Follow-up 188
3-12h Pearson (top panel) and Spearman (bottom panel) Correlation Coefficients of Nitrogen Dioxide Concentrations from Three Sampling Platforms during Baseline 189
3-12i Pearson (top panel) and Spearman (bottom panel) Correlation Coefficients of Nitrogen Dioxide Concentrations from Three Sampling Platforms during Follow-up 190
3-12j Principal Components of Benzene, Formaldehyde, PM10, and ETS Concentrations Measured on Four Sampling Platforms during Baseline 191
3-12k Pearson (top panel) and Spearman (bottom panel) Correlation Coefficients of Endotoxin Concentrations from Three Sampling Platforms during Baseline 193
3-12l Pearson (top panel) and Spearman (bottom panel) Correlation Coefficients of Endotoxin Concentrations from Three Sampling Platforms during Follow-up 194
3-12m Pearson (top panel) and Spearman (bottom panel) Correlation Coefficients of Glucan Concentrations from Three Sampling Platforms during Baseline 195
3-12n Pearson (top panel) and Spearman (bottom panel) Correlations of Glucan Concentrations from Three Sampling Platforms during Follow-up 196
3-12o Pearson (top panel) and Spearman (bottom panel) Correlations between ETS Concentrations from Three Sampling Platforms, Urinary Cotinine, and Urinary Cotinine Adjusted for Creatinine from Biospecimens and Samples Collected During Baseline 197
3-12p Pearson (top panel) and Spearman (bottom panel) Correlations between ETS concentrations from Three Sampling Platforms, Urinary Cotinine, and Urinary Cotinine Adjusted for Creatinine from Biospecimens and Samples Collected During Follow-up 198
3-12q Pearson and Spearman Correlations between Benzene Concentrations from the Personal Platform and Urinary Metabolite PMA from Biospecimens Collected During Baseline and Follow-up 199
3-12r Pearson and Spearman Correlations between Phthalate Concentrations from Dust and Urinary Metabolites from Biospecimens and Samples Collected During Baseline and Follow-up 200
4-1a Confidence of Interviewee Regarding Responses to Certain Modules of the Baseline Instrument 202

List of Figures

Figure		Page
2-1a	Process of Sampling Frame Development for Exposed Children Sample	18
2-1b	Map of Feasibility Study Counties/Parishes	19
2-6a	Personal Exposure Monitoring (PEM) Platform	48
2-6c	Residential Outdoor Air Measuring Platform	51
2-6d	LA DEQ Monitoring Site in Kenner, Louisiana	54
2-6e	Properly Packed Participant Tray Ready for Shipment to RTI	55
2-8a	Icepack Conditions as Indicator of Temperature Condition of Participant Boxes upon Receipt at RTI	66
3-1a	Gender Breakdown of Survey Respondents	76
3-1b	Survey Respondent Representation	76
3-1c	Breakdown of How Participants Reported Hearing about CHATS Forum	76
3-1d	Compiled Data from All Sites for Questions 1-7 of the Survey Regarding Forum Content	77
3-1e	Louisiana: Participant Willingness to Encourage Participation in the CHATS Study	78
3-1f	Mississippi: Participant Willingness to Encourage Participation in the CHATS Study	78
3-7f	Distribution of the Waking-Time Wearing Compliance of the PEM Platform during Baseline and Follow-up	107
3-10a	Comparison of Phthalate Metabolite Data Generated by LSU Interim Hospital and RTI for Frequently Measured Analytes (Other than DEHP Metabolites)	117
3-10b	Structure of Di(2-ethylhexyl) Phthalate (DEHP) and Various Metabolites	119
3-10c	Comparison of VOC Metabolite Data Generated by LSU Interim Hospital and RTI	125
3-10d	Control Chart for Formaldehyde Proficiency Testing Data	127
3-10e	Control Chart for BTX Proficiency Testing Data	128
3-10f	Chromatogram Showing Carbonyl DNPH Derivatives from Unexposed Badge Portions	130
3-10g	Concentrations of Selected DNPH Derivatives in Extracts (ng/mL) from Unexposed Badge Portions	131
3-10h	Carbonyls Measured in Extracts (Background-Corrected, ng/mL) from Badges Exposed for 7 Days Near a Roadway Following Storage	132
3-10i	Stability Testing of Archived Carbonyl Samples	133

3-10j Stability Testing of Archived VOC QC Samples 134
3-10j Carbonyls Continuing Calibration Check Standard Control Chart 139
3-10k Carbonyls Method Controls, Control Chart 140
3-10l VOCs Continuing Calibration Check Standard Control Chart – Low-Boiling Analytes 141
3-10m VOCs Continuing Calibration Check Standard Control Chart – Other Analytes (MTBE = methyl t-butyl ether; MEK = methyl ethyl ketone; TCE = trichloroethylene; p-DCB = p-dichlorobenzene) 142
3-10n VOCs Method Controls, Low-Boiling Analytes, Control Chart 143
3-10o VOCs Method Controls, Other Analytes, Control Chart 143
3-10p Dust Phthalates Method Controls, Control Chart 144
3-10q Dust Phthalate Continuing Calibration Check Standard, Control Chart 145
3-10r Spiked Extract Recoveries 150
3-10s QA-CPI_Low for NO_2 150
3-10t QA-CPI_Med-Hi for NO_2 150
3-10u QC-HIGH for NO_2 150
3-10v QC-Low for NO_2 151
3-10w QC-MED for NO_2 151
3-10x Duplicate Sample Analysis 151
3-10y Method Blanks 151
3-10z % Recovery NO_2 Lab Control Samples 151
3-10aa Distribution of Averaged Real-Time PM_{10} Concentrations Measured by Personal MicroPEM 153
3-10bb Distribution of Field Record Quality Indicators by Sample Type 154
3-10cc Distribution of Median Laboratory Record Quality Indicators by Sample Type 155
3-10dd Effect of Sample Collection Location on Field Record Quality Indicators by Sample Type 156
3-10ee Distribution of Laboratory Record Quality Indicators by Analyte for Air Aldehydes 157
3-10ff Distribution of Laboratory Record Quality Indicators by Analyte for Dust Phthalates 157
3-10gg Distribution of Laboratory Record Quality Indicators by Analyte for Air VOCs 158
3-10hh Distribution of Laboratory Record Quality Indicators by Analyte for Dust Microbiologicals 158
3-10ii Distribution of Laboratory Record Quality Indicators by Analyte for Air µPEM Dust Microbiologicals 159
3-10jj Distribution of Laboratory Record Quality Indicators for Air µPEM Environmental Tobacco Smoke 159

3-10kk Distribution of Laboratory Record Quality Indicators for Air µPEM Mass 159
3-10ll Distribution of Laboratory Record Quality I 160
3-10mm Distribution of Laboratory Record Quality Indicators for Air Hydrogen Sulfide 160
3-10nn Distribution of Laboratory Record Quality Indicators for Urinary Cotinine 160
3-10oo Distribution of Laboratory Record Quality Indicators for Air Nitrogen Dioxide 161
3-10pp Distribution of Laboratory Record Quality Indicators by Analyte for Urinary Phthalate Metabolites 161
3-12a Comparison of Indoor and Personal PM_{10} Concentrations 184
3-12b Principal Components 1 and 2 for Concentrations of Benzene, Formaldehyde, PM_{10}, and ETS Measured on Four Sampling Platforms during Baseline 191
3-12c Principal Components 1 and 3 for Concentrations of Benzene, Formaldehyde, PM_{10}, and ETS Measured on Four Sampling Platforms during Baseline 192

EXECUTIVE SUMMARY

On August 30, 2010, the Centers for Disease Control and Prevention (CDC) awarded a contract to RTI International to conduct the Feasibility Study phase of a longitudinal health study (known as the Children's Health after the Storms [CHATS] study). The purpose of the study was to assess the potential health effects of environmental exposures to Federal Emergency Management Agency (FEMA)-provided temporary housing units (THUs) among children who had resided in areas affected by Hurricanes Katrina and Rita. On May 7, 2012, the Office of Management and Budget (OMB) gave approval to conduct the CHATS study. The approved study had been reviewed and approved by the Institutional Review Boards (IRBs) of RTI, CDC, and the Louisiana State University Health Sciences Center. By design, the Feasibility Study was to include developing and accessing study materials and conducting field data collection, including Baseline and Follow-up health and environmental exposure data, on approximately 500 children. This target was later changed to 420 children. Field data collection for the Baseline Assessments began on May 8, 2012, and the 6-month Follow-up Assessments were completed on May 24, 2013; abstraction of medical records was completed on June 15, 2013. The purpose of the Feasibility Study was to collect data that CDC would use to determine whether a Full Study phase of the CHATS study would be practical and could be implemented. The objectives of the Feasibility Study were as follows:

- Assess the feasibility of locating, enrolling, and retaining study participants.
- Assess the feasibility of locating medical records.
- Evaluate operational issues of proposed data collection methods (e.g., data quality, selection bias, information bias, health, effectiveness of exposure assessment methodology).

RTI collected health and environmental exposure data for 174 children at the Baseline Assessment and 155 children at the Follow-up Assessment. In total, RTI abstracted 142 medical records based on the Baseline interviews and abstracted 49 records based on the Follow-up interviews.

Objective 1: Assess Feasibility of Locating, Enrolling, and Retaining Study Participants

The criteria set by CDC for this objective were the following:

- The pool of available participants is large enough to enroll the required sample size for the Full Study, including sufficient numbers of exposed and unexposed participants:

- At least 25% of persons or households identified in the sampling frame can be located and contacted to assess eligibility.
- At least 50% of eligible persons contacted agree to enroll.

- At least 75% of participants were retained in the study at 1-year Follow-up (<25% attrition rate).

Two different populations of children were included in the CHATS Feasibility Study: exposed children who had lived in the FEMA-provided THUs, and unexposed children who had never lived in the FEMA-provided THUs. FEMA provided a database of the adult applicants for THUs. This database was used as the sample frame to select 1,814 applicants for the exposed cohort for the Feasibility Study. RTI found that almost 100% of the selected sample included a Social Security Number for the individuals listed. RTI located 86% of the exposed sample using a combination of batch, interactive, and field tracing methods. We attempted to conduct screening interviews with applicants still living in the target areas who were not deceased or institutionalized. *RTI located and determined the eligibility of 69% of the individuals in the exposed sample.* The Feasibility Study unexposed sample included household addresses in Census blocks in which at least one exposed sample member resided (or Census Block Groups [CBGs] if the number of exposed was small in an area). A total of 1,153 addresses were selected randomly from the United State Postal Service's computerized delivery sequence file, and screening interviews were attempted to determine the eligibility of the household. Of these addresses, 18% were not usable, either because the site was a vacant lot or was not a dwelling unit. RTI screened more than 80% of the households and determined eligibility of 85% of the households in the unexposed sample. ***In summary, the overall locate rate was 92%, and RTI located and determined eligibility for 75% of the total sample, exceeding the criterion.***

The eligibility rates for the two cohorts—exposed and unexposed—differed substantially and were both lower than the rates expected based on Census data. The eligibility rate was 19% for the exposed cohort and 13% for the unexposed cohort. In total, 200 exposed children and 104 unexposed children were selected for Baseline Assessment. ***RTI was able to enroll 64% of the eligible children in the Baseline Assessment, and 61% completed the two-session assessment, exceeding the criterion.***

The period of time for field collection in the Feasibility Study was only 12 months. Consequently, retention was assessed using a 6-month Follow-up Assessment. A total of 174 children were eligible for the 6-month Follow-up Assessment, having completed the Baseline Assessment. ***RTI was able retain 90% of the children in the study for the 6-month Follow-up Assessment.***

Results of the Feasibility Study phase are shown in ***Table ES-1***.

Table ES-1. Targets and Observed Tracing and Recruitment Numbers, by Exposure Status

	Targets Recommended by RTI				Observed during Feasibility Study			
	Exposed		Unexposed		Exposed		Unexposed	
	%	No.	%	No.	%	No.	%	No.
Sample		1,814		1,116		1,814		1,153
Located	72	1,313	80	893	86	1,565	99.7	1,150
Screened or Determined Ineligible before Screening	85	1,116	85	759	80	1,249	85	979
Screened and Eligible	28	313	28	213	19	200	13	104
Enrolled/Completed Baseline Session 1	80	250	80	170	63	125	54	56
Completed Baseline Session 2	100	250	100	170	98	122	93	52
Completed 6-month Follow-up Session 1 and 2	90	225	90	153	87	106	94	49

The Community Advisory Panel (CAP) met throughout the Feasibility Study phase and was invaluable to the CHATS study with their advice on the approach to the community and their support facilitating the outreach campaign. The CAP members distributed posters, broadcast e-mails, and participated in television and radio interviews. E-mail was found to be the most effective means of communication to motivate people to attend the community forums (58% of attendees indicating that e-mail was their source of information).

Objective 2: Assess Feasibility of Locating Medical Records

The criterion set by CDC for this objective was the following:

- At least 25% of health records of participants can be located and reviewed.

A subset of parents were asked to provide permission for the Feasibility Study staff to contact up to three health care providers who had treated the child enrolled in the study. Parents of all children with reported asthma or other signature health outcomes at Baseline Assessment, plus a random selection of the other children, were asked to provide permission. At Follow-up Assessment, permission to access medical records was sought from those parents only under two conditions:

- The parent reported that the child was diagnosed with a signature health outcome since the Baseline Assessment; or
- The parent reported visits to the health care provider since the Baseline Assessment for a signature health outcome.

A total of 182 medical records were identified for abstraction during the Baseline Assessment. RTI found that 15% (27 out of 179 unduplicated records) of the health care providers had some of the target records destroyed by the flooding associated with Hurricanes Katrina and Rita. RTI was able to locate and abstract information for 78% of these. At Follow-up Assessment, 76 medical records were identified, and RTI was able to locate and abstract information for 63% of these records. ***In total, RTI was able to locate and abstract information for 73% of the medical records identified, exceeding the criterion.***

Table ES-2 provides a summary of the criteria for Objectives 1 and 2.

Table ES-2. Summary of Feasibility Study Criteria for Objectives 1 and 2

CDC Criterion	Determination	RTI Target
The pool of available participants (i.e., sampling frame) is large enough to enroll sufficient numbers of "exposed" and "unexposed" children.	***Met***	None specified
At least 25% of persons or households identified in sampling frame can be found and contacted to assess eligibility.	***Met*** The proportion of sampled households that completed a screening interview to assess eligibility (i.e., located and contacted) was 68% among exposed and 84% among unexposed.	62% among exposed; 68% among unexposed
At least 50% of eligible persons contacted agree to enroll.	***Met*** The proportion of eligible households who enrolled in the study was 63% among exposed and 54% among unexposed.	80%
At least 75% of participants are still in the study at 1-year Follow-up.* **Owing to the study design, we will evaluate based on a 6-month follow-up period.*	***Met*** The proportion of participants who completed the 6-month Follow-up was 87% among exposed and 94% among unexposed.	90%
At least 25% of health records of participants can be found and reviewed.	***Met*** The proportion of records that were found and reviewed was 78% at baseline and 63% at Follow-up.	80% of those providing permission for both exposed and unexposed

Objective 3: Evaluate Operational Issues of Proposed data Collection Methods

CDC did not identify specific areas or criteria for this objective. Although no criteria were set, RTI focused on four specific areas: effectiveness of identified biomarkers for assessing exposures, degree of selection bias, degree of information bias, and quality of data.

Effectiveness of Identified Biomarkers and Multiple Platforms for Assessing Exposures

A subset of children, aged 7 and older, were selected for additional testing to evaluate current exposure classification using a variety of methods (i.e., personal monitoring, indoor monitoring, dust collection, outdoor monitoring, central site monitoring [Kenner, Louisiana], and urine metabolite assessment). Comparisons could be made among: particulates, volatile organic compounds (VOCs), carbonyls, and nitrogen dioxide (NO_2) measured in the multiple platforms; VOC parent compounds measured with badges and VOC metabolites measured in urine; and between phthalate parent compounds measured in dust and phthalate metabolites measured in urine. Data were available from four sampling platforms to compare measurements of PM_{10}. As expected, strong positive correlations ($p < .05$) were noted for outdoor and central site concentrations for both the Baseline ($r = 0.661$) and Follow-up ($r = 0.488$) time periods. Consequently, it was concluded that a central site sampler could suffice for outdoor exposures to this pollutant. Similarly, significant positive correlations ($p < .002$) were noted between the personal and indoor samples for both time periods (Baseline $r = 0.853$ and Follow-up $r = 0.892$), indicating that a significant portion of exposure occurs inside the residence. However, the deviation of personal PM_{10} concentration from the indoor PM_{10} concentration often deviates by as much as 50% of the indoor value. Consequently, it was concluded that personal exposure monitoring is needed to accurately assess personal exposures.

Benzene concentrations were reviewed to represent the VOC comparisons, as one of the more frequent VOCs in the samplers. Data available from three sampling platforms indicated that outdoor monitoring is not a good measure of personal exposures to VOCs. Significant correlations ($p < .0001$) were noted between indoor and personal concentrations for both Baseline ($r = 0.663$) and Follow-up ($r = 0.437$). Because the Follow-up correlation was based on twice as many observations and the correlation was low, we conclude that personal measurement is important for VOCs, especially those released by vehicular emissions.

Because of the focus on formaldehyde in this study, formaldehyde concentrations were reviewed to represent the aldehyde comparisons. Concentrations were measured using personal, indoor, and outdoor platforms. Although the correlation between outdoor and personal platforms was significant at Baseline ($r = .326$, $p < .05$), it was low and not significant at Follow-up, leading to the conclusion that outdoor monitoring was not a good surrogate for personal monitors. As with the benzene results, significant ($p < .0001$) but modest correlations were noted at Baseline ($r = 0.643$) and Follow-up ($r = 0.787$). In conclusion, personal exposure measurements were the best measurements. NO_2 was measured using personal, indoor, and outdoor platforms. Personal NO_2 concentrations were significantly positively correlated with indoor concentrations ($p < .0001$) during both Baseline ($r = 0.916$) and Follow-up ($r = 0.957$).

Personal samplers were also significantly correlated with outdoor concentrations during Baseline ($r = 0.492$, $p < .0005$), but not during Follow-up. The Follow-up phase occurred during the winter and spring. Thus, indoor platforms are a good approximation for personal exposure to NO_2 for this population.

Three types of microbiologics (i.e., allergens, endotoxins, and glucans) were measured using two collection methods: MicroPEM™ filters (personal and indoor) and the collection of vacuum dust from participants' bedrooms.

- Allergens were rarely detected on filters, so comparisons of allergens between the filter and dust collection modes were not possible.
- For endotoxins, there was no association of either the personal filter or the indoor platform filter with dust, indicating the indoor dust measurements cannot be used to estimate exposure to endotoxins. Comparisons between the two filter types (personal and indoor) were not significant at Baseline and low but significant at Follow-up ($r = 0.421$, $p < .02$), indicating that the personal platform is the better measure of personal exposure.
- With regard to 1,3-β-diglucans, in general the findings were similar to those of endotoxins. That is, there were no significant correlations between dust and either personal or indoor filters. The conclusion was that dust cannot be used to estimate exposure to glucans. There was a significant correlation between the personal filter and indoor filter at Follow-up ($r = 0.489$, $p < .01$), but not at Baseline. The low correlation indicated that the personal platform is the better measure of personal exposure.

For environmental tobacco smoke (ETS), several platforms (personal and indoor) and urine were available to assess the correlation between ETS measured on the different platforms, and between each of the platforms and the urinary metabolite, cotinine. Personal and indoor ambient concentrations were significantly correlated ($p < .0001$) at both Baseline ($r = 0.913$) and Follow-up ($r = .802$), indicating that indoor ambient concentrations are an important source of exposure among these children. For comparisons between urinary cotinine and ambient ETS measured by either the personal or indoor platform, the Pearson correlations were seldom significant, but Spearman correlations were very significant. This finding may be caused by the different sample collection periods (ambient samples were integrated over 5 to 9 days, whereas the half-life of cotinine in urine is 20 hours). For an integrated assessment of ETS exposures in children, the ambient measure is superior to urine. However, urinary cotinine will be more reflective of recent exposure. Consequently, the desired use of the data, e.g., assessment of health outcomes facilitated by recent exposure to ETS, will drive the selection of integrated air measures vs. urinary cotinine measurements.

Ambient and urinary metabolites were also available for VOCs. The correlations were not significant. Investigations of the relationships between the concentrations measured by each of these modes and health outcomes of interest would be required to better identify the most appropriate measure of exposure.

Dust and urinary metabolites were available for phthalates. No significant associations were found. This result was expected, given the lack of association between indoor dust and personal monitoring filters for endotoxins and glucans.

Degree of Selection Bias

The screening response rate was higher among the unexposed sample (82%) than among the exposed sample (65%). In contrast, however, the participation rate once the household was determined to be eligible was higher among the exposed households. Participation rates for Baseline Session 1 were 67% of exposed households and 57% of unexposed households. Participation rate was high overall for Follow-up Session 1 among those who completed Baseline (91% of exposed households and 89% of unexposed households).

A number of concerns were expressed that certain social factors might affect the willingness of people in the Gulf Coast area to participate in the CHATS study. Among these factors were the extensive litigation activities, presence of asthma among children in the immediate family, and the health insurance status of the household. Responses were available from the screening interview for 25 households with eligible exposed children who elected not to participate. (Of these, seven refused to answer the questions regarding litigation and seven refused to answer questions regarding asthma among children in the household.) Responses were also available from the Baseline Assessment interview for 181 participants. The numbers responding to the screener were too few to make statistical conclusions. However, the percentage currently part of a lawsuit was less among nonparticipants and among participants (1 out of 25 [5%] and 32 out of 181 [18%], respectively). Having at least one child in the household diagnosed with asthma (not necessarily the participant child) may have been a motivating factor for participation (11% and 40% of families had at least one child with asthma, respectively). However, the number of nonparticipants with responses was small, so the degree of selection bias is not conclusive.

In terms of potential bias in the selection of the sample, the sampling probabilities for the exposed sample ranged considerably. However, the variation was largely due to intentional over- and undersampling in the various strata (county/parish and temporary housing unit type [group/private]) to achieve a minimum sample size within each substratum.

Another concern was whether the additional time burden of the exposure misclassification substudy might lead to reluctance by the participant to complete the two-session visit. The average length of Session 1 of the Baseline Assessment (in which the deployment of environmental monitors occurs) was 140 minutes (substudy sessions were longer because three platforms were deployed). Of the five participants who did not complete Session 2 of the Baseline Assessment, none was a substudy participant. Among the 174 participants who did complete both sessions of the Baseline Assessment, 49 were substudy participants. Four of the substudy participants (8%) refused the Follow-up Assessment, which is consistent with the refusal rate of 9% of the total. Therefore, the added time burden of the multiple platform deployment was not a deterrent to retention in the study.

Degree of Information Bias

Given the long time period between the Hurricanes and the CHATS interviews, information bias based on recall of the THU residence experience was a concern. From the FEMA database of applicants, it was determined that 8% of the participants (N = 33) and 8% of the applicants overall were given more than one THU. However, 28% of the participants reported that the child lived in more than one THU. Of the 33 participants reporting living in more than one THU, only 4 were indicated as having had more than one THU on the FEMA list. The extent of bias is difficult to assess because the child may have lived, at least part time, in THUs that were not the one allocated to the FEMA-list household applicant.

Given the widespread interest in litigation regarding the post-Hurricanes living experience, assessing information bias regarding health outcomes was also a focus of the Feasibility Study. The signature health outcomes reported with the highest frequency were asthma (31% of the participant children [55] were reported to have had a diagnosis of asthma prior to the Baseline Assessment). Medical records were accessed and abstracted for 53 of these participants. Of the self-reported asthma diagnoses, 53% were verified in medical records. This number may not reflect overreporting, however, because only up to three health care providers were contacted per participant for access to their medical records. In terms of underreporting, of the 87 participants who did not report asthma, but had medical records abstracted, a mention of asthma diagnosis was found on the records of 15% (13) of the children.

Quality of Data

The data from FEMA, which had been revised by CDC, were complete. Critical data elements, such as the Social Security Number, were 100% complete in the file.

RTI used three methods to ensure that the questionnaires were administered following standardized protocols, that all information collected was reflective of the intended answers of

each participant, and that no participant rights were violated during interactions with the CHATS field interviewers: computer-audio-recorded interview (CARI) reviews, verification calls, and field observations. In total, about 50% of Baseline visits and 40% of Follow-up visits were reviewed.

In a complex study such as CHATS, data quality depends on a number of factors, including staff training (field and laboratory staff); the instruments and operation of the equipment (ambient monitoring, dust collection, and physiologic testing); shipping and handling of biospecimens and samples; and conduct of laboratory tests. While field interviewers left training with a strong overall understanding of the CHATS instruments, they experienced some confusion between the various types of deployment and retrieval processes for the environmental equipment. This confusion accounted for some loss of usable data when the equipment was not deployed correctly. The complexity of the data collection protocols for the Health Assessment, combined with the challenge of collecting data in the home, meant that staff required education and capabilities of at least the registered nurse level. However, even with these skills and knowledge, the nurses did experience challenges obtaining usable tests with the equipment, most particularly the exhaled nitric oxide (eNO) measurements.

Compliance by participants is another critical issue affecting quality of the data. In this study, both fixed monitoring platforms (indoor and outdoor) and PEM platforms were utilized. There was only one report of tampering with any of the fixed platforms deployed for the 5- to 9-day periods; specifically, an indoor platform was moved from the kitchen to the child's bedroom. The personal monitoring required cooperation of the children aged 7 years and older to wear the PEM platform each day for the week. In three instances, the participants tampered with the platforms by removing the instruments and replacing them, and in one instance the family dog destroyed the platform. Waking-time wearing compliance could be assessed because each device included an accelerometer that indicated whether the device was being moved. More than 85% of the children wore the PEM platform more than 50% of the time they were awake. This compliance is well above previously reported data among children (40%–60%).

Samplers were shipped to RTI and biospecimens were shipped to the Louisiana State University Interim Hospital. There were external challenges to the shipping, such as Hurricane Isaac, which substantially delayed the arrival of shipped materials, and some operational issues, such as the inability of the hospital to accept courier drop-offs on Saturdays or Sundays. Although none of the environmental specimens were significantly impacted by delays, receipt by the laboratory was significantly delayed for about 13% of the biospecimens, to the extent that the data were of poor quality. Among the various environmental specimens, the most significant issues were handling problems, such as field interviewer operational errors for 17%

of the MicroPEM deployments, torn membranes on 4% of the VOC badges, or an inability to slide the bar in 2% of the aldehyde badges.

To help assess laboratory performance, duplicate samplers were deployed for carbonyl badges, hydrogen sulfide sorbent tubes, nitrogen dioxide (NO_2) badges, and VOC badges. For most analytes, the mean percentage difference between pairs of samplers was less than 10%, suggesting good agreement. For acetaldehyde and propanal, the differences were less than 20%. However, the acrolein analyte was found to be unreliable, because this aldehyde derivative is unstable on the badge. Field blank samplers were low and fairly consistent, suggesting that there was little contribution from sample handling and shipment. However, toluene gave some high values, suggesting that some contamination of the VOC badges occurs during sample handling and shipment. Three stability studies were conducted (for carbonyl badge storage, for formaldehyde extract storage, and for VOC extract storage). Guidelines for the assignment of record quality indicators were derived from these studies. For instance, for formaldehyde extracts stored for more than 12 weeks, a quality indicator of 1 was assigned, whereas for the other analytes, a quality indicator of 2 was assigned.

In terms of the laboratory findings for controls, all carbonyl analytes in all of the batches met control recovery objectives. Given the high volatility for both the VOC analytes and solvents, some analytes presented issues with regard to poor recovery from method controls. Vinyl chloride and butadiene yielded largely unreliable data (variable recovery and analysis results), but the consequences were relatively insignificant because these analytes were usually not detected in the study samples. Alpha-pinene also presented some recovery and variability issues that were resolved prior to the final testing. For phthalates in dust, the analytes were generally stable. However, the tests for benzyl butyl phthalate frequently failed on check standards, possibly due to the use of a nonanalogous labeled internal standard. Method controls were acceptable except for benzyl butyl phthalate and diethyl phthalate. For the microbiologics analyzed in dust samples, the R^2 for the positive and negative controls was higher than 0.95 for all analytes. For NO_2, the three methods of quality control/quality assurance testing (i.e., spiked extract recoveries, duplicate assessments for analytical precision and blanks) were all acceptable (agreement within 10%, recoveries between 90% and 110%). Individual MicroPEM filters were gravimetrically and optically analyzed, respectively, for PM_{10} mass and ETS. Most field blanks (N = 37) showed weight differences of less than 3µg for repeat measurements.

A comparison between results obtained in two laboratories was conducted for a subset of urine samples for phthalate and VOC metabolites. For phthalates, good agreement was found for most metabolites of frequently measured analytes. However, for di(2-ethylhexyl) phthalate (DEHP) metabolites, the best marker of exposure was mono(2-ethyl-5-oxohexyl)

phthalate (a DEHP metabolite, MEOHP). For VOC, many of the metabolites were consistently not found or found at very low concentrations. However, good associations were found between the two laboratories for those analytes present in higher concentrations.

Proficiency testing was conducted for formaldehyde and VOCs (benzene, toluene, and xylenes). The highest performance rating (= 1) was obtained for both throughout the testing period.

Recommendations for a Full Study

The following are recommendations for implementation in a Full Study.

Study Design

- Retain stratification based on site of the THU (private versus group).
- Include residential history and characteristics and regular activities in past homes, as well as the health history of the child, in the instruments.

Sample Design

- Use the same FEMA applicant file for the sample.
- Use a GIS approach to help link the sample design with the data collection process.
- Use the strategy of searching for unexposed households in close proximity to the exposed household.
- Use the lower level of eligibility rate found in the Feasibility Study for estimating sample size.

Subject Recruitment

- Plan for a longer time period to recruit and hire field interviewers. Hiring outside of the New Orleans Metro area, with field interviewers traveling into the area by car, should be considered.
- Extend training time focused on the deployment and retrieval of the various types of samples.
- Have training kits available from the beginning of data collection and schedule routine group calls throughout data collection as refreshers on procedures and any observed common errors to improve quality.
- Change the selection scheme to consider including more children in each family for selection.
- Shorten the interview sessions. Review items carefully to determine which could be dropped to reduce the burden.

Environmental Assessment

- Conduct more research to understand personal exposure monitoring (PEM) platform preferences in relationship to compliance.
- Engage children more throughout the study, so that they understand the purpose, value the sampling activities and compliance to protocols, and have ownership of research.
- Apply new informative tools for dissemination to schools and the participants.
- Use a just-in-time mail-out approach for the samplers to provide quality data.

Health Assessment

- Enhance screening during nurse recruitment to ensure a better understanding of the position requirements and avoid problems implementing the protocol.
- Simplify computer programs or allow manual recording of data from the NIOX machine to improve data quality.
- Conduct venipuncture at the beginning of the session.

Laboratory Analysis

- Use research clinical laboratories with automated reporting capabilities.
- Extract and analyze data from VOC badges at time of receipt to avoid storage impacts on some analytes.
- Evaluate linearity of collection efficiency for the VOC badges for periods of time from 5 to 9 days.
- Verify that nonlinear sampling behavior is not occurring for formaldehyde for sampling periods longer than 5 to 7 days if samplers will be deployed for longer than 7 days.
- Continue urine-based VOC metabolite determinations.
- Conduct research of the biomarkers and associated or parent analytes evaluated in CHATS to determine the most appropriate measures for the health outcome(s) under study.
- Include additional QA at the laboratory-level data preparation so more efficient processing can be accomplished.

Outreach

- Retain a Community Advisory Panel.
- Engage well-regarded community organizations and leaders as information dissemination channels and supports to expand the reach of awareness efforts.
- Schedule public forums outside of major festivals, holidays, and other key events. Also, partner with major family or health-related events scheduled by other leading organizations and collaborate with them to present the study.
- Increase the funding allocated to the media/campaign to help extend the reach and depth of public awareness.

1. BACKGROUND

1.1 Purpose of the CHATS Study

On August 29, 2005, Hurricane Katrina made landfall as a Category 3 storm on the U.S. Gulf Coast between New Orleans, Louisiana, and Mobile, Alabama. Soon after on September 24, 2005, Hurricane Rita made landfall as a Category 3 storm between Sabine Pass, Texas, and Johnsons Bayou, Louisiana. Families from the Gulf Coast were evacuated during the storms and later returned to severely damaged housing. The Federal Emergency Management Agency (FEMA) provided disaster-related housing along the Gulf Coast beginning in October 2005. At that time, FEMA typically addressed disaster-related housing requirements with a combination of different types temporary housing units (THUs), including travel trailers, mobile homes, and park models. FEMA uses THUs principally for short-term housing needs. These housing units are either placed on private sites while a homeowner's permanent residence is being repaired (i.e., private THUs), or placed in group configurations to support displaced renters (i.e., group THUs).

In the spring of 2006, several physicians along the Gulf Coast observed an increased reporting of upper respiratory illnesses among children who lived in FEMA-provided THUs following Hurricanes Katrina and Rita. Residents of FEMA-provided THUs expressed concerns about formaldehyde levels in their units and possible adverse health effects. In response to these concerns, the Centers for Disease Control and Prevention (CDC) conducted a case-series investigation in Hancock County, Mississippi, to assess the overall occurrence of respiratory diseases among children between August 2004 and August 2007. In 2007, FEMA requested that CDC conduct four studies related to indoor air exposures in FEMA-provided THUs and provided funding for doing so. Findings from the first three studies are available at http://www.cdc.gov/nceh/ehhe/trailerstudy/default.htm. The purpose of the fourth study was to determine whether there is an association between poor indoor air quality and adverse health effects among children who lived in FEMA-provided THUs following Hurricanes Katrina and Rita.

In May 2009, CDC released a Request for Proposals to design and conduct a longitudinal health study to assess the potential health effects of environmental exposures to FEMA-provided THUs among children who had resided in areas affected by Hurricanes Katrina and Rita. The study was designed in two phases: a base period 2-year Feasibility Study phase, and an optional 6-year Full Study phase. On August 31, 2010, RTI was awarded a contract—known as the Children's Health after the Storms (CHATS) study—to conduct the 2-year Feasibility Study of approximately 500 children in the affected areas. This report describes the Feasibility Study phase of CHATS and contains information on the development and assessment of study

materials and data collection procedures, including Baseline and Follow-up health and environmental exposure data.

1.2 CHATS Study Objectives

The primary objective of the Full Study is to determine if there is an association between prior occupancy[1] in FEMA-provided THUs [2] and adverse health effects among children who had resided in storm-affected areas at the time of Hurricanes Katrina or Rita. Three types of adverse health effects will be assessed in the Full Study: (1) short-term symptoms or diagnostic conditions that have since resolved, (2) long-term effects that are still present, and (3) increased sensitivity to current exposures. To accomplish this assessment, the CHATS study will obtain retrospective and prospective information on exposure and health. Specifically, information on the residential, exposure, and medical history of children through interviews with parents and medical record abstraction will be collected to assist in characterizing short-term symptoms or diagnostic conditions that have since been resolved. To assess the current health status and the development of any increased sensitivity to current exposures, the study will obtain information on children through a physical assessment and measurement of current and ongoing exposures to specific contaminants through (a) tests on biospecimens, (b) air and dust monitoring of the house and neighborhood, and (c) personal exposure measurements using a portable air samplers (such as the MicroPEM™).

To determine whether a Full Study is practical, the Feasibility Study, by design, included all of the same measurements and procedures proposed for the Full Study. However, given the small sample sizes of the Feasibility Study, associations between exposures and health outcomes were not assessed.

1.3 Purpose of the Feasibility Study

The primary purpose of the Feasibility Study was to determine the feasibility of conducting the Full Study phase of the CHATS study. Because Hurricanes Katrina and Rita occurred several years before the CHATS study began, CDC was concerned that researchers would not be able to locate participants and medical records. Further, CDC was concerned that participants would not remain in the study for the longitudinal visits. CDC will use the data collected during the Feasibility Study to determine whether a Full Study is practical. The objectives of the Feasibility Study were as follows:

- Assess feasibility of locating, enrolling, and retaining participants;

[1] "Prior occupancy" refers to the period after September 2005.

[2] A FEMA-provided temporary housing unit refers to a temporary housing unit that was provided to a resident whose home was impacted as a result of Hurricane Katrina or Rita.

- Assess feasibility of locating medical records; and
- Evaluate operational issues of proposed data collection methods (e.g., data quality, selection bias, information bias, health, and exposure assessment methodology).

CDC set several criteria for the Feasibility Study phase to determine whether the Full Study phase would be implemented. Those criteria included the following:

- The pool of available participants is large enough to enroll the required sample size for the Full Study, including sufficient numbers of exposed and unexposed participants:
  - At least 25% of persons or households identified in the sampling frame can be located and contacted to assess eligibility; and
  - At least 50% of eligible persons contacted agree to enroll.
- At least 75% of participants were retained in the Feasibility Study at a 1-year Follow-up Assessment (<25% attrition rate).
- At least 25% of health records of participants can be located and reviewed.
- Adequate funding is available for the Full Study phase.

The work described in this report addresses the first three criteria.

1.4 Study Design

The Feasibility Study phase included a Baseline Assessment and a 6-month Follow-up Assessment. Each assessment consisted of two home visits by field staff—referred to as Session 1 and Session 2 for both assessments—that occurred approximately 1 week apart. Before the Baseline Assessment began, a field interviewer went to the homes of potential participants to conduct a brief eligibility screening interview (using a handheld computer) with an adult at least 18 years of age. If the household had an eligible child, the field interviewer spoke with the adult parent/guardian about the study and asked for consent to participate.

During Session 1 of the Baseline Assessment, the field interviewer administered the health and environmental exposure questionnaire, performed a visual home inventory, set up the exposure assessment equipment, explained a procedure for the study's Time and Activity Diary, and instructed the parent on the use of a cell phone-sized personal exposure platform that included the MicroPEM and other instruments that children aged 7 years and older wore for 1 week. A stationary monitoring platform was placed in the homes of the younger children. During Session 2 of the Baseline Assessment, a registered nurse accompanied the field interviewer to administer a Health Assessment to the child, which included measuring the height and weight of the child, assessing the child for dermal rashes, conducting respiratory assessments, and obtaining biospecimens (blood and urine). The field interviewer administered

an exposure questionnaire, recorded all information gathered from the exposure assessment equipment, entered data from the Time and Activity Diary directly into the laptop, and collected global positioning system (GPS) information. Either at the time of Session 1 or Session 2, the field interviewer drove around the neighborhood, within a half-mile radius, and noted potential contaminant sources, such as dry cleaners. The procedures for the 6-month Follow-up Assessment were the same as the Baseline Assessment, except that blood was not collected during Session 2.

For a select group of the children aged 7 years and older, a substudy assessment was conducted in which—in addition to the personal exposure monitoring device the child wore—stationary monitoring devices were placed inside and outside the child's primary residence.

In addition, medical record abstraction was conducted on 30% of the children, which included all children with self-reported asthma or other signature health outcomes and a random sample (n = 50) of the other children. Health care providers were identified by the parent/guardian. Abstraction was conducted only with those providers for whom the parent/guardian provided consent for the study to contact.

Finally, a central location was chosen in the New Orleans area at a Louisiana Department of Environmental Quality (LDEQ) monitoring station in Kenner, Louisiana, where an outdoor monitoring station was established; ambient contaminants were then assessed for 90 days during each of the Baseline and 6-month Follow-up Assessments.

Further details of the study design and implementation are discussed in **Chapter 2**.

2. STUDY METHODS

2.1 Sample Design

This section describes the sampling plan for the CHATS Feasibility Study. The Feasibility Study sample was selected from two different populations of children: exposed and unexposed.

2.1.1 Exposed Sample

The sample of exposed children was based on a FEMA database that CDC provided. The FEMA database was a list of adult applicants for THUs, where each adult represents a household that lived in a THU. Consequently, exposed children were not sampled directly; rather, they were sampled indirectly by sampling adult applicants from the FEMA database list, and then sampling an eligible child in the eligible households.

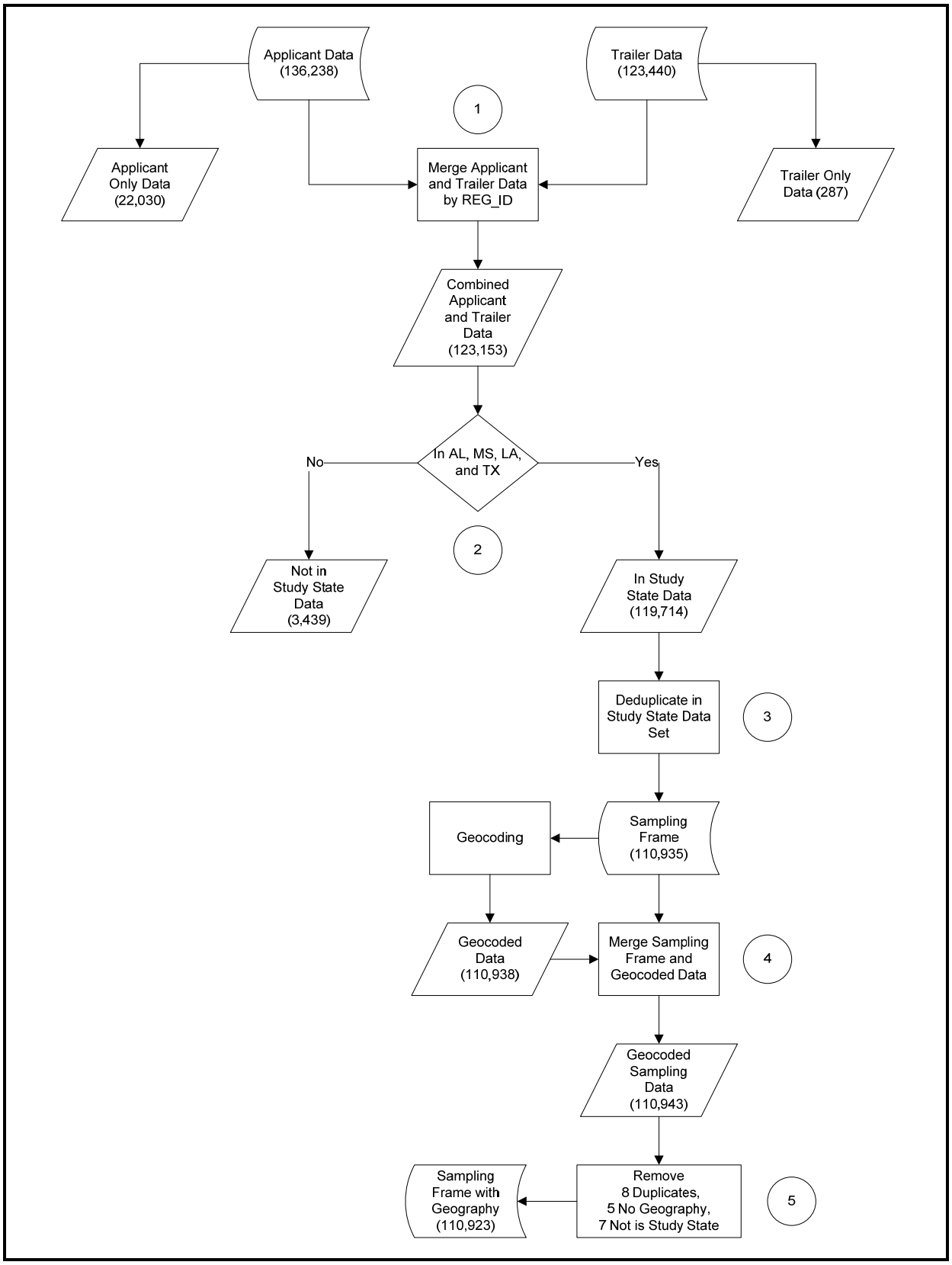
Sampling Frame Development for the Exposed Sample

The sampling frame development for the exposed sample began with the databases that RTI received from CDC and consisted of five steps:

1. Merge applicant and trailer datasets from CDC.
2. Restrict applicant/trailer dataset to most recent address in one of the study states (Louisiana and Mississippi).
3. De-duplicate the state dataset because some applicants had more than one trailer.
4. Geocode the de-duplicated dataset using the most recent address.
5. Remove anomalous observations (i.e., address fell on county/parish line and listed in multiple parishes, no geographic information, or state not one of the study states) from the geocoded dataset.

At the end of this process, there were 110,923 applicants. ***Figure 2-1a*** contains a detailed schematic of the sampling frame development process. Each of the five steps is denoted on the schematic with a number in a circle that corresponds to the steps in the sampling frame development process outlined above.

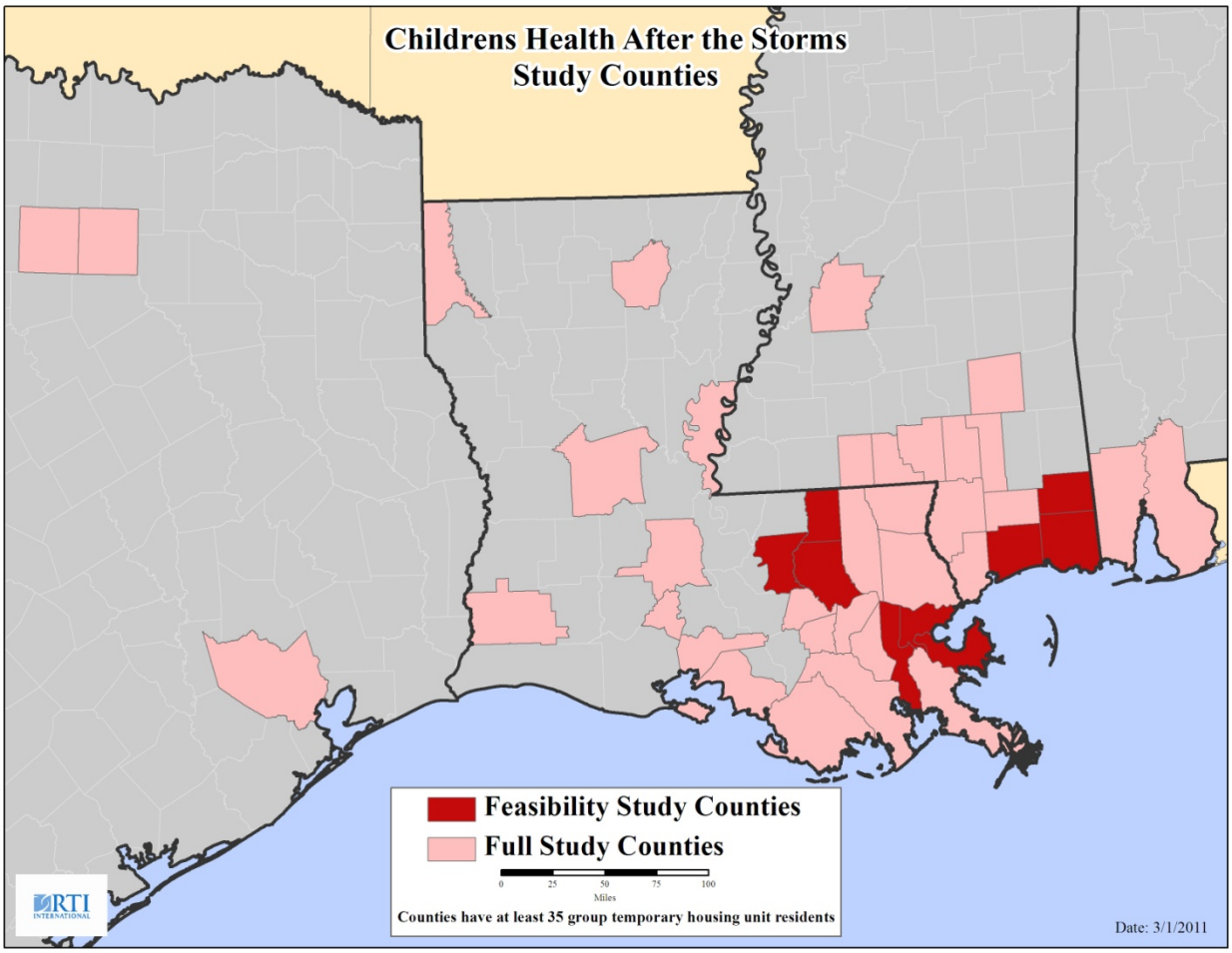
Figure 2-1a. Process of Sampling Frame Development for Exposed Children Sample



Stratification for the Exposed Sample

Six parishes in Louisiana (East Baton Rouge, Jefferson, Livingston, Orleans, St. Bernard, and St. Helena) and three counties in Mississippi (George, Harrison, and Jackson) were designated to be in the Feasibility Study. In Louisiana, the parishes were in two groups of three parishes each. In Mississippi, there was a single group of three counties. The counties/parishes represented a mix of rural and urban parishes/counties as well as a range of population densities. See ***Figure 2-1b*** for a map of the nine parishes/counties that were included for the Feasibility Study, as well as all the counties/parishes that appear on the FEMA frame. The counties/parishes were included as part of the stratification scheme. We further stratified each of these counties/parishes by THU type (i.e., group or private). Therefore, the explicit sampling strata were a cross-classification of county/parish and THU type. Finally, we stratified implicitly by sorting by Census tract to allocate the sample within the above-described explicit sampling strata.

Figure 2-1b. Map of Feasibility Study Counties/Parishes



Sample Size and Allocation for the Exposed Sample

The total sample size for the Feasibility Study was 1,814 exposed applicants. The sample was allocated proportionally across the sampling strata (i.e., cross-classification of county/parish and THU-type combination), with a minimum number of applicants sampled in each sampling stratum. For the group THU sampling strata, a minimum of 33 applicants per stratum was sampled. For the private THU sampling strata, a minimum of 33 applicants per stratum was sampled. If a sampling stratum had fewer applicants than the sample size, all applicants in the sampling stratum were selected. Once the sample was allocated across the sampling strata, it was proportionally allocated to the Census tracts within the sampling strata. ***Table 2-1a*** shows the Feasibility Study counties/parishes for the group THU residents (exposed) with sample allocation, and ***Table 2-1b*** shows the Feasibility Study counties/parishes for the private THU residents (exposed) with sample allocation.

Applicant Selection for the Exposed Sample

In general, sample selection was stratified using simple random sampling with proportional allocation (i.e., proportional allocation with a specified minimum number in each sampling stratum). In the first stage, an applicant's probability of selection was the number of applicants selected in his/her sampling stratum divided by the total number of applicants in that stratum. That is, the probability of selection for the ith applicant in the hth sampling stratum, p_{hi}, is

$$p_{hi} = \frac{n_h}{N_h},$$

where n_h is the number of applicants selected in the hth sampling stratum and N_h is the total number of applicants in the hth sampling stratum.

Table 2-1a. Feasibility Study Counties/Parishes for Group Temporary Housing Unit Residents (Exposed) with Sample Allocation

State	County, State	Group Count	County Proportion of Population	Group Sample Size	Required Sample Size (Proportional)	Adjusted Sample Size (Min = 4)	Sample Inflation Factor	Inflated Sample Size	Sampling Fraction	Design Weight
LA	Orleans, LA	3,664	0.2824	126	36	33	8.3367	275	0.0751	13.32
LA	Jefferson, LA	2,220	0.1711	126	22	20	8.3367	167	0.0752	13.29
LA	St. Bernard, LA	1,245	0.0959	126	12	11	8.3367	92	0.0739	13.53
LA	East Baton Rouge, LA	1,397	0.1077	126	14	13	8.3367	108	0.0773	12.94
LA	Livingston, LA	190	0.0146	126	2	4	8.3367	33	0.1737	5.76
LA	St. Helena, LA	36	0.0028	126	0	4	8.3367	36	1.0000	1.00
MS	Harrison, MS	2,591	0.1997	126	25	24	8.3367	200	0.0772	12.96
MS	Jackson, MS	1,491	0.1149	126	14	13	8.3367	108	0.0724	13.81
MS	George, MS	142	0.0109	126	1	4	8.3367	33	0.2324	4.30
Total		12,976	1.0000		126	126		1,052		

Table 2-1b. Feasibility Study Counties/Parishes for Private Temporary Housing Unit Residents (Exposed) with Sample Allocation

State	County, State	Group Count	County Proportion of Population	Group Sample Size	Required Sample Size (Proportional)	Adjusted Sample Size (Min = 4)	Sample Inflation Factor	Inflated Sample Size	Sampling Fraction	Design Weight
LA	Orleans, LA	13,583	0.2489	126	31	29	7.7809	226	0.0166	60.10
LA	Jefferson, LA	17,097	0.3133	126	39	36	7.7809	280	0.0164	61.06
LA	St. Bernard, LA	4,342	0.0796	126	10	9	7.7809	70	0.0161	62.03
LA	East Baton Rouge, LA	1,250	0.0229	126	3	4	7.7809	31	0.0248	40.32
LA	Livingston, LA	498	0.0091	126	1	4	7.7809	31	0.0622	16.06
LA	St. Helena, LA	232	0.0043	126	1	4	7.7809	31	0.1336	7.48
MS	Harrison, MS	9,359	0.1715	126	22	20	7.7809	156	0.0167	59.99
MS	Jackson, MS	7,928	0.1453	126	18	16	7.7809	124	0.0156	63.94
MS	George, MS	274	0.0050	126	1	4	7.7809	31	0.1131	8.84
Total		54,563	1.0000		126	126		980		

Within-Household Child Selection for the Exposed Sample

Once the applicant was selected and contacted, the number of eligible children was determined. Eligible children were defined as those who resided in designated geographic areas based on their FEMA trailer address provided immediately after the storm and who met the following eligibility criteria:

1. Aged 15 years old or less as of June 1, 2011, and born before December 31, 2007;
2. Resided in a household with at least one parent/guardian who was aged 18 years or older;
3. Resided in a household in which the parent/guardian speaks English, Spanish, or Vietnamese;
4. Currently lived in Louisiana and Mississippi;
5. Either resided in the storm-affected areas at the time of Hurricanes Katrina or Rita, or were born after the Hurricanes and have subsequently resided in the storm-affected areas; and

After the number of eligible children in the household represented by the applicant was determined, the probability of selection for the child was divided by the number of eligible children in the household. That is, the probability of selection for the jth child in the ith household in the hth sampling stratum, q_{hij}, was

$$q_{hij} = \frac{1}{c_{hi}},$$

where c_{hij} is the number of eligible children in the ith household in the hth sampling stratum.

Overall Child Probability of Selection and Design Weight for the Exposed Sample

The overall child probability of selection is the product of the applicant probability of selection and the child probability of selection. That is, the overall probability of selection for the jth child in the ith household in the hth sampling stratum, s_{hij}, is

$$s_{hij} = p_{hi} q_{hij}.$$

The design weight for a child is the inverse of the overall child probability of selection. That is, the design weight for the for the jth child in the ith household in the hth sampling stratum, d_{hij}, is

$$d_{hij} = \frac{1}{s_{hij}}.$$

The final probabilities of selection and corresponding weights are presented in ***Table 2-1c***. The number of sampled addresses reported in this table is slightly less than the numbers reported in Tables 2-1a and 2-1b, which presented initial calculations. Table 2-1c presents the final sample sizes.

Table 2-1c. Probability of Selection and Design Weight for the Exposed Children's Address by County/State and Temporary Housing Unit Type

County/State	Temp. Housing Unit Type	Total Number of Addresses	Number of Addresses Sampled	Address Probability of Selection[1]	Address Design Weight[1,2]	Sum of the Address Design Weights[3]
East Baton Rouge, LA	Group	1,397	88	0.0630	15.88	1,397
East Baton Rouge, LA	Private	1,250	17	0.0136	73.53	1,250
George, MS	Group	142	33	0.2324	4.30	142
George, MS	Private	274	31	0.1131	8.84	274
Harrison, MS	Group	2,591	173	0.0668	14.98	2,591
Harrison, MS	Private	9,359	132	0.0141	70.90	9,359
Jackson, MS	Group	1,491	98	0.0657	15.21	1,491
Jackson, MS	Private	7,928	110	0.0139	72.07	7,928
Jefferson, LA	Group	2,220	146	0.0658	15.21	2,220
Jefferson, LA	Private	17,097	258	0.0151	66.27	17,097
Livingston, LA	Group	190	34	0.1789	5.59	190
Livingston, LA	Private	498	30	0.0602	16.60	498
Orleans, LA	Group	3,664	237	0.0647	15.46	3,664
Orleans, LA	Private	13,583	208	0.0153	65.30	13,583
St. Bernard, LA	Group	1,245	82	0.0659	15.18	1,245
St. Bernard, LA	Private	4,342	70	0.0161	62.03	4,342
St. Helena, LA	Group	36	36	1.0000	1.00	36
St. Helena, LA	Private	232	31	0.1336	7.48	232
Total		67,539	1,1814			67,539

[1] Numbers rounded for presentation.

[2] Design weights are calculated for selected address. Addresses not selected have design weight equal to zero.

[3] In a sampling stratum, the sum of the address design weights should equal the number of addresses.

2.1.2 Unexposed Sample

The sample of unexposed children was based on a computerized delivery sequence file (CDSF), also known as address-based sampling. The CDSF database is a complete list of addresses. Consequently, unexposed children were not sampled directly; rather, they were sampled indirectly by sampling addresses from the CDSF list, and then sampling an eligible child in the eligible addresses.

Sampling Frame Development for the Unexposed Sample

The sampling frame for the unexposed sample was derived from the CDSF. The sampling frame was restricted to the household addresses in Census Blocks Groups (CBGs) in which at least one exposed sample member resided based on the updated address.

Stratification for the Unexposed Sample

Six parishes in Louisiana and three counties in Mississippi were designated for inclusion in the Feasibility Study. In Louisiana, the parishes were divided into two groups of three parishes each. In Mississippi, there was a single group of three counties. The counties/parishes represented a mix of rural and urban parishes/counties. See ***Figure 2-1b*** (above) for a map of study counties/parishes. The counties/parishes were included as part of the stratification scheme. Within each of these counties/parishes, we further stratified by THU type (i.e., group or private). Note that for the unexposed sample, THU type does not exist. The addresses were randomly assigned to a THU group type. Therefore, the explicit sampling strata were a cross-classification of county/parish and THU type. Finally, we stratified implicitly by sorting by Census tract to allocate the sample within the above-described explicit sampling strata.

Sample Size and Allocation for the Unexposed Sample

For the unexposed children in the Feasibility Study, we tried to match each exposed sampled unit with a corresponding unexposed unit within the same CBG, to try to implement the matching objective via geographic proximity. Selecting unexposed sample households within the same CBG as the exposed households increased the likelihood that the two households were similar in demographic and socioeconomic characteristics, which are often geographically clustered.

Address Selection for the Unexposed Sample

The sampling methodology was a stratified sequential random sampling whereby the sample was released in waves. In the sample design, CBGs served as strata, and samples were selected independently within each CBG. The goal was to match the number of unexposed sample members and the number of exposed sample members in a CBG; however, some CBGs had slightly fewer unexposed cases. Originally, the overall target samples sizes were 250 exposed and 170 unexposed cases. However, it was important that the unexposed cases be distributed widely across the CBGs with the exposed sample.

Approximately p_1 or 50% of the sample was released when data collection began. About 1 month after data collection started, the second wave of sample was released. The exact proportion of the sample released and its allocation in the second wave were determined

based on the data collection experience in the CBGs from the initial sample release. This process continued for several waves of sample release.

To actually select the sample in a CBG, we generated a uniform random number on the interval from 0 to 1 for each address in the CBG. We then sorted the uniform random number in ascending order. For a CBG, we followed these steps for the sample releases:

1. For the initial sample release, we selected the first $n_{h1} = p_1 * N_h$ addresses on the sorted list, where p_1 is the proportion to be sampled for the initial sample and N_h is the population size of the unexposed sample in h^{th} CBG.
2. For the second wave of sample release, we selected the next $n_{h2} = p_{h2} * N_h$ addresses on the sorted list starting with the $n_{h1} + 1$ address on the sorted list, where p_{2h} is the proportion to be sampled for the second wave in the in h^{th} CBG and N_h is the population size of the unexposed sample in h^{th} CBG.
3. For the third wave of sample release, we selected the next $n_{h3} = p_{h3} * N_h$ addresses on the sorted list, starting with the $n_{h1} + n_{h2} + 1$ address on the sorted list where p_{3h} is the proportion to be sampled for the third wave in the in h^{th} CBG and N_h is the population size of the unexposed sample in h^{th} CBG.
4. We continued this process until one of the following conditions was met: the required number of participants was achieved, the data collection time had expired, or the population in the stratum was exhausted.

Within-Household Child Selection for the Unexposed Sample

Once the address was selected and contacted, the number of eligible children was determined. Eligible children were defined as those who resided in designated geographic areas based on their current address and who met the following eligibility criteria:

1. Aged 15 years old or less as of June 1, 2011, and born before December 31, 2007;
2. Resided in a household with at least one parent/guardian who was aged 18 years or older;
3. Resided in a household in which the parent/guardian speaks English, Spanish, or Vietnamese;
4. Currently lived in Louisiana or Mississippi;
5. Resided in the storm-affected areas at the time of Hurricanes Katrina or Rita, or were born after the Hurricanes and have subsequently resided in the storm-affected areas; and

6. Either never resided in a THU *in utero* (e.g., pregnant mother must never have lived in a THU) or after birth.

After the number of eligible children in the address was determined, the probability of selection for the child was 1 divided by the number of eligible children at the address. That is, the probability of selection for the jth child in the ith address in the hth sampling stratum, q_{hij}, was

$$q_{hij} = \frac{1}{c_{hi}},$$

where c_{hij} is the number of eligible children in the ith address in the hth sampling stratum.

Overall Child Probability of Selection and Design Weight for the Unexposed Sample

The probability of selection for the addresses in a CBG is the total number of addresses released in the CBG divided by the population number of addresses in the CBG. That is, the probability of selection for the ith address in the hth CBG, pos_{hi}, is

$$pos_{hi} = \frac{\#(released\ addresses)_h}{\#(population\ addresses)_h},$$

where $\#(released\ addresses)_h$ is the number of released addresses in the hth CBG and $\#(population\ addresses)_h$ is the number of population addresses in the hth CBG. The design weight in a CBG is the inverse of the probability of section in the CBG. That is, the design weight for the ith address in the hth CBG, d_{hi}, is

$$d_{hi} = \frac{1}{pos_{hi}},$$

where pos_{hi} is the probability of selection for the ith address in the hth CBG.

The final probabilities of selection and corresponding weights are provided in ***Table 2-1d***.

Table 2-1d. Probability of Selection and Design Weight for the Unexposed Children's Address by Census Block Group

Census Block Group	Total Number of Addresses	Number of Addresses Sampled	Address Probability of Selection[1]	Address Design Weight[1,2]	Sum of the Address Design Weights[3]
220330007012	343	7	0.0204	49.00	343
220330040122	1,160	5	0.0043	232.00	1,160
220510206001	313	4	0.0128	78.25	313
220510275024	880	10	0.0114	88.00	880
220510278032	592	10	0.0169	59.20	592
220710006111	661	15	0.0227	44.07	661
220710017021	276	13	0.0471	21.23	276
220710017203	540	15	0.0278	36.00	540
220710017254	562	16	0.0285	35.13	562
220710017323	254	14	0.0551	18.14	254
220710017373	575	14	0.0243	41.07	575
220710017381	1,529	15	0.0098	101.93	1,529
220710017382	1,352	10	0.0074	135.20	1,352
220710017401	734	15	0.0204	48.93	734
220710017422	1,178	18	0.0153	65.44	1,178
220710030001	265	10	0.0377	26.50	265
220870302042	254	10	0.0394	25.40	254
220870302043	229	10	0.0437	22.90	229
220870302072	532	7	0.0132	76.00	532
220870306031	657	7	0.0107	93.86	657
280399501002	569	6	0.0105	94.83	569
280399501003	1,594	13	0.0082	122.62	1,594
280399503001	476	9	0.0189	52.89	476
280399503002	590	16	0.0271	36.88	590
280470003003	182	6	0.0330	30.33	182
280470024002	1,042	10	0.0096	104.20	1,042
280470027004	797	4	0.0050	199.25	797
280470032053	1,269	5	0.0039	253.80	1,269
280470032061	2,066	16	0.0077	129.13	2,066
280470033015	258	5	0.0194	51.60	258
280470033021	1,403	3	0.0021	467.67	1,403
280470033025	762	5	0.0066	152.40	762
280470035022	896	9	0.0100	99.56	896

(continued)

Table 2-1d. Probability of Selection and Design Weight for the Unexposed Children's Address by Census Block Group (continued)

Census Block Group	Total Number of Addresses	Number of Addresses Sampled	Address Probability of Selection[1]	Address Design Weight[1,2]	Sum of the Address Design Weights[3]
280590402022	371	11	0.0297	33.73	371
280590407004	691	3	0.0043	230.33	691
280590409002	1,667	4	0.0024	416.75	1,667
280590411004	663	19	0.0287	34.90	663
280590420003	500	8	0.0160	62.50	500
280590422003	567	10	0.0176	56.70	567
280590424001	306	9	0.0294	34.00	306
280590426003	322	10	0.0311	32.20	322
Total	29,877	396	N/A	N/A	29,877

[1] Numbers rounded for presentation.

[2] Design weights are calculated for selected address. Addresses not selected have design weight equal to zero.

[3] In a sampling stratum, the sum of the address design weights should equal the number of addresses.

2.2 Tracing Sample

Successful tracing efforts are critical to the overall success of a study such as CHATS. Because of the ongoing rebuilding in many areas of the Gulf Coast, many people were still displaced from their prehurricane residences at the time of the CHATS data collection 7 years later. As a result, some of the study population remained extremely mobile and, therefore, difficult to locate. CDC set a criterion of locating and contacting at least 25% of the persons or households identified in the sampling frame. The tracing plan was designed to approach tracing activities sequentially, moving from least expensive to more expensive strategies, to maximize results at the lowest cost. We combined batch tracing, interactive tracing, and field tracing to locate the sample members selected for the exposed cohort.

Only the exposed cohort required tracing because the exposed sample was selected from individuals who applied for and received temporary FEMA housing after Hurricanes Katrina and Rita, and the addresses on the FEMA list were not always current for the selected individual. We targeted all locating efforts toward the adult household member who was listed on the FEMA registry. The unexposed cohort did not require any advance tracing activities because the sample was selected from U.S. Postal addresses based on geographic proximity to a completed exposed case. The unexposed cohort did not have any individual person associated with the selected address; anyone who lived at the selected address was potentially eligible. Once a selected household was located, the focus of the study became the selected child, if an eligible child was identified.

2.2.1 Preliminary Review of Sample File

RTI received the sample file with the last known address for the selected individual. We implemented global checks on the sample file to examine the completeness of sample information, such as address and personal identification information. Our most significant observation was that the sample file included a Social Security Number (SSN) for 100% of the individuals listed. This component was critical for tracing activities.

2.2.2 Batch Tracing

RTI first conducted batch tracing on the entire exposed sample of 1,814 individuals. Batch tracing consisted of an automated service in which the individual's name, SSN, address, and/or phone number were matched to information in multiple vendor-supplied databases. The current address for each person was then determined by logic algorithms based on the information available. By using these services, we could match entire groups of sample members quickly at a relatively low cost. We used the following sources: National Change of Address (NCOA), Fast Data, and Accurint. All transmissions were handled as encrypted files.

National Change of Address: We sent the entire sample to NCOA first. Maintained by the U.S. Postal Service, NCOA provided updated addresses based on information provided when individuals moved within the previous 24 months.

FastData: For the second batch-tracing search, we submitted the most recent contact information available for a sample member to FastData. FastData returned matched records using header information from credit history reports to determine and match SSNs with updated addresses.

Accurint: As a confirmation check for the final batch-tracing search, we sent the same updated information NCOA provided to Accurint for an independent batch search. Accurint's search was similar to FastData's in terms of the information provided, but different logic was implemented to determine the most up-to-date information.

2.2.3 Interactive Tracing

RTI implemented interactive tracing for sample members for whom we could not confirm contact information via batch tracing. Professional tracing staff carried out these centralized tracing activities. During interactive tracing, tracers reviewed each case individually to determine which resources were most appropriate. The tracers had access to the databases of all three U.S. credit bureaus (Experian, Equifax, and Transunion), as well as consumer information databases and public records. Tracers made decisions about cases based on the information from these sources, along with information obtained from batch tracing and

database searches. Interactive tracing yielded updated addresses for the hard-to-find sample members not confirmed through batch tracing.

When the initial interactive tracing resources did not yield a confirmed address, tracers used additional resources to locate sample members, such as the Social Security Master Death Index Search, the Department of Motor Vehicles, Inmate and Military Locators, and other online resources.

2.2.4 Field Tracing

Even with the updated tracing information, some cases required field tracing because sample members had moved again since the last update. Field interviewers used leads developed through RTI's tracing efforts and developed new leads using contacts with local organizations and knowledgeable individuals, such as neighbors or relatives. Because of its higher cost, field tracing was reserved for the hardest-to-locate participants. During the field data collection period, interviewers could also send the cases back to RTI's Tracing Unit for further tracing if new leads were identified but required additional assistance to confirm.

2.2.5 Tracing Unit Systems Security

All RTI Tracing Unit supervisors and tracing staff were required to sign the project privacy agreement and affidavit before they could access participant information. Tracing Unit computer systems were configured to allow only assigned staff to access CHATS sample member information. In addition, all computer systems were double-password protected. Each staff member was given one user ID and a password to log onto the computer, and a separate user ID and password to access the tracing control system as added data security. All Tracing Unit data transfer folders were protected with specific permissions giving read and write access only to assigned Tracing Unit staff and assigned project staff directly involved in batch tracing activities.

During all batch and interactive tracing activities, sample member data were sent electronically to the batch service or the database search vendor. All locating information (names, addresses, telephone numbers, and case IDs) sent to and from external tracing sources were transmitted electronically by an access-controlled FTP server using a password-protected login. Study identifiers used on data files sent to external batch tracing services were different from study identifiers used on final data files destined for release, whether restricted or public use.

2.2.6 50-HouseholdTest of Tracing Protocol for Four States

To determine if the tracing protocol we used for the Feasibility Study would be effective in all four of the states in the Full Study—Louisiana, Mississippi, Alabama, and Texas—we randomly selected 50 households from each state for the Feasibility Study tracing test. The locate rates from each of the steps listed above, including batch tracing (NCOA, Fast Data, and Accurint), were documented and analyzed for effectiveness. We compared the percentage of cases that were not located at all from any of the batch tracing steps and found that only 1% were not located at all. This result was consistent for the 50-household test and the Feasibility Study sample of 1,814. Our findings from the 50-household test indicated that the batch-tracing protocol implemented for the Feasibility Study in Louisiana and Mississippi was also effective for Alabama and Texas.

2.3 Screening and Interviewing Instruments

The CHATS Feasibility Study was complex; multiple instruments had to be programmed on both hand-held and laptop computers for field interviewers and registered nurses. The field interviewers used a hand-held computer called an iPAQ for three of the instruments that required mobility in the field. Initially, they used the iPAQ to screen each household to determine eligibility. They also used the iPAQ to complete two additional interview instruments if a child was selected: the Home Assessment program, which was part of Session 1 of the Baseline Assessment, and the Neighborhood Source Survey, which was administered after the Baseline Session 1 or before the field interviewer left the neighborhood after the Baseline Session 2. The field interviewers and nurses used the laptop computers to administer the questionnaires and conduct the health and environmental assessments in the participants' homes. On-screen instructions for deploying and collecting environmental devices and collecting biospecimens for environmental and Health Assessments were programmed for the laptops. Details about the environmental and Health Assessments are addressed in ***Sections 2.6*** and ***2.7***. This section provides information about the screening and other Baseline and Follow-up questionnaires conducted on the iPAQ and laptop computers.

All study materials and instruments were prepared in English and translated to Spanish. Introductory materials, such as letters, brochures, and consent forms, were also translated into Vietnamese to encourage participation and to answer questions the potential participants might have. RTI ensured that the translations of the materials reflected the intent of the English documents while remaining appropriate to the native speaker. We hired and training bilingual field interviewers for Spanish interviews. For Vietnamese-speaking participants, we hired interpreters to translate the questions and assessments to facilitate communication between monolingual Vietnamese participants and field interviewers and nurses.

To provide current status reporting for supervisors and project staff, each night interviewers securely transmitted the data collected on the iPAQ and laptops in the field. For daily reporting, the iPAQ data were first synced with the project laptop and then transmitted to RTI each evening. Data from completed questionnaires were removed from the laptop computers during transmission after having been verified as received intact at RTI. All iPAQ and laptop files containing project data were encrypted and inaccessible without the appropriate passwords, even if the hard disk from the laptop was removed and connected to another computer.

2.3.1 Screening Instrument

The screening instrument was designed to collect information about a selected household to determine if its residents were eligible for the study. We chose the iPAQ handheld computer to administer the screening instrument because its small profile facilitated data collection at the doorstep. The screening instrument included a scripted introduction and preliminary screening questions appropriate for the selected household based on the sample source—exposed or unexposed. Once a household was determined to be eligible, the screener provided scripted questions designed to collect data about any children in the household and then determined eligibility for the selected child. When a child was selected, the iPAQ displayed screens to invite the parent or guardian to participate in the study, then provided a transition to the laptop computer to obtain consent and conduct Session 1 of the Baseline Assessment.

The iPAQ was also programmed as a case management system for managing assigned screening cases and offered a record of calls and comments that the field interviewers could use to track the status of cases assigned to them.

2.3.2 Informed Consent

RTI developed multiple versions of consent forms for the interviews, medical records abstraction, and biological specimen collection. We developed parent or guardian versions for Sessions 1 and 2 for the Baseline and Follow-up Assessments. We used age-appropriate assent forms for children aged 8 to 11 years and 12 years or older. To complete the informed consent process, the parent/guardian, or child aged 8 years or older, was handed a paper copy of the consent or assent form. While the field interviewers read a script from the computer, the participant could follow along with the paper form. The parent/guardian or child aged 12 years and older then signed the paper copy. Children aged 8 to 11 years received a paper assent form but were not asked to sign the form. For children aged 7 years and younger, only the script in the laptop was required for child assent (i.e., for older children, written assent was required; for younger children, only verbal assent was required). Nurses also used separate consent forms for the Health Assessment. Each consent or assent form (or script) described the purpose of the

study, its sponsorship, privacy provisions, the voluntary nature of the study, compensation, and contact information if participants had questions. The forms also documented the cash (incentive) payments of up to $65 for the child and up to $75 for the parent, depending on the child's age and level of participation in the different study elements.

2.3.3 Administering the Interview

The Baseline and Follow-up Assessments consisted of two sessions the interviewer conducted in the home; sessions were held 5 to 9 days apart. During Session 1, the field interviewer used a laptop computer to administer the questionnaire to the parent, and then set up the environmental assessment equipment and requested that the parent and/or child complete a daily log of activities, called a Time and Activity Diary. If the child was old enough to wear the personal exposure monitor (PEM) platform, the field interviewer instructed both the parent and the child on its use. In addition, at the initial visit and at any subsequent visit after the study family had moved, the field interviewer completed a home inspection survey using the iPAQ.

At Session 2, after reviewing data collected from the first session, the nurse performed a Health Assessment and collected biospecimens from the selected child. At the same time, the field interviewer interviewed the parent, recorded data from the Time and Activity Diary, and collected the environmental assessment equipment. The field interviewer also completed a brief electronic instrument on the iPAQ that collected the GPS coordinates of the home and information about contaminant sources in the neighborhood. This instrument, called the Neighborhood Source Survey, was completed sometime after the Session 1 and before the field interviewer left the neighborhood after completing the Session 2.

2.3.4 Baseline Assessment

The Baseline Assessment was conducted at the earliest opportunity following the screening, but nearly always required a scheduled appointment for a later date after the screening was completed. The scheduling allowed the field interviewer to select a time when the parent and child were both available to complete the assessment. All Baseline Assessments were conducted between May 8 and December 9, 2012.

Session 1

The field interviewer used a computerized questionnaire to collect demographic information, family characteristics, detailed residential history, medical history of the child, family history of signature adverse health conditions, and exposures. Exposure questions covered such subjects as environmental tobacco smoke; use of household cleaners; home heating, cooling, and ventilation; and classes in school temporary buildings. An age-appropriate

version of the Pediatric Quality of Life Inventory (PedsQL) was administered for children without a reported history of asthma or asthma symptoms. For children with a diagnosed history of asthma or symptoms consistent with asthma, the field interviewer administered the age-appropriate version of the PedsQL Asthma Module. At the end of the questionnaire, participants were asked to update their own contact information in preparation for the Follow-up Assessment visit 6 months later.

The field interviewers requested written parental consent to release the child's medical records if the child was selected for medical record abstraction. They requested medical records for all children with a reported signature health outcome based on the responses to the child's medical history, and for a randomly selected subset of children with no reported signature health outcome. (See ***Section 2.7*** for a listing of the signature health outcomes.) The expectation was that one third of the children would have reported signature health outcomes to trigger the collection of the medical records and an additional 50 children would be randomly selected from among the children with no reported signature health outcomes.

The home inspection assessment, collected on the iPAQ, gathered information about specific rooms in the home and physical characteristics of the home. The environmental assessment included a request for all children aged 7 years or older to wear a PEM platform during the time between sessions. If the child was younger than 7 years old, a fixed-location platform was deployed in the room where the child spent most of his or her time. For a select sample of cases, known as the substudy, three devices were deployed: personal, indoor, and outdoor. Each device recorded airborne pollutants during the week between Sessions 1 and 2. For all children selected to wear the PEM platform, we provided a letter explaining the study and requesting permission for the child to wear the device while attending class and school functions. (***Section 2.6*** provides additional details about the environmental sampling.)

Participants were asked to provide or confirm complete contact information and provide information for an additional person for future contacting. The field interviewer also instructed the parent and child on how to complete the Time and Activity Diary to report the child's physical activity level and location in 30-minute increments during the week between Sessions 1 and 2. Before leaving the home, the field interviewer scheduled the appointment for the Session 2 and provided cash payments to the parent for the study elements completed during Session 1. The field interviewer scheduled the return visit for Session 2 when both the parent and child were available so that a study nurse could arrive with the field interviewer to collect biospecimens and conduct the necessary Health Assessments.

Session 2

Session 2 took place between 5 and 9 days after the Session 1 interview. Whenever possible, the field interviewer and nurse arrived at the household at the same time. The field interviewer asked about recent environmental exposures the child experienced and collected the child's Time and Activity Diary. The field interviewer also retrieved, packed, and shipped all environmental assessment equipment that had been deployed. The field interviewer also dispensed the cash incentives to the child and parent. If the Neighborhood Source Survey was not completed prior to Session 2, then the field interviewer also recorded the GPS coordinates of the home and documented any neighborhood sources of contaminants before leaving the neighborhood.

Before beginning the Health Assessment, the nurse obtained the parent/guardian's written consent for the release of medical data collected during the Health Assessment. If the parent did not authorize the release of these data, the nurse did not complete a Health Assessment with the child. If the parent/guardian did consent to the release of the Health Assessment data, the nurse began the assessment, collecting blood and urine and assessing pulmonary function if the child was old enough and agreed to the assessment. The blood collection and pulmonary function tests were performed only with children aged 5 years and older. The pulmonary function assessments included spirometry and exhaled nitric oxide tests. The nurse also conducted facial and dermal assessments, measured the child's height and weight, and administered an age-appropriate asthma control assessment. The nurse labeled, packaged, and shipped the blood and urine samples for analysis, in accordance with the study protocol.

2.3.5 Follow-up Assessment

The Follow-up Assessment took place 6 months after the Baseline Assessment, and for the most part, included the same elements as those completed during the Baseline Assessment for both Session 1 and Session 2. The field interviewer contacted the parent by phone to schedule the interview date and time. All Follow-up Assessments were conducted between December 6, 2012, and May 24, 2013.

Session 1

The field interviewer administered the same quality-of-life measures used during the Baseline Assessment interview, repeated the home inspection if participants had moved to a new residence since the Baseline Assessment, and deployed the environmental assessment equipment. The interviewer requested parental permission to release the child's recent medical records if selected for medical record abstraction. Medical records were requested for children based on similar criteria as those used for the Baseline Session 1, with the additional

requirement that the child had been seen by a doctor or medical provider during the 6-month timeframe since the Baseline interview. Before leaving the home, the interviewer paid the appropriate cash incentives and scheduled a return visit for Session 2 at 5 to 9 days later. Just as in Session 1 of the Baseline interview, participants were asked to update their own contact information, as well as to provide information for an additional person for future contacting.

Session 2

In Session 2 of the Follow-up Assessment, the field interviewer administered key health-related questions to identify health changes since the Baseline Assessment, asked about environmental exposures, collected the Time and Activity Diary, conducted another environmental assessment, and retrieved all equipment left during Session 1. The field interviewer provided the final cash payment appropriate for completed elements of this session.

The nurse accompanied the field interviewer and first obtained parental permission to release the data collected during the Health Assessment to RTI's CHATS researchers. If consent was obtained, the nurse conducted the Health Assessment by assessing pulmonary function on participants aged 5 years and older, conducting a facial and dermal assessment, measuring the child's height and weight, administering an age-appropriate asthma control assessment, and collecting a urine sample. Blood was not collected at the Follow-up Assessment. The nurse packaged and shipped the urine sample to the laboratory for analysis.

2.3.6 Central Site Assessment

Environmental samples were collected at the home, and a central site was also identified for daily collection of specific air samples during a set time period for both the Baseline and Follow-up Assessments. Each time period occurred in the middle of the data collection period and lasted approximately 90 days. The centralized outdoor sample collections took place at an U.S. Environmental Protection Agency (EPA) monitoring station known as a State-Local Air Monitoring Systems (SLAM) in Kenner, Louisiana. Field interviewers collected outdoor air samples, which were then analyzed for the levels of particulate matter with an aerodynamic diameter smaller than 10 micrometers (PM_{10}), formaldehyde and VOCs. In addition, ozone, PM_{10}, $PM_{2.5}$ and NO_2 concentrations were obtained from the public datasets. The concentrations measured at centrally located SLAMS sites were compared for a subset of the residential collections, which took place during the same collection period. (See ***Section 2.6.4*** for additional information about the central site collection.)

The field interviewers who deployed and retrieved the central site samplers used a computerized questionnaire similar to the Baseline instrument for the outdoor environmental platform deployment and retrieval. The outdoor platform for the central site was identical to the outdoor platform used for the residential collection.

2.4 Staff Recruitment and Training

2.4.1 Field Interviewers

RTI's Field Operations management team worked closely with the field supervisors to establish interviewer hiring goals, determine targeted average pay rate, and assist in making all final hiring and pay rate decisions. Field staff were located through standard sources of field interviewer candidates, which included referrals from other studies, field interviewers who had worked successfully on similar studies and were known to the supervisors through their contacts in the survey research community, and candidates responding to Internet postings who had relevant experience.

Selection criteria for field interviewers emphasized the following: (1) geographic locations in or near the selected sample sites in the Louisiana and Mississippi area; (2) successful experience in survey research or closely related face-to-face interviewing; (3) preferable experience in collecting health and environmental data from children; (4) experience in tracking participants; and, (5) willingness to commit their time for the duration of the Feasibility Study.

RTI hired two bilingual field interviewers (Spanish- and English-speaking) in anticipation of encountering sample members who preferred to complete the interview in Spanish (according to the Census, 9% of the population in the area of recruitment spoke Spanish, but spoke English less than "very well"). Both bilingual candidates passed an RTI-administered Language Skills Assessment and completed an additional 4-hour training in Spanish. Although the CHATS questionnaire was not translated into Vietnamese for the Feasibility Study, local interpreters were hired to assist with administering the English questionnaire to participants whose primary language was Vietnamese and who were not comfortable completing the interview in English.

Prior to attending training, all field staff completed RTI's Institutional Review Board (IRB) ethics tutorial and assessment via a web-based system. Staff also received a CHATS manual for review. Each trainee completed and passed a home study to ensure knowledge of project procedures.

At training, all field staff signed a privacy pledge. By signing the pledge, field staff entered into a contractual agreement to keep private all data they collected for CHATS. Their signature on the document also certified that they would carry out all project procedures precisely as presented in their field manuals and at training.

The training sessions focused on key project protocols and procedures needed to successfully perform job duties. The trainees participated in sessions that stressed the

importance of remaining culturally sensitive while working in ethnically diverse communities. The CHATS training program used web-based assignments, self-study manuals, classroom instruction, demonstrations, and hands-on exercises. All field staff who worked on CHATS passed a project certification before beginning fieldwork. During training, project staff evaluated each trainee's ability to follow all data collection procedures correctly and as presented during training. Trainees who demonstrated proficiency in these procedures received a letter of authorization and identification badge, allowing them to begin work on CHATS.

As part of the initial training, two field interviewers were selected to deploy and retrieve the outdoor samplers from the central site. Because the protocol for the deployment and retrieval was very similar to the outdoor residential assessment, the training for the central site took place one afternoon at the site of the central site collection in Kenner, Louisiana.

For the CHATS Feasibility Study, the staff was divided into two teams, each managed by a field supervisor. One team primarily covered the New Orleans metro area. The other covered the Mississippi Gulf Coast and Baton Rouge metro area.

Initial Training Session

Recruiting for field interviewers began in March 2012. In total, 17 field interviewers were recruited and hired. Two field interviewers, however, resigned prior to being trained; thus, only 15 field interviewers completed the training. The initial training was conducted over a 5-day period from April 19 to April 23, 2012, in Metairie, Louisiana. All field interviewers who completed the initial training session were trained to conduct substudy assessments; however, only staff who were working cases assigned as substudy cases actually completed Baseline substudy assessments. ***Table 2-4a*** shows the breakdown of the total number of field interviewers recruited.

Screening-only Training Session

During the initial weeks of data collection, the New Orleans area field interviewing staff experienced an unexpected and dramatically high level of attrition. More than half (60%) of the original 10 field interviewers hired in this area left the project. Half of this attrition was involuntary due to a failure to follow project protocols, lack of work, and an attempt to falsify data. To temporarily alleviate the shortfall, three experienced field interviewers from Louisiana were hired to bolster the effort in New Orleans during July and August 2012. Following a 3-day training session on the study overview and screening protocols, these three staff members used their extensive field experience to conduct screenings and set appointments for any selected Baseline participants, which other staff who were training in the full assessment would

complete. With the addition of new staff hired for the attrition training in August, these extra field interviewers were no longer needed to maintain progress in New Orleans.

Table 2-4a. Field Interviewer Recruitment by Location and Training Session

Location	Hired for April Training	Attrition (April – August)	Total Working (August)	Hired for August Training	Attrition (August – End of Baseline)	Total Working (End of Baseline)	Attrition (Follow-up)	Total working (end of Follow-up)
New Orleans Metro	10	6	4*	5	4	5	0	6**
Baton Rouge Metro	2	0	2	1	1	2	0	2
MS Gulf Coast	5	1	4	1	1	4	1	3
Total	17	7	10*	7	6	11	1**	11**

*= Does not include 3 screening-only interviewers.

** = One interviewer returned from medical leave for the conclusion of Follow-up.

Attrition Training Session

Recruiting for field interviewers to offset attrition began in early July 2012. In total, we hired seven field interviewers. The primary geographic focus of this replacement training was New Orleans. Although we hired two additional field interviewers outside of the New Orleans metro area, the expectation was that almost all field interviewers—both April and August hires—would conduct some of their work in New Orleans.

Training was conducted over a 5-day period from August 15 to August 19, 2012. Two of the six field interviewers completed an additional 2-hour substudy training component and were certified for conducting substudy interviews. Because technical complications occurred and Hurricane Isaac made landfall, these newly trained field interviewers did not begin work until September 2012.

Follow-up Training

Prior to the beginning of the Follow-up Assessment data collection, all active field interviewers participated in a phone training held on November 30, 2012. The purpose of this training call was to review the slight changes in administering the instrument between the Baseline and Follow-up Assessments and to refresh staff on general CHATS procedures and protocols. Shortly after Follow-up data collection began, we held an in-person session on December 12, 2012. The purpose of this session was to reiterate many of these changes and to address challenges experienced during Baseline data collection.

For the Follow-up Assessment, the remaining four field interviewers who had not conducted substudy interviews during the Baseline Assessment were trained or retrained and certified for the substudy deployment. This training session took place on December 12, 2012.

Before the central site data collection resumed in February 2013, the field interviewer who deployed and retrieved the outdoor platform completed a 2-hour refresher training by telephone.

2.4.2 Nurse Training

Initial Training

Table 2-4b shows the breakdown of the total number of nurses recruited. Initially, RTI collaborated with both the Louisiana State University Health Science Center (LSU HSC) and Coastal Family Health Clinic (CFHC) to recruit and employ nurses using their established resources, including Internet job postings, local newspapers, and professional publications. Key qualifications for the selected nurses included (1) state-specific licensure as a registered nurse (RN); (2) Bachelors of Science in Nursing (preferred); (3) at least 1+ years of experience in providing care to children and families and providing care in the home or in other independent practice settings; and, (4) willingness to commit their time for the duration of the study.

Table 2-4b. Registered Nurse Recruitment by Location and Training Session

Location	Hired for April Training	Hired for May Training	Attrition (July)	Hired for July Training	Attrition (Aug)	Total Working (End of Baseline)	Attrition (Follow-up)	Total Working (end of Follow-up)
New Orleans Metro	4	0	0	0	1	3	0	3
Baton Rouge Metro	1	0	0	0	0	1	0	1
MS Gulf Coast	1	2	2	3	2	2	0	2
Total	6	2	2	3	3	6	0	6

Prior to attending a 3-day training course, all nurses successfully completed the aforementioned IRB ethics tutorial and a home study of procedures specific to the Health Assessment. The course held April 19–21, 2012 in Metairie, Louisiana, provided the nurses an opportunity for protocol familiarization and hands-on experience with the various devices and equipment required for data collection. At the conclusion of the training course, each nurse was certified by project staff to begin working on CHATS.

Additional Training

Since CFHC was able to hire only one nurse prior to the April training, two additional nurses were later hired to cover visits in the Mississippi coast area. These nurses were trained

in a 3-day course, May 5–8, 2012 in Biloxi, Mississippi. Because two of three CFHC staff members left in July, additional staff had to be recruited. ATEN Solutions, the subcontractor initially providing the medical record abstraction staff, recruited and hired three nurses who were trained in Biloxi, Mississippi, August 1–3, 2012. Only one of these staff remained with the study.

Follow-up Training

Prior to the beginning of the Follow-up data collection period, RTI held a conference call on November 28, 2012, with all nurses. In this call, we reviewed the elements of the data collection protocol and introduced the minor changes in the Follow-up Assessment.

2.4.3 Abstractor Training

RTI subcontracted with ATEN Solutions to hire and manage two medical records abstractors and one medical records abstraction supervisor who conducted quality control (QC). All abstractors held certification as Certified Coding Assistants (CCA) from the American Health Information Management Association (AHIMA), had to be meticulous and well organized, and willing to commit the time required for the task for the duration of the study.

Prior to attending training, the abstractors successfully completed a home study of procedures specific to CHATS medical record abstraction that included a description of the study and an overview of the types of medical record data to be abstracted. The 4-hour training course conducted in July 2012 emphasized the procedures for contacting providers, examples of the specific types of data required for abstraction, a field-by-field review of the abstraction collection form, a review of managing data requests and abstraction status, procedures for dealing with refusals for record requests, and processes for maintaining confidentiality of records and abstracted data. At the mid-point in the Baseline data collection, ATEN's clinical team completed an independent reabstraction for QC purposes. No retraining needs were identified from this review.

2.5 Subject Recruitment

2.5.1 Screening

Sample member households became available to each field supervisor for assignment to a field interviewer on a rolling basis throughout the Baseline Assessment (May – November 2012). Exposed cases were made available as they were released from tracing. Unexposed cases were released at regular intervals to correspond with the exposed cases that had completed Session 1 visits in the area. The field supervisors then assigned each case to a locally

based interviewer. These assignments were made based on geographic proximity and availability of staff.

Prior to making an in-person visit, RTI sent a letter and brochure introducing the study and its objectives to all selected households with valid mailing addresses. The assigned interviewer visited the household, attempted to speak to an adult resident, explained the purpose of the visit, answered any questions about the study, and conducted the screening questionnaire. If a child in the household was selected for the Baseline Assessment, the field interviewer discussed that child's participation with a parent or guardian and attempted to set an appointment to return in 3 to 7 days. Initially, the field interviewers were prepared to conduct the Baseline Assessment immediately following the screening, but found that parents and children were rarely available to begin the interview the same day as the screening. Typically the Baseline was scheduled 3 to 7 days after the screening was completed to allow time for the preparation and shipment of the environmental assessment platforms on an as-needed basis and reduce the risk of any environmental samplers expiring.

Field interviewers were directed to visit households primarily on weekends and during evenings when sample members were more likely to be at home. They were also instructed to plan their visits to coincide with other appointments in the area and to attempt to visit all households in the same area to improve efficiency. Field interviewers visited sample member households until the screening was completed, someone in the household firmly refused participation, or it became logistically too complicated and expensive to continue planning future visits.

RTI coached field interviewers regularly on the best ways to address potential participant concerns and avert refusals. Field supervisors referenced Record of Call reports regularly to determine when each interviewer was working and when they had visited each selected household. Using these reports, the field supervisors drafted weekly work plans for each field interviewer to ensure that all households were visited on different days of the week at different times.

To expedite the screening effort in New Orleans, we hired and trained three experienced field interviewers to complete screenings and set appointments for Baseline Assessments that other field interviewers would complete.

2.5.2 Baseline Assessment

Field interviewers approached the parent or guardian of the selected children at the time of screening to set an appointment for the Baseline Assessment if they were available. If they were not available or not willing to commit to an appointment at the time of screening,

the field interviewer returned to the household until an appointment time was set. The field interviewer then reported the appointment date and time via the case management system, which alerted the RTI staff to prepare and ship the appropriate environmental assessment platform base on the child's age.

In most cases, the same field interviewer who conducted the home visit would also conduct the screening. If the participant refused to participate, a different field interviewer was assigned to the case. RTI then sent refusal letters to the household urging them to participate and reiterating the purpose of the study. Then this second field interviewer made a return visit to attempt to convert the refusal and schedule a firm appointment. If the initial field interviewer repeatedly visited the home without encountering the parent or guardian, or if the parent or guardian failed to appear at the scheduled appointment time and made no attempt to reschedule, the household would also be transferred to a second field interviewer.

At the conclusion of the Baseline Session 1, the instrument directed the field interviewer to make an appointment for the second session. This session had to be scheduled at least 5 days and no more than 9 days after the first session. The field interviewer often had developed significant rapport with the participants by this point and generally had little difficulty gaining cooperation. The field interviewer typically returned with the nurse at the appointed time for the second session. On a few occasions, none of the nurses were available at the scheduled time. In these rare instances, the field interviewer made another appointment with the parent or guardian for the nurse to conduct the Health Assessment separately and proceeded with the interview and environmental platform retrieval.

To address initial concerns that the nurse would not be able to conduct the Health Assessment in the home, both Louisiana State University and CFHC offered the use of mobile health units as alternative locations for the Health Assessment. However, the nurses did not indicate the need for these units, so they were not used.

2.5.3 Follow-up Assessment

During the Baseline Assessment, field interviewers asked each participant to provide updated contact information, including alternate persons with whom to speak if the participant moved. Interviewers used this updated contact information to recontact each participant who completed the Baseline Assessment for the Follow-up Assessment.

The Follow-up households were assigned to each field interviewer on a rolling basis. Each household record was made available in the case management system to the field interviewer 5 months from the date of completion for the Baseline Session 1. The household was considered eligible to pursue until 7 months from the completion of the Baseline Session 1.

There were a few exceptions to this 2-month Follow-up data collection window. Because the Follow-up Assessments did not begin until December 2012, which was already close to the 7-month mark of the first completed Baseline households, all the early households were granted extensions to completing the Follow-up Assessment. A total of six households from the initial December release were completed outside the 7-month window. Other situations arose later in the Follow-up where a participant expressed interest in completing the Follow-up Assessment but was unavailable during this window. We evaluated each situation was evaluated on a case-by-case basis, and in all, 13 (or 8% of the total work completed) interviews were permitted to be completed after the 7-month mark. None exceeded the 7-month mark by more than 4 weeks.

Follow-up households were assigned to locally based field interviewers, largely based on proximity to the selected address. Whenever possible, these cases were reassigned to the field interviewer who completed the Baseline Assessment. We provided the field interviewer with the phone number the participant had offered during the Baseline sessions, and the field interviewer attempted to recontact the participant a minimum of three times using that number. If these attempts proved unsuccessful, we gave the field interviewer alternate phone numbers for the household, which were either offered during the Baseline Assessment or uncovered through tracing. If the participant still could not be reached, we gave the field interviewer contact information for any alternate persons the participant had mentioned during the Baseline Assessment. In some rare instances, the field supervisor attempted to contact the participant via e-mail, if an e-mail address had been provided. If no contact was made via any of these means, the interviewer made an unscheduled visit to the home to secure an appointment.

If the participant refused the Follow-up Assessment, or the initial field interviewer failed to schedule an appointment to complete the interview after several weeks, the household was transferred to another field interviewer. The initial field interviewer completed most Follow-up Assessments, but some were transferred to as many as four different field interviewers before completion. Ultimately, just over half of the Follow-up interviews (52%) were completed by the same field interviewer who completed the Baseline Assessment.

Because the Baseline Assessments was extended through December 9, 2012, just over 5 months from the end of data collection on May 19, 2013, interviewers did not have the full 2-month window to complete the Follow-up Assessments for the final batch of cases. Nevertheless, the field interviewers were able to contact and schedule the last group of cases before the end of data collection.

Because of the smaller number of cases to be worked relative to Baseline, no overnight travel was required to complete Follow-up Assessments.

2.6 Environmental Assessment

2.6.1 Environmental Sample Box Preparation and Shipping to Field Interviewers

RTI shipped "participant boxes" containing environmental assessment supplies and different combinations of outdoor, indoor, and personal air sampler boxes to each field interviewer. The different age-related protocols, multiple possible platforms, and the presence of QC samples determined the participant box contents. Each household was eligible to receive the personal platform, the indoor platform, the outdoor platform, or a combination of the three. Most participants older than 7 years received only the personal platform, but a selected subgroup of these children received all three. The type of platform deployed depended on the age of the selected child and the contents of the participant box. Households with children younger than 7 years old were not eligible to receive the personal platform or the outdoor platform; they received the indoor platform only. ***Table 2-6a*** summarizes the usage frequency of each box type for the two study phases of Baseline and Follow-up; the parenthesized values indicate the percentage of a box type that contained a QC sample. ***Table 2-6b*** summarizes the types of environmental samples collected.

Table 2-6a. Participant Box Usage Frequency and Inclusion of QC Samples by Box Type

Box Type (Description)	Number of Participants (% that Received a QC Sample)	
	Baseline	Follow-up
Substudy (>7 Years—Indoor, Outdoor, and Personal Samples)	46 (4.3)	103 (71.8)
Older Child Normal (>7 Years—Personal Samples Only)	105 (17.1)	33 (75.8)
Younger Child Normal (<7 Years—Indoor Samples Only)	29 (0.0)	18 (0.0)
Central Site (Outdoor Samples Only, daily sample collection)	97 (8.2)	83 (4.8)

RTI shipped four primary types of participant boxes to the field: three types for participants and one type for the central site. The three participant box types shipped reflected the child's age and whether the child participated in the substudy. An additional nine sub-box types tracked deployment and collection of QC samples. RTI shipped participant boxes for delivery the day prior to a scheduled appointment. Upon receipt, the field interviewer reviewed and confirmed that the contents of the box(es) were correct.

Table 2-6b. Types of Environmental Samplers

Item Name	Platform (P=Personal; I=Indoor; O=Outdoor)	Purpose of Item	Picture of Item
RTI MicroPEM™	P, I, O	Actively measures PM_{10} mass (filter and nephelometer), secondhand smoke (SHS), 3-axis accelerometer, temperature, relative humidity (RH)	
3M single-stage VOC Badge	P, I, O	Passively measures exposure to volatile organic compounds (VOCs) emitted from household furnishings, consumer products, and found in ambient air.	
SKC Aldehyde Badge (also referred to as "Carbonyl badge")	P, I, O	Passively measures exposure to formaldehyde and other carbonyls emitted from pressed wood materials and found in ambient air.	
Ogawa NO_2 Badge	P, I, O	Passively measures exposure to nitrogen dioxide (NO_2), a combustion by-product	
H_2S Badge	I	Passively measures hydrogen sulfide (H_2S), a gas emitted from some types of drywall and sewer gas	
HOBO	I	Passively measures temperature and relative humidity	

2.6.2 Week-Long Sample Collections at the Household—Exposure Measuring Devices

As noted above, CHATS participants consented to have up to three exposure platforms deployed over the course of 5 to 9 days between Session 1 and Session 2. These platforms

could include a residential outdoor platform, a residential indoor platform, and a personal platform. Each device is discussed in more detail in the following sections.

During Session 1, the field interviewer deployed the appropriate environmental sampler while at the participant's home according to the platform's Standard Operating Procedure (SOP). Each platform contained samplers that were precalibrated and labeled at the RTI laboratory. The field interviewer removed the environmental samplers from each participant box for placement in an appropriate protection or carrying mechanism: a small custom-designed pouch for PEM samplers, an unobtrusive wire box for indoor measuring devices, and an unobtrusive wire box with weather shield for outdoor measuring devices.

Personal Exposure Monitoring (PEM) Platform

As shown in ***Figure 2-6a***, the PEM platform consisted of four samplers: MicroPEM, SKC Aldehyde badge, 3M VOC badge, and Ogawa NO_2 badge.

Figure 2-6a. Personal Exposure Monitoring (PEM) Platform

During Session 1, the field interviewer offered the child a few options for wearing the PEM platform (i.e., on a bandolier-type belt, shoulder strap, or lanyard) and let him or her decide. The field interviewer turned on the MicroPEM, removed the caps from the passive samplers, and recorded the time of deployment. The field interviewer encouraged the child to wear the device with the PEM platform at all times and to follow the rules below:

- The PEM platform should be worn over the child's top layer of clothes.

- When the child is sleeping or bathing, the child or parent can place the PEM platform on a table or chair, or hang it on a hanger or door knob near the child without the samplers being blocked.
- While the child is participating in sports activities, someone can place the PEM platform in a locker or it can remain in a car. The child should also note on the Time and Activity Diary when the PEM is not being worn and the location of the PEM.

The child wore the PEM from Session 1 until Session 2, when the field interviewer returned to the home. During Session 2, the field interviewer retrieved PEM platform samplers in the same manner as for outdoor samplers during Session 2, placed them in the participant box, and then shipped to the RTI laboratory.

Residential Indoor Platform

As shown in ***Figure 2-6b***, the indoor air monitoring platform consisted of the same four samplers used above in the personal platform (MicroPEM SKC Aldehyde badge, 3M VOC badge, Ogawa NO_2 badge) and a HOBO for taking temperature and humidity measurements. For a subset of homes, a H_2S badge was also included in the platform.

Figure 2-6b. Indoor Air Measuring Platform

During Session 1, the field interviewer entered the participant's ID and participant box ID codes using a scanner into a sample datasheet in his or her laptop and confirmed individual sampler IDs in the indoor measuring device. The field interviewer removed the samplers from their containers, turned on the measuring device, removed the caps from the samplers, and

then placed the measuring device in an unobtrusive wire box and recorded the time of deployment.

The wire box containing the measuring device and samplers was placed on a table in a common area that met the following criteria:

- Away from foot traffic;
- Away from doors and windows;
- Away from HVAC vents;
- Away from strong light/heat sources;
- Away from fireplace;
- Located a minimum of 12 inches from wall; and
- If positioned in the corner of a room, box was angled so all samplers were directed toward the center of the room.

During Session 2, the field interviewers confirmed the sampler IDs by again scanning them into the computer; the interviewers then powered off the measuring device and recapped the samplers. The computer recorded the time of retrieval for each platform. The field interviewers removed the MicroPEM from the wire box and covered the inlet using a fingercot provided in the sampler box. The field interviewers placed all aldehyde, VOC, and NO_2 badges in their own containers and in an insulated foam tray with ice packs. They then placed all loaded insulated tray(s) into the original participant's box(es), and shipped the participant box(es) back to the RTI laboratory.

Residential Outdoor Measuring Platform

As shown in ***Figure 2-6c***, the outdoor air monitoring platform consisted of the following four samplers within the wired cage: MicroPEM for PM_{10}, SKC Passive Aldehyde badge for formaldehyde, 3M VOC badge, and Ogawa NO_2 badge.

Figure 2-6c. Residential Outdoor Air Measuring Platform

During Session 1, the field interviewer entered the participant's ID and participant box ID codes using a scanner into a sample datasheet in his or her laptop and confirmed individual sampler IDs in the outdoor measuring device. The field interviewer removed the four samplers from their containers, turned on the measuring device, removed the caps from the samplers, and then placed the measuring device outdoors in an unobtrusive wire box with weather shield. Criteria for the location of the outdoor platform were:

- Located in a common area, usually in a backyard, balcony, or parking lot;
- Located where it was not obstructed by fences or shrubbery;
- Located 10 to 50 feet from residential structures;
- Located a minimum of 1 meter above ground;
- Located away from HVAC units; and
- Located away from outdoor pet's play area.

During Session 2, field interviewers retrieved the samplers in the same manner as the indoor samplers during Session 2, placed them in the participant box, and then shipped the box to the RTI laboratory.

2.6.3 Other Sample Collections at the Household

In addition to the environmental sample collections detailed in the previous section, field interviewers also completed a one-time indoor dust sample collection at each household during the Baseline and 6-month Follow-up Assessments.

Indoor Dust Sample Collection

To assess dermal exposures, field interviewers collected a residential floor dust sample in order to measure various allergen levels indoors, which can trigger asthma. To conduct the indoor dust sampling, we used the Housing and Urban Development (HUD) 2004 method "Vacuum Dust Sample Collection Protocol for Allergens."[3] This method used an electric canister micro-vacuum equipped with an autoclaved HEPA filter sock to collect the vacuumed dust.

During Session 2, field interviewers used a handheld vacuum cleaner with prelabeled special filter inserts to collect a dust sample from the child's bedroom floor, bed, and bedding. The field interviewer was instructed to avoid disturbing or walking in the area to be sampled. After sampling, the field interviewer removed the filters from the vacuum cleaner and placed them in a pyrogen-free sterile glass jar and in the participant's box for transport. The field interviewer then shipped the filters to the RTI laboratory with other environmental samples from the same participant.

Air Exchange Rate (AER) Calculation

We initially proposed to perform a carbon dioxide (CO_2) decay-derived AER measurement in individual homes and to compare the accuracy with another AER measurement using a PFT-CAT (**P**er**F**luoro**T**oluene emitters in combination with **C**hemical **A**dsorbent **T**ubes) method from a subset of homes. During data collection preparations, we learned that EPA had evaluated and validated various air exchange rate models as potential alternatives for direct measurement. RTI decided to model the air exchange rates after extensive discussions with EPA scientists, which helped to reduce the session/visit time.

We chose to use a mechanistic model developed by Lawrence Berkeley Laboratory, including natural ventilation (LBLX)[4], to calculate the air exchange rate for the current residences of study participants. The EPA evaluated this model, comparing it with PFT-CAT-derived measurement data collected from a panel study conducted in North Carolina.[5] The LBLX

[3] US Department of Housing and Urban Development. (2008). Vacuum dust sample collection protocol for allergens. http://portal.hud.gov/hudportal/documents/huddoc?id=DOC_12539.pdf

[4] *2009 ASHRAE Handbook: Fundamentals* (2009). American Society of Heating, Refrigeration and Air-Conditioning Engineers, Atlanta, GA.

[5] Breen, M. S., Breen, M., Williams, R. W., & Schultz, B. D. (2010). Predicting residential air exchange rates from questionnaires and meteorology: model evaluation in central North Carolina. *Environmental science & technology*, *44*(24), 9349-9356.

model assumes the building is a single, well-mixed compartment. Air-flow rates by air infiltration and natural ventilation were calculated separately and combined as overall air-flow rate, and then divided by house volume to calculate the air exchange rate. The CHATS residential questionnaires and indoor measurements of temperature and relative humidity provided data for many of the required model input parameters for the LBLX model. We did not collect meteorological data (wind speed, ambient temperature) and home facts (house age, type, volume, local sheltering) necessary for the model during the study, but we obtained from various Internet sources.[6],[7],[8] The window opening area input was not available from CHATS questionnaires. We assumed that occupants' behavior of opening windows would be similar between the Gulf Coast and North Carolina and used published data from the EPA.[9]

2.6.4 Additional Environmental Sample Collections (Off-Site)

SLAM (State-Local Air Monitoring Systems)

RTI's initial proposal was to sample at three central sites as part of the substudy to address the representativeness of central, ambient air quality monitoring for personal exposure. The central sites were to be located at SLAM sites in New Orleans, Baton Rouge, and southern Mississippi. As sampling frame development progressed, however, we realized that the low number of expected participants in Baton Rouge and Mississippi would not provide a sufficient quantity of data for assessing central site representativeness. Therefore, we decided to concentrate resources and operate a single central site in New Orleans. We considered two SLAM sites in New Orleans, but the Louisiana Department of Environmental Quality (LA DEQ) would grant access to only the Kenner site in Louisiana (GPS coordinates 30° 02' 27.476" N, 90° 16' 21.866" W). This site was also close to the participants' residences and the field interviewers could access it easily. Similar to residential outdoor sampling, we collected daily air samples using precalibrated and labeled MicroPEM, SKC Aldehyde badge, and 3M VOC badge (***Figure 2-6d***). RTI collected central site air samples for 96 days in the Baseline Assessment and 83 days in the Follow-up Assessment.

On the first day of sampling, the field interviewer placed the wire box with weather shield on a RTI-provided stake, took the samplers from the participant box and placed them in the wire box, turned on the equipment, removed the caps from the samplers, and recorded the time of deployment.

[6] www.wunderground.com
[7] www.zillow.com
[8] www.google.com/maps
[9] Breen et al., 2010.

Figure 2-6d. LA DEQ Monitoring Site in Kenner, Louisiana

Approximately 24 hours later, the field interviewer returned to the site, confirmed the sampler IDs by scanning them into the computer, powered off the measuring device, and recapped the samplers. The computer recorded the time of retrieval for the platform. The field interviewer removed the MicroPEM from the wire box and covered the inlet using a fingercot provided in the sampler box. The field interviewer placed the SKC Aldehyde badge and 3M VOC badge in their own containers and in an insulated foam tray with ice packs. The field interviewer then placed the loaded insulated tray into the original central site box and shipped the participant box back to the RTI laboratory. He/she repeated this same procedure with new samplers every day.

RTI also obtained ambient level PM_{10}, ozone, and NO_2 data from the EPA Air Quality Database of other SLAM locations in the Baton Rouge area (Baton Rouge-Capital), New Orleans area (Chalmette-Vista and Kenner), and Mississippi coast area (Jackson Metro, Pascagoula, and Hinds County) for every day of the Baseline and Follow-up Assessments. Due to the data availability in the SLAM sites in New Orleans and Mississippi areas, multiple sites in these areas were used to obtain the data.

2.6.5 Sample Handling and Shipping to RTI

Once the Session 2 retrieval was completed, the field interviewer prepared and shipped the participant or central site box(es) back to RTI. Upon completion of the Session 2 appointment, the field interviewer was instructed to load three frozen gel packs into the

sampler tray to keep the aldehyde and VOC samplers cold during transport. (See ***Figure 2-6e*** for an image of a properly prepared box.) Once the sampler tray was properly packed, the field interviewer placed it into the participant box and sealed the box with clear packing tape. The field interviewer attached a return label to the participant box and delivered it to a FedEx station. All boxes were marked for overnight delivery to ensure prompt sample receipt. Special handling of the participant or central site box was required if the Session 2 retrieval occurred on a national holiday, Friday, or during the weekend, or if the field interviewer was not able to take the participant box to the FedEx location the same day the Session 2 appointment was completed. If special handling procedures were needed, the field interviewer was required to continually cycle fresh, frozen gel packs in the sampler tray until the box could be shipped back to RTI. Field interviewers were provided with additional gel packs for such an occurrence.

Figure 2-6e. Properly Packed Participant Tray Ready for Shipment to RTI

2.7 Health Assessment

To assess health outcomes and evaluate the effects of environmental contaminants on these outcomes, RTI collected health data from all participants. For data collection activities we followed the Health Assessment protocol, which was developed with input from the clinician members of the Technical Advisory Panel (TAP), Drs. Ralph Delfino and David Tinkelman. Following the 3-day training session to ensure protocol adherence, each nurse was accompanied on his/her first visit by a nurse educator from the LSU HSC, who provided first-line supervision of the nurses. Throughout the data collection period, to further monitor quality, the nurse educator periodically accompanied the nursing staff on visits. As described earlier, the field interviewers who administered several of the health-related and quality-of-life instruments also received training on best practices to obtain these types of data.

RTI collected Health Assessment data in participant homes during two visits, one week apart. During Session 1, the field interviewer administered health and quality-of-life questionnaires. During Session 2, the nurse performed a physical assessment of the participant's face and skin for evidence of allergic symptoms, measured height and weight, performed pulmonary function testing (PFT) (spirometry and exhaled nitric oxide measurement), and collected biospecimens. The nurse administered the Asthma Control Test (ACT) to participants who reported an asthma diagnosis. Although similar data were collected at the Baseline and Follow-up Assessments, the Follow-up Assessment emphasized collection of data that reflected changes in health status and healthcare utilization since the Baseline Assessment. Another difference between the two visits was the biospecimen collection; at the Baseline Assessment, the nurse collected blood and urine; at the Follow-up Assessment, only urine was collected.

Since the health effects of living in the Gulf Coast area after the Hurricanes were a primary interest of CHATS, questionnaire data identified those participants with an asthma diagnosis and other signature health outcomes. The signature health outcomes included wheezing or extended dry cough without an asthma diagnosis; hay fever; allergies without an asthma diagnosis; rhinitis; eczema; itchy rash; and cancer. Because atopic facial and dermal symptoms are associated with asthma and other allergic responses, nursing staff also assessed CHATS participants for these outcomes.

Pulmonary function testing included a measurement of the fractional exhaled nitric oxide (eNO) using the NIOX MINO (Aerocrine) instrument. The eNO measurement is increasingly used in clinical practice for diagnosing asthma. An eNO value that reflects airway inflammation that exceeds 40 ppb is considered suggestive of asthma. Although the nursing staff demonstrated the procedure and provided an animated guide to assist in obtaining the required inhalation/exhalation, many participants of all ages had difficulty performing the required maneuver. Early in the study, the data collection instrument did not capture some successful maneuvers because some nurses were unable to follow the somewhat counter-intuitive program instructions. This problem resolved as the study progressed and the nurses became increasingly comfortable with the programming instructions. The measuring device was also quite sensitive to temperature. In a few instances, measurements were unsuccessful in homes in which the ambient temperature was hot or in which intense heat was generated from cooking. We measured all participants who self-reported an asthma diagnosis and/or respiratory symptoms suggestive of asthma. Only a random sample of participants without self-reported asthma and/or asthma symptoms were measured.

We assessed the pulmonary function of all CHATS participants, regardless of asthma status, with spirometry using the Easy-On PC (ndd Medical Technologies). Like the eNO

measurement, many participants had difficulty performing spirometry. Nurses also demonstrated this procedure and provided an animated presentation as a visual guide to the procedure. Although an approximately 15-minute interval occurred between the eNO measurement and the spirometry, the difference in the performance requirements between the two may have contributed to the difficulty. For instance, the eNO measurement required the child to blow in a slow, even rate, whereas the spirometry required the child to exhale hard and fast. Some children, especially younger children, were confused by the different methods of blowing. American Thoracic Society (ATS) standards were used to assess test quality.

During Baseline and Follow-up Assessments, we collected urine from each child to assess for exposure to phthalates, VOCs, and cotinine. We assessed creatinine to permit normalization of the measures. We also collected blood during the Baseline Assessment: total and specific IgEs and a Complete Blood Count (CBC). Protocols based on professional and regulatory standards guided nurses in obtaining specimens and preparing them for shipment delivery to the laboratory. We discuss results from these analyses in ***Chapter 3***.

2.8 Laboratory Analysis and Data Handling

2.8.1 Laboratory Protocols

Appendix A lists the analytes and their abbreviations used in tables in this report, media, and the source of samples and biospecimens. Protocols were prepared for sample analysis for the environmental samples and biological specimens. ***Table 2-8a*** shows an overview of the methods***,*** and full protocols are included in ***Appendix B***. All biospecimens (two blood and one urine) were logged into the clinical data system at the CLIA-certified LSU Interim Public Hospital's laboratory. The CBC, total IgE, and urinary creatinine were determined within 48 hours using an automated analyzer. The remaining urine was divided into aliquots for later analysis of cotinine, and for the substudy samples, analysis of VOC metabolites and phthalate metabolites. Biospecimens were stored frozen until further analyses. Environmental samples were distributed to the analytical laboratories at RTI for analyses. Routine QC steps (e.g., field and laboratory blanks, laboratory controls, and continuing calibration checks) were incorporated into procedures as described in each protocol. Each carbonyl badge (synonymous in all instances with Aldehyde badge) had its own background (unexposed portion) and each section was analyzed; the mass measured in the unexposed side was subtracted from the mass in the in the exposed side. VOC badge extracts were corrected for a method blank, prepared and analyzed with each batch. All results presented in the data deliverables keep samples and duplicates distinct. In addition, Quality Assurance (QA) analyses were included for VOC and formaldehyde badges through ongoing demonstration of proficiency as part of proficiency testing programs administered by American Industrial Hygiene Association (AIHA), and for VOC and phthalate metabolites by independent analysis of a subset of the urine samples at RTI.

Table 2-8a. Summary of Procedures (Research Operating Protocols [ROPs] and Standard Operating Procedures [SOPs]) Used for Analysis of CHATS Samples

Matrix and Analyte Group	ROP Name and Number	Method Overview	Primary Lab	QA Lab or Proficiency Organization
PM Mass	Gravimetric Analysis of Mass Collected on Filter Media in Children's Health after the Storms (CHATS), CANE-CHATS-009	Aerosols were collected onto Pall Teflo filters and the mass of the aerosols was determined by weighing the filters before and after sample collection.	RTI	None
PM Filter ETS	Sampling and Analysis of Aerosols for Black Carbon and Environmental Tobacco Smoke Using Optical Absorbance, CANE-CHATS-010	Aerosols were captured onto Pall Teflo filters, and the black carbon (BC) and environmental tobacco smoke (ETS) content was determined using an optical absorbance method. The filter is sequentially irradiated with multiple wavelengths of light. The amount of light of each wavelength absorbed is correlated to BC and ETS content on the filter at each wavelength.	RTI	None
PM Filter Allergens	Extraction and Analysis of Dust and PM Filter Samples for Environmental (Asp f 1, Bla g 1, Der f 1, and Der p 1) Antigens for CHATS, ROP 03, rev 1	Dust and filter samples were weighed for dust mass. The procedures in the ROP followed the package insert guidelines in the ELISA test kits commercially available from Indoor Biotechnologies, Inc. (Charlottesville, VA). Antigens were quantitated based on antigen-antibody binding, using a known monoclonal antibody reference for comparison.	RTI	None
HOBO Measure of Temperature and Relative humidity (Data can also be obtained from the MicroPEM)	Temperature and Relative Humidity Collection Using the HOBO U10 Data Logger in CHATS, CANE-CHATS-012	The HOBO was designed to measure relative humidity and temperature, providing direct and continuous readout as well as electronic recording of the information. In CHATS, field interviewers deployed the unit in the participant's home, and indoor temperature and relative humidity measurements were collected on a 5-minute basis for 5 to 9 days of record of the participant's indoor conditions. The unit was shipped back to RTI, and RTI staff downloaded the relative humidity and temperature data from these units. The HOBO monitors were factory calibrated and subject to on-site verification prior to field placement. Batteries for these monitors were replaced every 6 months. The monitor was located away from heating zones, zones of air movement, and fixed lighting sources. Attaching the monitor to the indoor sampling cage met these requirements. MicroPEM temperature and relative humidity sensor readings were verified prior to field placement.	RTI	NA

(continued)

Table 2-8a. Summary of Procedures (Research Operating Protocols [ROPs] and Standard Operating Procedures [SOPs]) Used for Analysis of CHATS Samples (continued)

Matrix and Analyte Group	ROP Name and Number	Method Overview	Primary Lab	QA Lab or Proficiency Organization
PM Filter and Bulk Floor Dust for (1,3)-β-di-glucan	Extraction and Analysis of Dust and PM Filter Samples for (1,3)-β-di-Glucan for Children's Health After the Storms (CHATS), ROP 02, rev 1	Dust and filter samples were weighed for dust mass. (1-3)-β-D-glucan was quantitated using Glucatell® (Associates of Cape Cod, Inc., Falmouth, MA), a commercially available assay. Quantitations were based on the reaction of glucan in the specimen with lysate, producing a color change over time at 540 nm, as compared to similar reactions of a standard reference of known glucan content.	RTI	None
PM Filter and Bulk Floor Dust For Endotoxin	Extraction and Analysis of Dust and PM Filter Samples for Endotoxin for Children's Health After the Storms (CHATS), ROP 01, rev 1	Dust and filter samples were weighed for dust mass. Endotoxin was quantitated based on the sample reaction with Pyrochrome® (Associates of Cape Cod, Falmouth, MA), a commercially available Limulus Amebocyte Lysate (LAL) assay. The reaction causes a color change at 405 nm over time, and is compared to similar reactions of a known standard endotoxin reference.	RTI	None
Bulk Floor Dust, Pre-Analysis Processing	Sieving of Dust Samples for CHATS, Research Operating Procedure 04	The collected HEPA sock dust sample was opened and placed in the sterile sieve. It was sieved for 30 minutes, and the collected dust was weighed and saved for analysis.	RTI	NA
Air Carbonyls	Procedure for Determining Carbonyls from Passive Samplers for Children's Health after the Storms (CHATS), EAR-CHATS-001	The procedure was taken from EPA's Compendium Method TO-11A, Determination of Formaldehyde in Ambient Air Using Adsorbent Cartridge followed by High Performance Liquid Chromatography (HPLC) and SKC Update to EPA Compendium Method IP-6A, Determination of Formaldehyde and Other Aldehydes in Indoor Air Using a Solid Adsorbent Trap. DNPH-coated filters from passive badges (both the exposed and unexposed or blank portions) were extracted in acetonitrile. The filter was removed from each extraction vial and the extract is analyzed by HPLC-UV. QC samples included reagent blanks, matrix blanks, matrix spikes, calibration checks, and second source checks. The HPLC was calibrated using a minimum of a five-point standard curve. Chromatograms were processed using Empower2 data system and data were output as individual electronic files using an export macro. After QA review of the individual data files, data were read electronically into the study database from the output files per data management protocol.	RTI	AIHA

(continued)

Table 2-8a. Summary of Procedures (Research Operating Protocols [ROPs] and Standard Operating Procedures [SOPs]) Used for Analysis of CHATS Samples (continued)

Matrix and Analyte Group	ROP Name and Number	Method Overview	Primary Lab	QA Lab or Proficiency Organization
Air VOCs	Procedure for Determining Volatile Organic Compounds from Passive Samplers for Children's Health after the Storms (CHATS), EAR-CHATS-002	The procedure was developed for use in conjunction with methods for the analysis of VOCs extracted from 3M 3500 Organic Vapor Monitors (OVM) badges (3M, Minneapolis, MN), SOP EAR-GLC-001 and SOP EAR-GLC-002. The charcoal filters were extracted directly inside the passive badges in 2:1 acetone:carbon disulfide extraction solution. The extraction solution was analyzed by GC-MSD (Agilent Technologies 6890 gas chromatogram with a 5973N mass selective detector and ChemStation software). QC samples included reagent blanks, matrix blanks, matrix spikes, calibration checks, and second source checks. The GC-MSD was calibrated using a minimum of a five-point standard curve. Chromatograms were processed using Agilent Technologies ChemStation data system and data were output as individual electronic files. Each analyte in each batch was corrected for method background though use of the method (matrix) blank for that batch. After a QA review was conducted on the individual data files, data were read electronically into the study database from the output files	RTI	WASP
H_2S	Procedure for Determining Hydrogen Sulfide (H_2S) from Passive Samplers for Children's Health After the Storms (CHATS), ROP 09	The procedure was taken from the manufacturer of the passive sampler (Radiello®, Fondazione Salvatore Maugeri, Padua, Italy). The cartridge contained zinc acetate, which adsorbs hydrogen sulfide, transforming it into stable zinc sulfide. The sulfide was recovered by extraction with water then reacted with the N,N-dimethyl-p-phenylendiammonium ion in a strongly acidic ferric chloride solution (an oxidizing agent) to yield methylene blue. Methylene blue was quantified by visible spectrometry. The primary QC samples were laboratory blanks, which were averaged and subtracted from the samples to correct for background. The spectrophotometer was calibrated using a minimum of a four-point standard curve, which is prepared from dilutions of a methylene blue solution obtained from the manufacturer of the passive samplers. Absorbance measurements were saved in files generated by the spectrophotometer software then processed using a spreadsheet to generate a calibration curve and quantitate sample concentrations. After QA review of the individual data files, data were read electronically into the study database from the output files.	RTI	None

(continued)

Table 2-8a. Summary of Procedures (Research Operating Protocols [ROPs] and Standard Operating Procedures [SOPs]) Used for Analysis of CHATS Samples (continued)

Matrix and Analyte Group	ROP Name and Number	Method Overview	Primary Lab	QA Lab or Proficiency Organization
NO_2	Research Operating Procedures for Handling and Analysis of Passive NO2 Samplers, for the Children's Health after the Storms (CHATS) Study, EC-CHATS 001	Each Ogawa passive sampler consisted of one TEA-coated pad (for NO_2 sampling) mounted in one end of a barrel-shaped holder attached to a badge. Each sampler was delivered to the sampling location inside a zip-closure plastic bag that is placed inside a screw-top, airtight storage container. At the sampling location, the passive sampler was removed from the protective shipping container and bag and exposed to ambient air for a carefully selected and documented period of time, usually 1 day to 1 week. The sampler was then returned to its bag and shipping container for storage until it was processed for analysis. Blank, unexposed TEA-coated pads from the same lots as the study samples were provided for use as extraction Method Blanks (MBs). Results were reported in µg NO_2- per sample. The average NO_2 exposure concentration was then calculated based on the nitrite content measured by ion chromatography, the exposure duration, and the appropriate collection factors. This SOP document contains the RTI Ion Analysis Laboratory procedures for handling the exposed NO_2 pads as received from the field.	RTI	None
Blood CBC	Automated Complete Blood Count (CBC) for CHATS on the Coulter LH750, CORE-HEME-1	The COULTER® LH 750 was a quantitative, automated hematology analyzer for In Vitro Diagnostic use in clinical laboratories. The LH 750 provided automated complete blood count, leukocyte differential, reticulocyte analysis, and nucleated red blood cell (NRBC) enumeration. The purpose of the LH 750 was to separate the normal patient, with all normal system-generated parameters, from the patient who needs additional studies of any of these parameters. These studies included further measurements of cell size and platelet distribution, biochemical investigations, manual WBC differential or any other definitive test that helps diagnose the patient's condition.	LSU	None
Serum, Specific IgEs	Core Laboratory Immunochemistry Manual – Immulite, CORE-Immuno-50	IMMULITE 2000 3gAllergy™ Specific IgE is a solid-phase, two-step, chemiluminescent immunoassay that exploits liquid phase kinetics in a bead format. (U.S. Patent No. 4,778,751) It represents a significant advance over conventional methods relying on allergens attached to a solid-phase support, such as a paper disk. The allergens were covalently bound to a soluble polymer/co-polymer matrix, which in turn is labeled with a ligand. The use of an amino acid copolymer amplifies the amount of allergen that the matrix can support.	LSU	None

(continued)

Table 2-8a. Summary of Procedures (Research Operating Protocols [ROPs] and Standard Operating Procedures [SOPs]) Used for Analysis of CHATS Samples (continued)

Matrix and Analyte Group	ROP Name and Number	Method Overview	Primary Lab	QA Lab or Proficiency Organization
Serum, Total IgE	Determination of Total IgE in CHATS using the Beckman Coulter Access Immunoassay System, CORE- Total IGE Revision 0	The Access Total IgE assay is a sequential two-step immunoenzymatic ("sandwich") assay. A sample is added to a reaction vessel along with paramagnetic particles coated with goat antimouse: mouse anti-IgE complexes. The IgE in the sample binds to the mouse anti-IgE on the particles. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Equine anti-IgE conjugated to alkaline phosphatase is then added and binds to the previously bound IgE on the particles. A second separation and wash step removes unbound conjugate. Then, the chemiluminescent substrate Lumi-Phos* 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of IgE in the sample. The amount of analyte in the sample is determined from a stored, multipoint calibration curve.	LSU	None
Urine, Creatinine	Quantitative Determination of Creatinine in Human Plasma, Serum, for CHATS Using the SYNCHRON® System(s), CORE-CHEM-DXC-24	CR-S reagent was used to measure the creatinine concentration by a modified rate Jaffé method. In the reaction, creatinine combines with picrate in an alkaline solution to form a creatinine-picrate complex. The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into the cuvette. The ratio used was one part sample to 11 parts reagent for serum and one part sample to 73 parts reagent for urine. The System monitored the change in absorbance at 520 nanometers. This change in absorbance was directly proportional to the concentration of CR-S in the sample and the System used it to calculate and express CR-S concentration.	LSU	None
Urinary Phthalate Metabolites	Phthalate Metabolites in Urine by High Resolution-Accurate Mass Spectrometry for CHATS, PHTHMET-U01	A modification of CDC Method No. 6306.03 for urine phthalate metabolites (2010) was used. This method measured individual total phthalate metabolites with the addition of labeled internal standards after enzyme hydrolysis. Compound separation and identification were achieved with an ultra-high performance liquid chromatography-mass spectrometer system (UHPLC-MS). High resolution and accurate mass (HR/AM) feature of mass spectrometer was used to select the target compounds specifically. Sample processing is simplified and a quick method is achieved.	LSU	RTI (with the method shown below)

(continued)

Table 2-8a. Summary of Procedures (Research Operating Protocols [ROPs] and Standard Operating Procedures [SOPs]) Used for Analysis of CHATS Samples (continued)

Matrix and Analyte Group	ROP Name and Number	Method Overview	Primary Lab	QA Lab or Proficiency Organization
Urinary VOC Metabolites	Mercapturic Acid Metabolites in Urine by High Resolution-Accurate Mass Spectrometry for CHATS, MERACIDVOC-U01	A modification of the method of Alwis et al. (2012) was used. This method used urine with the addition of labeled internal standards. Compound separation and identification were achieved with an ultra-high performance liquid chromatography-mass spectrometer system (UHPLC-MS). High resolution and accurate mass (HR/AM) feature of mass spectrometer was used to select the target compounds specifically. Sample processing was simplified and a quick method was achieved.	LSU	RTI (with the method shown below)
Urinary Cotinine	Nicotine - Cotinine in Urine by Turboflow LC-Electrospray Tandem Mass Spectrometry	This method used urine with the addition of labeled internal standards. Separation and concentration of the hydrolyzed metabolites was performed by turboflow liquid chromatography on a Thermo Scientific Aria TLX2 multiplex LC system which allows for a vigorous cleaning cycle of the column to reduce the possibility of carryover and also allows diversion of waste while directing sample to the tandem mass spectrometry. The use of tandem mass spectrometry improves specificity and sensitivity in determination of nicotine and cotinine. Turboflow HPLC eliminated many of the problems created by ion suppression in tandem mass spectrometry.	LSU	None
Urinary Phthalates Metabolites	Determination of Phthalate Metabolites in Urine for Children's Health after the Storms (CHATS), EAR-CHATS-22	The method was based on two procedures from the Centers for Disease Control and Prevention (CDC). The sample preparation method was taken from the procedure developed by Dr. Dana Barr (NHANES 2001-2002). The instrumental method was taken from the procedure developed by Dr. Antonia Calafat (NHANES 2007-2008). Urine samples were processed using enzymatic deconjugation of the glucuronides followed by automated solid phase extraction (auto-SPE) and concentration of the resulting eluate. The phthalate metabolites were then chromatographically resolved by reversed phase high performance liquid chromatography-electrospray ionization-tandem mass spectrometry (HPLC-ESI-MS/MS) and quantified by isotope dilution. QC samples include reagent blanks, reagent controls, matrix blanks, matrix spikes, and calibration checks. The HPLC was calibrated using a minimum of a six-point standard curve. Chromatograms were processed using Analyst 1.4.2 data system and data were output as Microsoft Excel Spreadsheets (*.xls). The data required to be entered into the CHATS database were done by the laboratory supervisor, using CHATS ROP #21 and uploading it to the Enhanced Security Network using FileZilla.	LSU (using method shown above)	RTI

(continued)

Table 2-8a. Summary of Procedures (Research Operating Protocols [ROPs] and Standard Operating Procedures [SOPs]) Used for Analysis of CHATS Samples (continued)

Matrix and Analyte Group	ROP Name and Number	Method Overview	Primary Lab	QA Lab or Proficiency Organization
Urinary VOC Metabolites	Determination of VOC Metabolites in Urine for Children's Health after the Storms (CHATS), EAR-CHATS-23	The method was adapted from a procedure from CDC, developed by K. Udeni Alwis et.al. Urine samples are diluted tenfold and filtered. The VOC metabolites were then chromatographically resolved by reversed phase ultrahigh performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UHPLC-ESI-MS/MS) and quantified by isotope dilution. QC samples included reagent blanks, reagent controls, matrix blanks, matrix spikes, and calibration checks. The HPLC was calibrated using a minimum of a six-point standard curve. Chromatograms were processed using Analyst 1.4.2 data system and data were output as Microsoft Excel Spreadsheets (*.xls). After QA review of the individual data files, data were uploaded electronically into the study database from the output files using FileZilla.	LSU (using method shown above)	RTI

2.8.2 Sample Shipping and Handling

RTI shipped blood and urine samples or dropped them off at the LSU laboratory; receipt and condition were provided to RTI via PDF files of the hardcopy shipping manifests. The remaining sampler types were shipped to a receiving laboratory at RTI. Upon receipt, staff logged the samples, inspected them for damage that might have influenced the quality of results obtained through their analyses, and stored them briefly according to their respective analytical protocol prior to transfer to the appropriate analytical laboratory for analysis. Sample transfer was documented via chain-of-custody forms signed by a representative of each analytical laboratory.

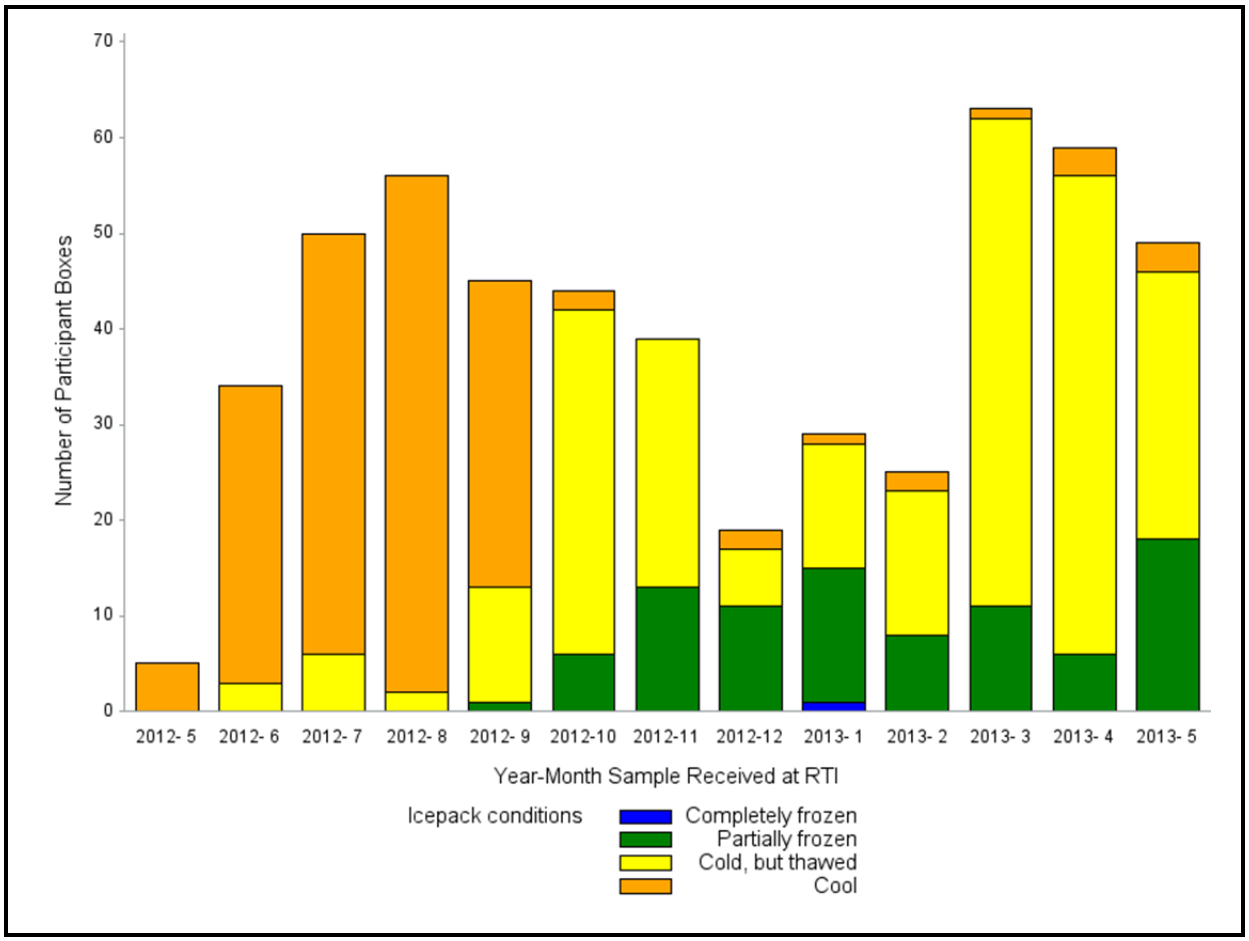
When the participant boxes arrived at RTI, staff inspected the physical condition of each environmental sampler to evaluate whether field interviewers had properly handled and shipped the boxes and their contents. The application of quality indicators is described in detail in ***Section 2.8.3***; however, we also mention it here in an abbreviated form to indicate how the concept was applied to sample integrity prior to analysis. As an integral part of sample receipt, our staff assessed the integrity of each sample and recorded that information electronically. We assigned record quality indicators (RQIs) to each sample record using a numeric indicator value of 0, 1, or 2. We assigned an RQI of 0 to records where the sample lacked any evidence of compromise, either during deployment or shipping; these samples were considered "acceptable" without reservation. Alternatively, an RQI of 1 indicated that some aspect of the sample was not ideal (e.g., the sample storage temperature during shipment was outside the specified range or the sample was delayed during shipment) and the data derived from the analysis of that sample, while still acceptable, carried an increased level of uncertainty. Finally, we assigned an RQI of 2 to sample records when evidence (physical or recorded) indicated the sample integrity had been compromised and analysis would not yield useable results. Examples of such conditions included samplers with lids improperly secured (e.g., VOC and vacuum dust) or samplers with clear evidence of damage to critical membrane coverings (e.g., VOC badges). We assessed the temperature of the box based on the physical condition of icepacks that were shipped together with samplers; temperature was recorded in four categories (i.e., completely frozen; partially frozen; cold, but thawed; cool). Samplers in participant boxes that were shipped during or right before holidays or weekends were not always maintained at reasonable temperatures because the icepacks had typically melted. The higher temperatures did not affect some of the samplers in the box. However, the temperature did affect some samplers; we assigned an RQI of 1 to those because of potential exposure to higher-than-desirable temperatures. ***Table 2-8b*** provides frequencies of box return over weekends or holidays.

Table 2-8b. Frequencies and Percentages of Participant Box Return to RTI over Weekends or Holidays

	Number (Percent of Total)	
	Baseline	Follow-up
Frequency and Percentage of Participant Box Return	9 (5.0)	4 (2.6)

Figure 2-8a shows the temperature condition of the participant boxes when RTI received them. Because of insufficient insulation and high ambient temperature during the summer, many participant boxes were returned with thawed icepacks during the first half of Baseline Assessment. At the end of September 2012, RTI modified the participant box by adding another layer of insulating foam. This modification effectively kept the temperature lower, which improved the condition of shipped environmental samples.

Figure 2-8a. Icepack Conditions as Indicator of Temperature Condition of Participant Boxes upon Receipt at RTI



RTI collected biological samples 7 days a week, at the participant's convenience, but storms and problems with couriers delayed the delivery of some samples. Delivery delays of more than 72 hours can cause degradation of whole blood and questionable CBC results from the purple-top vacutainer tube, so RTI assigned those results a laboratory RQI of 2 for poor quality (15, or 13% of the total number collected, were affected). Most delays were caused by weekend collections (9, or 60% of the delays); for example, the nurse collected the sample on a Saturday or Sunday but the courier was not able to pick up the shipment until Monday or Tuesday delivery. Problems also occurred when the courier delivered samples directly to the LSU laboratory on Saturday morning because the receiving dock of the LSU Interim Public Hospital was closed. We resolved this issue by speaking to a supervisor at the courier's business. ***Table 2-8c*** quantifies the Baseline samples affected by weather, delays, or other reasons that adversely affected the sample quality or resulted in a sample that could not be analyzed. (No blood was collected during the Follow-up Assessment.)

Table 2-8c. Baseline Samples Adversely Affected by Weather, Delays in Delivery, or Other Reasons

	Purple-Top Tube (for CBC)	Gold-Top Tube (for IgE)	Urine Samples
Number delayed by Hurricane Isaac	2	—	—
Number delayed by FedEx attempt of Saturday delivery	4	—	—
Number delayed by weekend sample collection/similar reason	9	—	1
Clotted blood	1	1	—
Urine leaked completely out of specimen container	—	—	2
Total collected	113	112*	154

*Although a purple-top tube was collected, a gold-top tube was not collected from one child.

During the Baseline Assessment a small number of urine samples leaked during shipment, and two samples leaked completely out of the urine specimen container. RTI promptly contacted nurses who had shipped leaking urine specimens and reminded them to ensure that the urine specimen cap was tightened before they packed the sample for shipment. During the 6-month Follow-up Assessment, nurses collected a total of 138 urine samples. Although a few urine samples leaked slightly during shipment, none of them leaked completely.

2.8.3 Data Submission and Processing

The Data Management Protocol (see ***Appendix B***) was the primary guidance document for data delivery from the analytical laboratories to the Laboratory Data Manager and subsequent data processing steps. This document contains the framework for data delivery structure, filenames, and content and describes the general scheme under which analytical

data were processed with other requisite information (e.g., sample deployment times, and sample RQIs). RTI delivered all laboratory data to predefined electronic folders within our Enhanced Security Network (ESN) and processed the data exclusively in that environment using SAS (v 9.3). Because each sample type required different processing procedures based on the type of measurement and requirement for ancillary data, we developed individualized processing programs for each laboratory module. In this regimen, we needed customized data processing routines for air VOCs, air carbonyls, air H_2S, air NO_2, air particles (PM_{10} mass and ETS), dust phthalates, dust allergens (vacuum and MicroPEM filter), urinary cotinine, clinical data (CBCs, IgEs, and urinary creatinine), urinary metabolites (VOC and phthalate), and urinary cotinine. To the extent possible, we used a common processing structure that we modified to accommodate each module's specific needs. In addition to the SAS programs for processing individual modules, RTI created an overarching macro-driven program, *StatDataReview*, to assign SAS libraries, to call the specific program needed for a particular data processing event, and to create graphical and tabular data summaries.

Processing the results for the environmental samples required us to deploy the sample deployment and retrieve metadata from the questionnaire datasets to facilitate computation of final concentrations and to allow comprehensive sample accounting. We constructed SAS programs to extract selected variables from these primary datasets and configure a metadata dataset for these purposes.

When RTI received each data delivery, our staff reviewed the delivered files to check that format and content were correct and consistent and that filename was specified in the Data Management Protocol. If we needed to make modifications to facilitate processing of a particular delivery file, we saved the file under a nominally revised filename. After adding the filename to the *StatDataReview* program, we ran a program to incorporate the new data into the final datasets (Baseline and Follow-up) for that module. We retained intermediate datasets containing all original and created variables as permanent SAS datasets. The structures of both the intermediate and final datasets are one record per sample ID-analyte combination. In addition to the final measurement concentration (FINLCONC), we assigned values for three corroborative variables for each data record. Two of these variables, NDIND and FINAL_RQI, characterize the magnitude and quality, respectively, of the final measurement concentration. NDIND is a dichotomous numeric variable to which a value of 1 is assigned if the final concentration measurement is at or above the method detection limit; otherwise, a value of 0 is assigned. FINAL_RQI is computed logically from the maximum of the "field" RQI (FIELD_RQI) or "lab" RQI (ARQI). The third corroborative variable, FINLCONCUNIT, contains the units of the final concentration measurement.

Because of the multiple stages involved that could impact the quality of the sample—i.e., shipping, deployment, collection, shipping, transfer, testing—RTI developed the concept of RQI and applied it to the CHATS study to inform subsequent data analysts about the uncertainty of each measurement, which resulted from the sample collection and shipment processes as well as the sample analysis process. RTI assigned RQIs according to the following scheme:

- FINAL_RQI = 0: This RQI was assigned to records where both the sample and the analytical result lacked any evidence of compromise during deployment, shipping, or analysis. These samples are "acceptable" without reservation. This RQI includes samples with a nondetect reported for a given analyte.
- FINAL_RQI = 1: This RQI indicates that some aspect of the sample or its analytical result was not ideal. The data derived from the analysis of such a sample, while still acceptable, had an increased level of uncertainty. This RQI includes detectable values that are below the lowest calibration curve point; extrapolation beyond curve limits, either above or below the curve, increases the uncertainty of such values.
- FINAL_RQI = 2: This RQI was assigned to sample records where evidence indicated the sample integrity had been compromised or the analysis result did not meet the QA criteria specified in the analytical protocol. These measurements are deemed outside the acceptable range of uncertainties.
- FINAL_RQI = 3: This RQI was assigned to sample records where a mismatch was found between the sample ID associated with the analytical result and the sample ID in the metadata dataset. RTI investigated and resolved records with FINAL_RQI = 3 so that the RQI ultimately reassigned only included 0, 1, 2, 4, or 5 in the final dataset.
- FINAL_RQI = 4: This RQI was assigned to records that had metadata (i.e., the sample had been successfully collected), but had no analytical results exist because the sample was deemed unanalyzable (FIELD_RQI = 2) upon receipt at RTI, most often due to shipping issues. In most cases, samples with this condition were not distributed to the laboratory for analysis.
- FINAL_RQI = 5: This RQI was assigned to records that had incomplete metadata (i.e., the field interviewer did not successfully collect the sample and did not record a sample collection date) and had no analytical results.
- FINAL_RQI = 6: This RQI was assigned to records that had metadata (i.e., the sample was successfully collected), but had no analytical results; and we could find no reason for the absence of these results. Samples with FINAL_RQI = 6 were assumed to be in the analytical queue, and results would be forthcoming. All records with FINAL_RQI = 6 were thoroughly investigated before the final datasets were completed, and the RQI ultimately reassigned to 0, 1, 2, 4, or 5 in the final dataset.

2.9 Outreach

The purpose of the CHATS outreach effort was to build a broad partnership to obtain input and agreement and to engage trusted Gulf Coast organizations and community leaders' support for the study. The primary goal of the public outreach was to support the recruitment, enrollment, and retention of eligible participants for the Baseline and 6-month Follow-up Assessments.

CDC and RTI established three objectives for the outreach effort:

1. Raise awareness and inform Gulf Coast residents and eligible families of the study and its purpose.
2. Encourage eligible families to support and participate in the study.
3. Provide a means for ongoing, two-way communication between the study team and affected residents to maintain a flow of accurate, timely information to communities, and for communities to share any concerns and/or questions with the study team.

RTI developed the public outreach plan and implemented it after receiving approval from the IRBs for CDC and RTI. This plan reflected the established target areas for the Feasibility Study and the audiences identified for recruitment and enrollment. The plan focused on establishing and achieving the public outreach objectives for the study through three primary activities: a Community Advisory Panel (CAP) to help inform and guide the outreach effort and to serve as a voice to and advocate for the community; a broad community-based partnership effort to support public awareness and dissemination of news and promotional materials about CHATS to area families; and limited advertising to support broad, local awareness about CHATS through trusted mainstream and digital channels, including radio, print, and online media.

After the plan was approved and when the other major activities were ready, RTI worked with the Louisiana Public Health Institute (LPHI) to develop and implement the public outreach and communication effort. LPHI supported RTI with communication and public involvement—specifically, outreach to recruit, enroll, and retain eligible participants for data collection. In addition, LPHI conducted an evaluation of the outreach effort covering the Feasibility Study. RTI also provided recommendations for revising/enhancing future outreach efforts, as warranted, for a possible Full Study. RTI carried out the public outreach effort concurrently with two other major tasks: frame development and sample selection strategies, and data collection.

2.9.1 Establishment of a CHATS Community Advisory Panel (CAP)

To facilitate the broader community engagement, our first outreach effort was to establish a CAP of leaders of respected organizations to serve as a voice for and to the community on CHATS. RTI and LPHI worked together to identify and recruit eight (five from Louisiana and three from Mississippi) key community leaders and community organizations known to deliver needed human and social services in the aftermath of the storms and who also had the trust of the Gulf Coast residents in the communities they served. CAP members were to provide local expertise and input on all aspects of the outreach efforts, including messages, materials development, dissemination, and public forums, as well as any other special event. CAP members also provided critical insights on particular sensitivities of area residents to research studies and the design and purpose of CHATS, as well as anticipated receptivity to the study. CAP members helped establish the CHATS name and acronym as the brand for public awareness and education. They served as invaluable representatives of the community and brought questions, concerns, and recommendations that arose prior to, during, and after the Feasibility Study to the study team. RTI held regular meetings with the CAP members throughout the Feasibility Study.

2.9.2 CHATS Partnership Development and Public Forums

RTI launched the CHATS partnership effort by participating in the Mississippi Disaster Task Force's annual wellness conference, which allowed us to meet with and introduce CHATS to more than 150 social service organizations serving Gulf Coast families throughout the targeted areas for the study. Working with LPHI and based on recommendations by CAP members, RTI developed a comprehensive list of more than 1,000 community organizations and leaders and conducted outreach to these organizations to engage them as partners with CHATS. The list was composed of a diverse array of organizations and individuals, primarily focusing on those that served families with children (e.g., state and local health departments, schools, religious organizations, social service, and civic organizations, recreation centers, community centers, neighborhood leaders, neighborhood associations) reflecting children as the target population. The majority of organizations continued to work with individuals who lived in FEMA-provided trailers in Louisiana and Mississippi, including representatives of different ethnic groups. CHATS outreach efforts specifically focused on outreach to schools in the targeted sites, and RTI organized in-person and conference call meetings with area school officials to inform them of the study and secure their support. This support was particularly critical, since children who participated in the study were required to wear a small air-measuring device in their daily routine, which included time spent at school. Schools that the participating children attend needed to agree to the use of the device on their premises.

RTI distributed e-mail invitations to the public forums approximately 3 weeks prior to the first forum, and made more than 900 follow-up calls to encourage attendance at the event, both the week prior to the first forum and during the week of the forum.

To address the possibility of lower turnout due to reported study fatigue and the Easter break, RTI also promoted the forums with live-read announcements on local television stations and placed the forum invites on public calendars in each area. The week of the forums, staff also distributed over 300 forum invitations to customers at the local Wal-Mart in St. Bernard's Parish, and CAP members broadly distributed the forums invitations to their constituencies and through their own extensive listserv, community newsletters, and weekly e-mails.

To further promote the forums and encourage public attendance, RTI also placed announcements in the Community News sections of the following publications:

- *The New Orleans Times Picayune*
- *The New Orleans Tribune*
- *The Louisiana Weekly*
- *Data News Weekly*
- http://jeffersonchamber.org/2012/04/childrens-health-after-the-storms-chats-forumapril-11/
- *The Advocate*
- *The Baton Rouge Journal*
- *St. Helena Echo*
- *The Livingston Parish News*
- *The Mississippi Sun-Herald*
- Biloxi's *D'Iberville Press.*

RTI also contacted local pediatrician practices to place CHATS public forum announcements in their lobbies and waiting areas. In addition, New Orleans Medical Association President, Dr. Jola Creer-Perry, distributed the CHATS forum invitation to her list of area pediatricians with a note encouraging them to distribute the announcements to their patient-parents.

The New Orleans Neighborhood Partnership Network (NPN) distributed the CHATS forum invitation to their database of over 3,000 individuals and organizations on April 3 and April 9, 2012. Jefferson Parish Public Information Officer, Kris Fortunato, sent the CHATS forum invitation to the 60 Neighborhood Association presidents in Jefferson Parish. Finally, the team worked with the publishers of the *New Orleans (NOLA) Baby & Family Magazine* and *The New*

Orleans Agenda to deliver CHATS public forum invitations to their respective databases, which represented over 11,000 individuals.

Community forums were used to create awareness of and educate local community leaders and influencers on the CHATS study in an effort to gain support. The RTI and LPHI team planned and managed five CHATS Community Forums; responded to two requests for presentations by area organizations, and conducted two interviews with local media. We developed presentation content, as well as collateral materials (posters, community information cards, specialty items, and related materials), which were disseminated to attendees. The Community Forums were held April 9 to April 13, 2012, just before data collection began, in the Louisiana parishes of Orleans, St. Bernard, East Baton Rouge, Jefferson, and St. Helena, and the coastal counties of Mississippi, Harrison, George, and Jefferson. The forums were open to the public and publicized via the local news media, which aired news stories on the forums, and specific invitations were extended to key community leaders and stakeholders, including the Mayor's Office, parish presidents, local health department officials, city council members, neighborhood association, Faith-based Leaders, school superintendents, and others.

RTI also administered a participant survey, developed in collaboration with LPHI, and designed to assess the usefulness of the content provided at the forums and participant willingness to support the CHATS study.

2.9.3 Design and Implementation of a Local Media/Advertising Campaign

RTI selected 9 counties and parishes throughout Mississippi and Louisiana as the target sites for the Feasibility Study. RTI and LPHI worked with mainstream media outlets serving the news and information needs of the communities (newspapers, radio stations, billboards, and a CHATS newsletter) to announce the study and to share news on its progress to encourage participation by eligible families. RTI coordinated with the communications representative for CDC to develop and release public messages to the media, and to arrange interviews of CHATS spokespersons. RTI contracted with Morgan & Co of New Orleans to create outdoor billboards, posters, and bulletins.

The campaign launched on May 25, 2012, and finished on June 18, 2013. In total, CHATS received more than 270 airings of the radio spots and television interviews. Two interviews aired with Dr. Diane Wagener (RTI) at the public forums in St. Bernard Parish, Louisiana, and in Hancock County, Mississippi. The local CBS-affiliate aired interviews with Ms. Timolynn Sams of the Neighborhoods Partnership Network, who represented the CAP.

RTI harnessed social media, including web-based tools and communities, to promote the study, address questions, and encourage support and participation by the target communities. The team geo-targeted online ads on Facebook to reach area families in the communities targeted for the Feasibility Study at street and neighborhood levels. Other social media opportunities included creating linkages for the CHATS website to the websites of CAP and other partner organizations serving the target communities, setting up CHATS Twitter and Facebook accounts with links from the CHATS website, and posting daily updates. A CHATS profile and presence was established in the community by joining online communities of neighborhood associations and community development corporations in the target areas.

3. FINDINGS

3.1 Public Outreach Results

The CHATS Community Advisory Panel (CAP) supported the CHATS study throughout the duration of the Feasibility Study. Their contributions were invaluable to the CHATS study and its success and include the following:

- Assistance in establishing a name that became the brand for the study;
- Critical insights into the public's reaction to the hurricane response, including weariness and lingering anger, and their perceptions of post-event housing and Federal assistance; and
- Guidance on incorporating this knowledge into how the study could be positively presented to prospective participants and the community at large.

Lastly, the CAP's active participation in the public forums and subsequent media outreach activities and interviews during the data collection and follow-up phases of CHATS were essential to keeping the study in front of the public's attention.

In addition to key partnerships with the organizations that composed the CAP, RTI reached out and established informal information dissemination partnerships with hundreds of public, private, and community-based organizations serving the needs of Gulf Coast families. These partners showed their support by displaying CHATS posters, disseminating the CHATS newsletter and information cards in their client waiting areas and at their special events (e.g., health fairs, booths at festivals), and directing inquiries from the public to the RTI Team and/or the CHATS website.

CAP members, staff from the Louisiana Public Health Institute (LPHI), and other CHATS partners also helped to provide advance publicity for the public forums that were held in April 2012. Despite the need to hold the forums during Easter break, when area families might be traveling, a number of organizations sent representatives to attend one or more of the forums. All the attendees expressed support for CHATS and a commitment to support public awareness efforts. ***Table 3-1a*** provides a summary of the number of attendees at each of the forums.

Table 3-1a. Snapshot of Forum Attendance

East Baton Rouge Parish	St. Bernard Parish & Chalmette Neighborhood Advisory Council	Orleans & Jefferson Parishes	Gulfport, Mississippi	Biloxi, Mississippi, & Mississippi Coast Interfaith Disaster Task Force	Total
17	17	21	11	32	98

Attendees at the public community forums were given surveys to capture their opinions on the content of the forums, as well as the logistics of the meetings. Only 48 forum attendees completed surveys, and there were missing items for some participants. Of the completed surveys, 9 participants were male and 39 were female (see ***Figure 3-1a***, below, for a gender breakdown of survey respondents). There was an even representation of state of residency, with 24 Louisiana resident participants and 24 Mississippi resident participants. Participants represented a wide array of organizations. Six participants reported being representatives of schools; 13 were community members; 6 were representatives of businesses; 4 were representatives of CBOs; and 19 responded "other," with responses for "other" ranging from state government officials to Department of Health employees (see ***Figure 3-1b*** for an organizational breakdown of survey respondents).

Figure 3-1a. Gender Breakdown of Survey Respondents

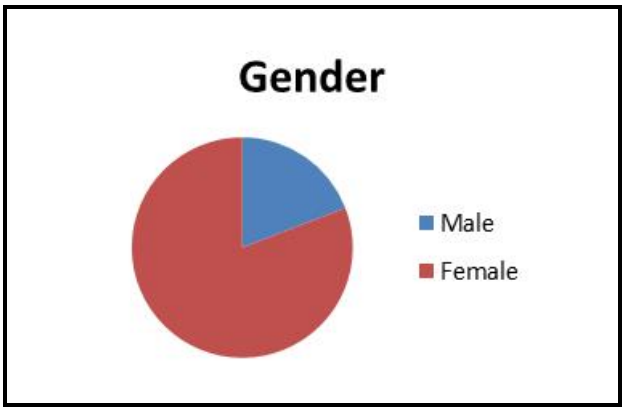
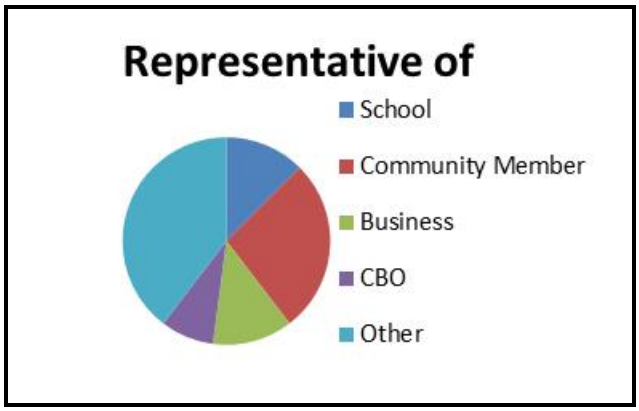
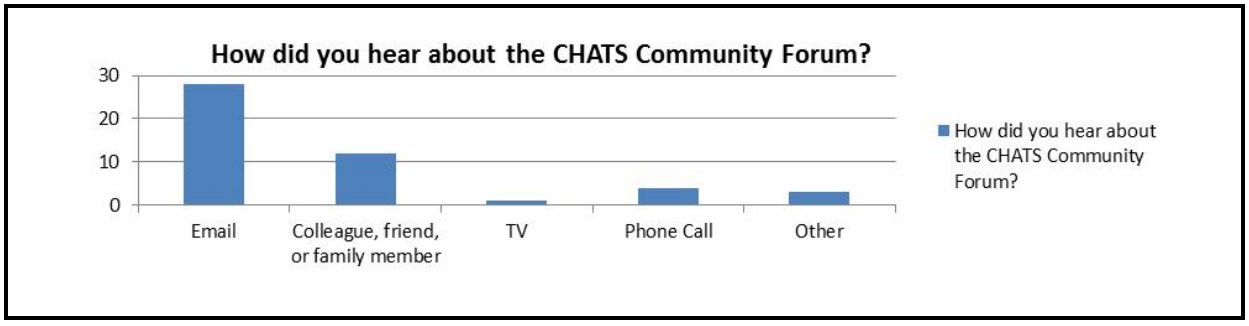


Figure 3-1b. Survey Respondent Representation



Participants reported hearing about the forums from a variety of sources, with e-mail being the most common at 58.3% (28). Other responses included a colleague, friend, or family member; TV; phone call; and other unspecified means (see ***Figure 3-1c*** for a breakdown of how participants reported hearing about the CHATs forum).

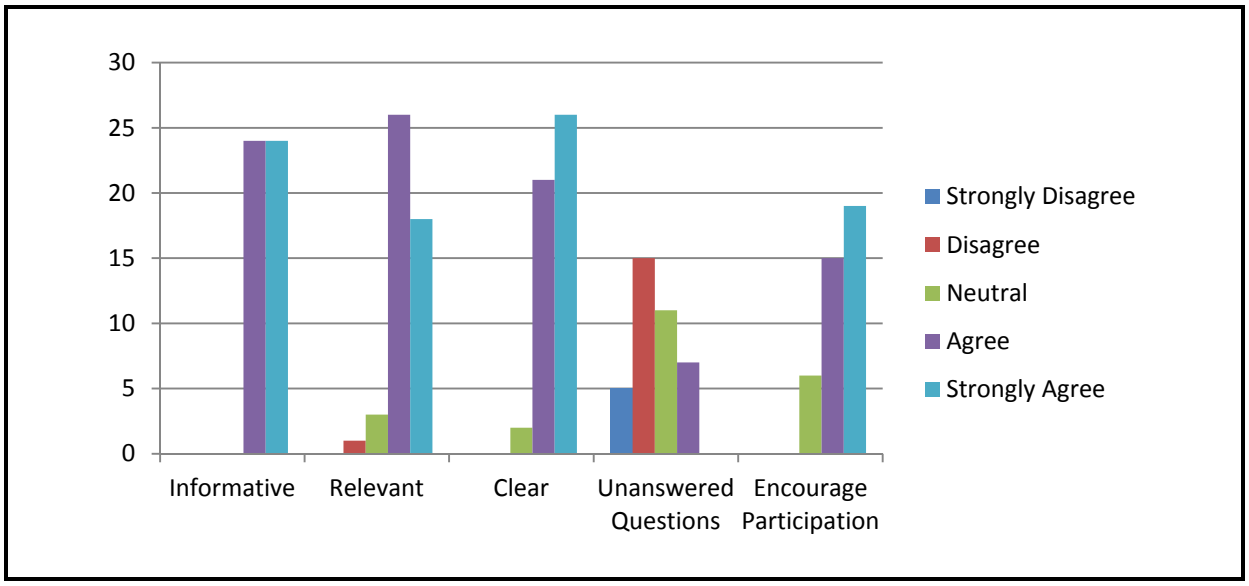
Figure 3-1c. Breakdown of How Participants Reported Hearing about CHATS Forum



The results for questions regarding meeting content were extremely positive. When asked if the meeting was informative, 100% of attendees either agreed (50%) or strongly agreed (50%). The pattern of response with regard to relevance of information was similar, with 92% of community forum participants agreeing or strongly agreeing. Again, 98% of community forum participants agreed on the clarity and ease of understanding the materials. When asked if they had any unanswered questions after the presentation, 10.4% strongly disagreed (5), 31% disagreed (15), 22% were neutral (11), and 15% agreed (7). Finally, when asked if they would encourage family members, community members, and other people in their lives to participate in the project if contacted, 12% were neutral (6), 31% agreed (15), and 40% strongly agreed (19).

Attendees of the two Mississippi forums reported slightly higher perceived meeting quality (averaging a rating of 4.68 out of 5 for overall meeting quality) than attendees of the two Louisiana forums (averaging a rating of 4.15 out of 5 for overall meeting quality). Attendees of the two Mississippi forums also said they would be likely to encourage family members, community members, and other people in their lives to participate in the project, with 54.2% strongly agreeing (13), 16.7% agreeing (4), and 8.3% being neutral (2). Attendees of the two Louisiana forums were slightly less positive, with 25% strongly agreeing (6), 45.8% agreeing (11), and 16.7% being neutral (4) (see ***Figure 3-1d***).

Figure 3-1d. Compiled Data from All Sites for Questions 1-7 of the Survey Regarding Forum Content



Participants at the Louisiana sites as well as Mississippi community forum sites indicated that if they were contacted, they would be likely to encourage fellow community members to participate in the project (see ***Figures 3-1e*** and ***3-1f***).

Figure 3-1e. Louisiana: Participant Willingness to Encourage Participation in the CHATS Study

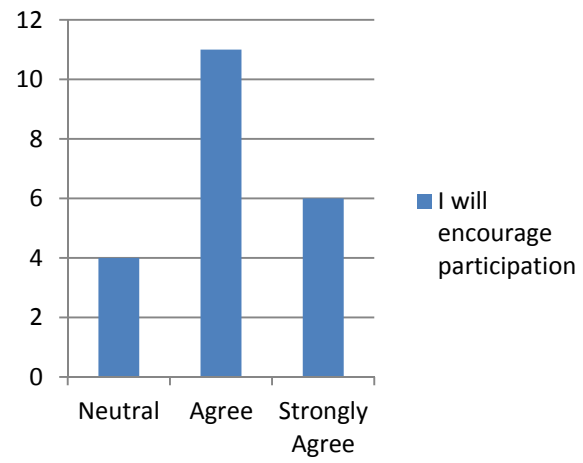
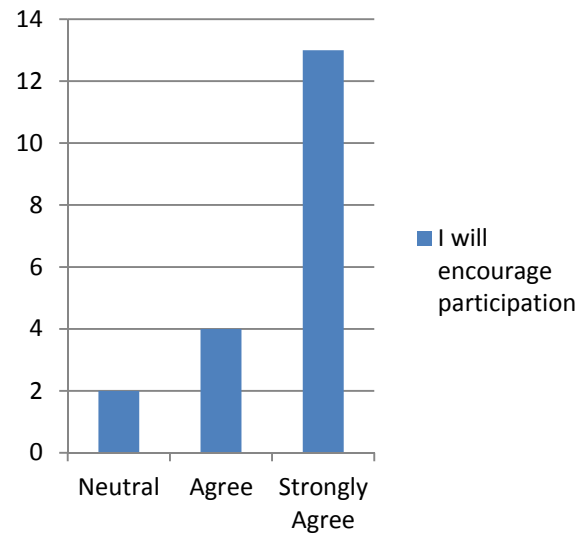


Figure 3-1f. Mississippi: Participant Willingness to Encourage Participation in the CHATS Study



3.1.1 Media Campaign Results

The media campaign was scheduled to run for 2 weeks prior to the start of data collection to introduce the campaign to the public before field interviewers began making calls and home visits. The campaign was to continue for 2 to 4 weeks following the start of data collection. However, delays in the start of data collection resulted in the campaign overlapping the start of data collection by 1 to 2 weeks.

RTI began tracking the paid media campaign immediately after its launch on May 25, 2012. This included placements of a radio public service announcement (PSA), community billboards, a newspaper advertisement, and Facebook Banner advertisements. ***Table 3-1b*** shows the advertising space purchased for CHATS' print and radio PSAs, the run-dates and length of exposure (e.g., 2 weeks, 3 weeks) for each ad, and the frequency each ad (radio, billboard, newspaper) was shown/heard. ***Table 3-1c*** shows the placement of CHATS Community Bulletin and Billboards.

RTI prepared a final report detailing the results of the media campaign, including numbers of exposures to the targeted audience, location, and air times. In total, RTI secured 126 airings of the CHATS radio PSA in New Orleans and 144 airings in Biloxi and Gulfport, Mississippi, and the surrounding areas. These numbers represent both the actual purchased airings of the ads (72 in New Orleans, and 63 in Mississippi), combined with in-kind or donated air-time RTI was able to secure at no cost from area radio stations, which saw the CHATS study as a worthwhile public service effort. When asked if they had heard about CHATS before being approached to participate, only 6% (11 of 181) of the participants indicated "yes," but most of them (9) had heard through friends, rather than through media.

Table 3-1b. CHATS Public Service Advertisements

Tactic	2nd Quarter													Freq
		April					May				June			
	26	2	9	16	23	30	7	14	21	28	4	11	18	
NEW ORLEANS, LA														
Radio: WBOK (:60)										20	19	1		**54**
Trumpet Newspaper										1				**1**
Posters										14				**14**
GULF COAST, MS														
Radio: WJZD (60)										20	19	1		**54**
6 Posters									5/25 6					**6**
BATON ROUGE, LA									5/25					
2 Posters												2		**2**
1 Bulletin												1		**1**
REGIONAL														
Facebook										1				**1**
PRODUCTION														
Poster Production (21)														**22**
Bulletin Production														**1**

Table 3-1c. CHATS Billboard and Community Bulletin Board Placements

CHATS Bulletin-Billboard Placement SPEC SHEET

Market	Media	Size	Location	Weekly Impressions	Zip Target
	Radio	:60			
New Orleans	Poster #7-0188	10'6 x 22'9	2900 Cleary Ave W/S F/S	23144	70006
New Orleans	Poster #7-0403	10'6 x 22'9	6560 Lapalco N/S F/E	79,625	70072
New Orleans	Poster #7-0469	10'6 x 22'9	2145 Williams Blvd	67,068	70065
New Orleans	Poster #7-0506	10'6 x 22'9	Behrman Hwy & Northbrook	63,152	70056
New Orleans	Poster #7-0576	10'6 x 22'9	Westbank Expwy & Allo	218912	70058
New Orleans	Poster #7-0776	10'6 x 22'9	3412 S Claiborne &LA Ave	61345	70115
New Orleans	Poster #7-0831	10'6 x 22'9	8610 Oak	12703	70118
New Orleans	Poster #7-0942	10'6 x 22'9	E Judge Perez & Valmar	58293	70043
New Orleans	Poster #7-1031	10'6 x 22'9	3501 Tulane & Clark	88792	70119
New Orleans	Poster #7-1065	10'6 x 22'9	Chef Hwy W/o Paris Rd	35988	70128
New Orleans	Poster #7-1078	10'6 x 22'9	Washington Ave & Jeff Davis	50785	70125
New Orleans	Poster #7-1107	10'6 x 22'9	St Claude E/O Delery	44782	70117
New Orleans	Poster #7-1539	10'6 x 22'9	Chef Menteur E/O Jourdan	111,168	70126
New Orleans	Poster #7-1602	10'6 x 22'9	Chef Hwy & Flake	50565	70127
Biloxi-Gulfport	Poster #7589	10'6 x 22'9	E/S 25th Ave S/O 34th St	81938	Gulfport
Biloxi-Gulfport	Poster #7719	10'6 x 22'9	WE/S Popps Ferry Rd s/o Bridge	44499	Biloxi
Biloxi-Gulfport	Poster #7857	10'6 x 22'9	E/S Lorraine Rd S/o Seaway Canal	79417	Gulfport
Biloxi-Gulfport	Poster #7867	10'6 x 22'9	S/S Pass Rd E/o Veterans	41772	Biloxi
Biloxi-Gulfport	Poster # 7775	10'6x 22'9	E/s Hwy 63 N/o 23/63	16757	Lucedale
Biloxi-Gulfport	Poster #6302	10'6 x 22'9	N/S Us 90 w/o Hwy 63	57445	Pascagoula
Baton Rouge	Poster #16045	10'6 x 22'9	Scenic Hwy w/s Hwy 19	60900	70807
Baton Rouge	Poster #16056	10'6 x 22'9	Jefferson Hwy @ Parkforest	48094	70816
Baton Rouge	Bulletin #375	10' x 40'	W/S I-55 .2 Mi S/O Hwy 38	29291	70441
New Orleans	Trumpet Newspa	10"w x 6"h			
	Facebook	110pixels w x 80 pixels h			
	Facebook	135 characters of copy			

3.2 Study Participant Locating Results

3.2.1 Locating Outcomes

As described in ***Section 2.2,*** RTI concentrated locating efforts on the exposed cohort, because those were the individuals who were known to have lived in FEMA-provided THUs and who may not have had their current address listed on the FEMA records. We selected the unexposed cohort from U.S. Postal Service addresses based on close geographic proximity to the exposed cohort. RTI located nearly 100% of the unexposed sample. The three cases not located appeared to have incomplete or difficult-to-locate addresses.

For the exposed sample, the key to the locating success for this mobile population was the combination of advance batch and interactive tracing followed by field tracing and, in some

cases, additional interactive tracing to follow up on new leads identified in the field during data collection. All 1,814 exposed cases were included in the initial batch tracing. ***Table 3-2a*** provides an overview of the locate rates that were calculated based on the results from the two sources used for batch tracing. (See ***Section 2.2*** for additional information about the batch tracing process.)

Table 3-2a. Outcomes of Batch Tracing – Summary Findings Based on Two Sources

Status	Frequency	Percentage
Address match: within LA or MS	1,368	75
Address match: out of state	86	5
Deceased	113	6
City: nonmatch between sources	89	5
State: nonmatch between sources	31	2
Address: nonmatch between sources	111	6
Not found in either source	16	1
Total	1,814	100

The two batch tracing sources returned the same address for 75% of the exposed sample. These 1,368 households were sent directly to field interviewers to contact. A total of 113 (6%) of the households were confirmed as deceased by both tracing sources and were final coded as ineligible without being released to the field. The same "out-of-state" address was returned for an additional 5% of the households. Overall, 86% of the exposed households had tracing outcomes that were consistent between the two batch tracing sources. Only 1% was not located by either source, and 14% had conflicting locate results between the two sources. The households that had partial matches, no match, or were not found were all sent for interactive tracing, which was completed by the RTI centralized tracing staff. Additionally, the households that were located out of state were confirmed by RTI in order to ensure the correct individual was traced by the vendors. In total, 333 households were sent for interactive tracing before they were sent to the field.

The advanced interactive tracing effort yielded 87% of the households (291) located in Louisiana or Mississippi. An additional 12% (39) were also located but found to be living outside of the target states for the Feasibility Study. An additional 1% (3) was confirmed as deceased. The 291 households located in the target states were released to field interviewers to be confirmed and screened at the updated address or returned to the tracing unit for additional interactive tracing as necessary. The total number of households returned to RTI for additional tracing was 444 exposed households. These households could have been from the group of

households that were initially located through the batch tracing or through the advance interactive tracing.

It is important to note that we considered the results from advance tracing preliminary until we confirmed the addresses in the field. As evidenced by the final locate rates presented in ***Section 3.2***, sample members continued to move between the time the batch tracing was conducted and the field interviewers attempted to contact the household. ***Table 3-2b*** presents the final locate results based on advanced batch, interactive, and field tracing. Ultimately, we located 86% of the exposed sample, and we located nearly the entire unexposed sample. The overall locate rate was 92% for the Feasibility Study. This outcome far exceeded RTI's target of locating 70% to 75% of the exposed sample and 80% of the unexposed sample.

Table 3-2b. Locating Outcomes by Exposed and Unexposed Cohort

	Total Sample	Located	Unable to Locate	Locate Rate, %
Exposed	1,814	1,565	249	86
Unexposed	1,153	1,150	3	99.7
Total	2,967	2,715	252	92

Locating Outcomes for Test Cases in Alabama and Texas

As part of the Feasibility Study, we tested the effectiveness of the batch tracing in 50 households in both Texas and Alabama. The combined results of batch testing for all 100 households (Alabama and Texas) are presented in ***Table 3-2c***. As with the batch tracing effort for Louisiana and Mississippi, the two-source tracing comparison approach yielded only 1% of the households not located. Among the 100 test cases, a higher nonmatch rate occurred; if these cases were pursued, additional households would have to be sent to interactive tracing before being released to field interviewers. However, the overall tracing plan used for the Feasibility Study in Louisiana and Mississippi seemed to be effective for these two additional states based on the overall batch locate rate of 99% of the cases by one of the two sources. Based on these findings, if a Full Study were to be completed, we could follow the same model for advance tracing in Alabama and Texas as we did for the Feasibility Study. We would simply plan to do additional interactive tracing to confirm the address before sending it to field interviewers.

Table 3-2c. Outcomes of Batch Tracing for Test Cases (Alabama and Texas)

Status	Frequency	Percentage
Address match	22	22
Deceased	5	5
City: nonmatch between sources	18	18
State: nonmatch between sources	41	41
Address: nonmatch between sources	13	13
Not found in either source	1	1
Total	100	100

3.3 Study Participant Recruitment Results

3.3.1 Screening

During the Baseline data collection period, field interviewers contacted and completed screenings with 1,851 households. Of the total sample of 2,967 households, field interviewers conducted screenings for 62% of the households, and eligibility was determined for 75%. ***Table 3-3a*** summarizes the total counts of eligible, ineligible, and unknown screening outcomes by sample cohort (exposed and unexposed). ***Table 3-3b*** breaks down the nonresponse into categories by sample cohort (exposed and unexposed).

Table 3-3a. Summary of Screening Outcomes

	Total Sample	Determined Ineligible Prior to Screening	Screening Completed, Ineligible, No Child Selected for Baseline	Screening Completed, Eligible Child Selected for Baseline	Nonresponse, Eligibility Not Determined	Screening Response Rate, %*	Eligibility Rate, %**
Exposed	1,814	208	841	200	565	65	19
Unexposed	1,153	169	706	104	174	82	13
Total	2,967	377	1,547	304	739	71	16

* Among households eligible to be screened (i.e., “total sample” minus “determined ineligible prior to screening”).

** Among households that were screened.

Table 3-3b. Reasons for Screening Nonresponse among Exposed and Unexposed

	Unable to Locate	Unable to Contact	Refusal	Other	Total
Exposed	249 (44%)	236 (42%)	75 (13%)	5 (1%)	565 (100%)
Unexposed	3 (2%)	121 (70%)	46 (26%)	4 (2%)	174 (100%)
Total	252	357	121	9	739

Based on the screening stage results, we determined that the vast majority of these households were ineligible. A total of 1,547 households completed the screening and were found to be ineligible. Of those, 1,457 (or 79% of those screened) were ineligible because no child was selected based on the exposed or unexposed criteria. For an additional 90 sample members (or 5% of those screened), a child was initially selected but later determined to be ineligible. These 90 sample members were primarily among the unexposed sample in which the child had lived in a FEMA-provided trailer, the mother had been living in the FEMA-provided trailer while pregnant with the child, or the child had not lived in the Gulf Coast region for the past 3 years. Eligible children were selected from the remaining 304 households, and the case progressed to the Baseline Session 1. Overall, these 304 households represented an eligibility rate of 16% of those who completed the screening.

Feasibility Study Objective Met

Objective: CDC set a criterion for locating and contacting at least 25% of the persons or households identified in the sampling frame.

Outcome: Of the 2,967 cases selected from the sampling frame, we located, screened, and determined eligibility for 2,228 cases, for a locating/screening rate of 75%.

The two cohorts—exposed and unexposed—experienced very different eligibility rates. The number of households screened from the exposed sample was 1,041, of which 200 had a child selected for the Baseline Assessment, making the eligibility rate among the exposed households 19%. A total of 810 households was screened from the unexposed sample, of which 104 had a child selected for the Baseline Assessment, making the eligibility rate among the exposed households 13%. This outcome was lower than RTI's predicted eligibility rate of 28% for both exposed and unexposed samples.

In 377 of the households without completed screeners, the household was determined to be ineligible outside of the screening process. The primary reasons for this determination among the exposed sample was that the selected sample member was deceased (125) or had moved out of the sampled area (71). The primary reasons for this determination among unexposed households were that the address was either vacant (143) or not a dwelling unit where people reside (22). Individuals in the remaining cases (16) were incarcerated, institutionalized, or were other nonresponse cases. These 377 cases composed 13% of the total sample and were never contacted because there was no household to screen.

Finally, in 739 cases no screening took place and eligibility could not be determined. The primary reason for these nonresponse cases was that the sample members were never home or available when the field interviewer arrived to conduct the screening. This issue occurred with 352 of the selected households, accounting for 12% of the total sample. Another 121 households refused to participate in the screening process, representing 4% of the total sample. One final significant factor that affected screening response rates was difficulty in

locating the households. In total, 249 of the exposed households could not be located despite RTI's and field staff's tracing efforts. This challenge affected only the exposed sample, but represented nearly 14% of the exposed cohort.

Litigation

For the Feasibility Study, we included questions about participation in lawsuits related to Hurricanes Katrina and Rita. We asked these questions to see if a correlation existed between participation in the study and participating or thinking about participating in a lawsuit. We asked these questions only of (a) screening respondents who refused to participate in the screener, (b) screening respondents who completed the screener but refused to participate in the Baseline Assessment, and (c) participants in the Baseline Assessment. Of the 121 refusal cases, 80% (n = 96) refused to complete the screener at all and, thus, did not answer the questions about litigation. Of the 304 households that completed the screener and agreed to the complete the Baseline interview, only 181 actually completed the Baseline. The remaining 123 households were never asked the litigation questions because they did not participate in the Baseline. Only the 25 screening respondents who completed the screener and then refused to participate were asked the questions about possible litigation. Of those 25, 1 confirmed being part of a lawsuit related to Hurricanes Katrina or Rita, and 18 indicated they were not part of a lawsuit. Six participants refused to answer the question. Of the 18 who reported they were not part of a lawsuit, 17 indicated they did not plan to join a lawsuit, and 1 reported being unsure. The same 6 participants also refused to answer the follow-up question about considering joining a lawsuit.

Of the 181 participants who completed the Baseline Session 1, 32 (18%) were part of a lawsuit related to Hurricanes Katrina or Rita and 9 (5%) more were considering joining one. Most participants (137, or 76%) indicated they were not part or a lawsuit nor intending to join a lawsuit, with 3 refusing to answer questions related to this topic or reported as not knowing.

Asthma

As with the litigation questions, we asked the question about a child in the household with an asthma diagnosis only of the 25 participants who completed the screener but refused to participate in the Baseline Assessment. Of the 25 who were asked, 2 reported that a child in the household had been diagnosed with asthma, and 16 indicated that no child in the household had been diagnosed with asthma. Seven participants refused to answer the question.

Of the 181 participants who completed the Baseline Session 1 Assessment, 73 (40%) indicated that a child in the household had been diagnosed with asthma by a medical

professional and 108 (60%) indicated that no child in the household had been diagnosed with asthma. None of the participants reported they did not know or refused to answer the question.

3.3.2 Baseline Assessment

> **Feasibility Study Objective Met**
>
> *Objective*: CDC set a criterion for enrolling at least 50% of the eligible sample.
>
> *Outcome*: Of the 285 eligible households, we completed 181 Baseline Session 1 Assessments for a response rate of 64%. We completed 174 Baseline Session 2 Assessments for a response rate of 61%.

In total, 304 children were selected through the screening process to participate in the CHATS Baseline Assessment. Of this sample, 181 (60%) completed Session 1 and 174 (57% of total, 96% of those completing Session 1) completed both sessions of the assessment. The response rate (those completing Session 1) among the 285 eligible households was 64%. This participation rate was substantially above the criterion CDC set that at least 50% of eligible persons contacted agree to enroll. However, this participation rate was below RTI's target rate of 80% enrollment. ***Table 3-3c*** summarizes the breakdown of outcomes by session.

Of the 304 cases selected through screening, 123 (or 40%) did not participate. The primary reason for this nonresponse was refusals (80, or 26% of the eligible sample). The secondary reason for nonresponse was that several participants (24, or 8% of the eligible sample) were never available to be recontacted after the screening.

During the Baseline, 19 households were discovered to be ineligible for participation in CHATS after they were selected through the screening. While setting up the appointment for the Baseline, the field interviewers determined that the parent had misunderstood the screening questions and inadvertently screened in as eligible. During the Baseline, 8 children (2%) could not be interviewed because information about them had been misreported during the screening. The other contributing factor involved households that moved out of the area after screening but before a field interviewer could schedule and complete the Baseline Assessment. We suspect that many households moved out of the area when Hurricane Isaac occurred. This storm made landfall on the Gulf Coast on August 28, 2012, devastated portions of the sampled area, and suspended field work for several weeks. All but two of the participants who had moved were screened before Hurricane Issac and had left their homes prior to the resumption of field work in September. Children selected from either of the above cited categories were not eligible to be included in the Baseline sample.

Although the Baseline Assessment was conducted over two sessions a week apart, there were minimal issues in maintaining cooperation between the sessions. Nearly all sample members who participated in the first session also completed the second. Only seven (4%)

could not or chose not to participate in Session 2. Two of these did complete Session 2, but the data could not be counted because the date for Session 2 was outside the 9-day window. This timing rendered the exposures collected from the environmental samplers inaccurate.

Additional components were conducted as part of the Baseline Assessment. The Home Assessment, conducted during the first session, was completed for 180 of the 181 Baseline Session 1 Assessments. The Neighborhood Source Survey, conducted at either the first or second session or at some other time when the field interviewer was in the area, was completed for 178 of the 181 Baseline Session 1 Assessments. A nurse also administered the Health Assessment during the second session. The Health Assessment was completed for 170 (94%) of the 181 Baseline Session 1 Assessments. The participants in the remaining four cases completing Session 2 declined to permit this assessment of their children.

Table 3-3c summarizes the total counts of ineligible, completed, and nonresponse outcomes by session.

Table 3-3c. Summary of Baseline Assessment Outcomes by Session

	Selected for Participation	Ineligible	Completed	Nonresponse	Enrollment Rate, %
Session 1	304	19	181	104	64
Home Assessment			180	105	63
Neighborhood Source Survey			178	107	63
Session 2	181	0	174	7	96
Health Assessment			170	4	94

3.3.3 Follow-up Assessment

By design, RTI recontacted the 174 participants who completed the initial Baseline Assessment approximately 5 months later regarding participation in the Follow-up Assessment. Similar to the Baseline Assessment, the Follow-up Assessment was divided between two sessions and had most of the same associated components. In total, 155 of the participants contacted for the Follow-up Assessment completed it, representing 90% of the eligible sample. Seventeen participants elected not to continue in the study, and two households were ineligible to do so. ***Table 3-3d*** summarizes the total counts of ineligible, completed, and nonresponse outcomes by session.

Table 3-3d. Summary of Follow-up Assessment Outcomes by Session

	Selected for Participation	Ineligible	Completed	Nonresponse	Retention Rate, %
Session 1	174	2	155	17	90
Session 2	155	0	155	0	100

The primary reason for nonresponse was again refusals. Sixteen of the selected participants refused to participate, comprising 9% of the total and 94% of nonresponse. Two participants expressed interest in the study but could not participate. In one situation, the child selected was institutionalized during the data collection period. In another, the household had moved out of the sample area. Discounting these two cases that were not eligible for participation, field staff had an effective retention rate of 90% among eligible cases. Of the sixteen refusals, four were substudy participants during the Baseline Assessment. Although the Follow-up Assessment was conducted at 6 months, this retention rate was consistent with a rate substantially above the CDC criterion of 75% at a 1-year Follow-up. RTI's target retention rate of 90% at 6 months was met.

Although the Follow-up was also conducted over two sessions roughly a week apart, all participants in the first session also completed the second session. Three households elected not to take part in the Health Assessment, ordinarily scheduled with a nurse for the second session. Two of these refused, but the third was unable to participate because the child became severely ill after Session 1. Overall, 98% of participants in the Follow-up Assessment also completed the Health Assessment.

During the Follow-up Assessment, the field interviewer was directed to complete a new Home Assessment and Neighborhood Source Survey when the participants had moved to a new home since the Baseline. This situation occurred 14 times (9% of Follow-up Assessments) and both assessments were completed in each case.

3.4 Feasibility of Enrolling and Retaining Participants

3.4.1 Enrolling Participants for Baseline

Contacting eligible households (i.e., those that were screened in and found to have an eligible child in the home) was not a significant issue in recruiting participants. Every household that was selected for inclusion in the Baseline received multiple visits by field interviewers, and by the end of the data collection period, only 8 cases (3%) had never been available to speak

with the field interviewer after the initial screening visit. Seven of these eight households occurred in the New Orleans metro area.

Refusals

Refusals were the primary encumbrance to gaining participation. Overall, 95 households selected for participation refused to ever participate, accounting for 33% of the 285 cases that were eligible to complete the study. Refusal rates were consistently high throughout the data collection period, but varied significantly geographically. Refusals were the most problematic in the New Orleans metro area, where they accounted for 37% of eligible households, and least problematic along the Mississippi Gulf Coast, where they accounted for 28%. The refusal rate in the Baton Rouge metro area fell in between the other two, at 35%.

Most eligible participants consented to receiving more information regarding the study at the time of screening. However, 26 households (9%) refused at this point. Another field interviewer made conversion attempts for all refusal cases. This effort resulted in 10 additional Baseline completions, for a conversion rate of 10%.

Field interviewers anecdotally reported the primary reason for participant refusals was sheer indifference. A secondary reason was concern over the amount of time involved. The estimated time expected ranged from 2 hours, 15 minutes to 3 hours, 15 minutes. The average of 2 hours, 45 minutes was shared with the participants as part of the consent process. The actual time required for Baseline Session 1 was 140 minutes, on average. The average amount of time for Session 2 was 88 minutes for the field interviewer portion and 68 minutes for the nurse portion, though these more often than not could be conducted simultaneously.

The high rate of refusals seriously impaired inclusion in the Baseline, but did not prohibit data collection; however, we had to devote time (which affected costs) to each of these refusal cases. ***Table 3-4a*** shows the total counts of ineligible, completed, and nonresponse outcomes by geographic region. ***Table 3-4b*** shows the total counts of ineligible, completed, and nonresponse outcomes by geographic region for the exposed cohort. ***Table 3-4c*** shows the total counts of ineligible, completed, and nonresponse outcomes by geographic region for the unexposed cohort.

Table 3-4a. Baseline Session 1 Outcomes by Region

Location	Total Sample	Ineligible	Completed	Unable to Contact	Refused	Rate, %	
						Participation	Refusal
New Orleans*	152	12	80	8	52	57	37
Baton Rouge**	24	1	15	0	8	65	35
MS Gulf Coast***	121	6	82	1	32	71	28
Outliers****	7	0	5	0	2	71	29
Total	304	19	181	9	95	64	33

*= Louisiana parishes of Jefferson, Orleans, and St. Bernard

**= Louisiana parishes of East Baton Rouge, St. Helena, and Tangipahoa

***= Mississippi counties of Harrison, Jackson, and George

****= All others sampled (Jones county, MS, and Plaquemines and St. Tammany parishes, LA)

Table 3-4b. Baseline Session 1 Outcomes among Exposed Participants

Location	Total Sample	Ineligible	Completed	Unable to Contact	Refused	Rate, %	
						Participation	Refusal
New Orleans*	99	8	56	7	28	62	31
Baton Rouge**	21	1	13	0	7	65	35
MS Gulf Coast***	72	5	50	0	17	75	25
Outliers****	7	0	5	0	2	71	29
Total	199	14	124	7	54	67	29

*= Louisiana parishes of Jefferson, Orleans, and St. Bernard

**= Louisiana parishes of East Baton Rouge, St. Helena, and Tangipahoa

***= Mississippi counties of Harrison, Jackson, and George

****= All others sampled (Jones county, MS, and Plaquemines and St. Tammany parishes, LA)

Table 3-4c. Baseline Session 1 Outcomes among Unexposed Participants

Location	Total Sample	Ineligible	Completed	Unable to Contact	Refused	Rate, %	
						Participation	Refusal
New Orleans*	53	4	23	1	25	47	51
Baton Rouge**	3	0	2	0	1	67	33
MS Gulf Coast***	49	1	32	1	15	67	31
Outliers****	0	0	0	0	0	n/a	n/a
Total	105	5	57	2	41	57	41

*= Louisiana parishes of Jefferson, Orleans, and St. Bernard

**= Louisiana parishes of East Baton Rouge, St. Helena, and Tangipahoa

***= Mississippi counties of Harrison, Jackson, and George

****= All others sampled (Jones county, MS, and Plaquemines and St. Tammany parishes, LA)

Broken Appointments

Broken appointments were also a significant hurdle to Baseline data collection. By design, nearly all Baseline Assessments had to be scheduled a minimum of 3 to 6 days in advance to accommodate the time to prepare and ship the environmental assessment platforms. Unfortunately, this lag time allowed participants additional time to reconsider or reschedule their appointment time. Session 1 appointments took place only 45% of the time they were initially scheduled.

Most participants who failed to be available at the time of their initial appointment did reschedule and complete the interview. Overall, 71% of participants who made appointments did eventually complete them. The frequency of cancelled and rescheduled appointments was consistent throughout the data collection period, but varied geographically. Overall, appointments were more successful along the Mississippi Gulf Coast, where 82% of these cases were later completed. In the New Orleans and Baton Rouge metro areas, the success rate was much lower at 63% and 61%, respectively (see ***Table 3-4d***).

Table 3-4d. Summary of Baseline Session 1 Appointments by Geographic Region

Location	Household Initially Making Appointments	Baseline Session 1 Completed at Initial Appointment	Percentage of Initial Appointments Held	Baseline Session 1 Completed Eventually	Response Rate Among Households with Appointments, %
New Orleans*	100	43	43	63	63
Baton Rouge**	18	4	22	11	61
MS Gulf Coast***	83	43	52	68	82
Outliers****	7	3	43	5	71
Total	208	93	45	147	71

*= Louisiana parishes of Jefferson, Orleans, and St. Bernard

**= Louisiana parishes of East Baton Rouge, St. Helena, and Tangipahoa

***= Mississippi counties of Harrison, Jackson, and George

****= All others sampled (Jones county, MS, and Plaquemines and St. Tammany parishes, LA)

A total of 34 Session 1 participants (19%) essentially completed the Baseline Assessment interviews with no appointment needed. These interviews almost all occurred right at the beginning or toward the end of data collection. At these points, additional environmental assessment platforms were already in the field so the field interviewers did not need to request the platforms.

Although participants often told the field interviewer that the reason for a broken, cancelled, or rescheduled appointment was that they had a sudden change of schedule or

simply forgot, in many cases, the field interviewers felt that the time to complete each session was a contributing factor.

Because such a large number of participants made appointments, and those who did largely completed the Baseline Assessment, broken appointments did not prohibit data collection. However, the time, effort, and logistical concerns raised by these rounds of cancellation and rescheduling created ever-present hurdles to data collection.

3.4.2 Retaining Participants for Follow-up

Contacting households again for the Follow-up Assessments was not a significant issue. Several participants had moved or changed phone numbers since they were first contacted for the Baseline Assessment, but the additional contact information (alternative phone numbers, e-mail address, alternative contact person) requested at the conclusion of the Baseline Assessments provided enough alternatives to reach these participants with generally minor inconvenience to the field team. A field interviewer was able to speak with the participants about the Follow-up for every eligible case.

Only 19 participants failed to be retained for the Follow-up. The primary reason for nonresponse during the Follow-up was refusals. Seventeen participants refused at least one field interviewer, for a refusal rate of 10%. Field interviewers anecdotally reported that most participants who chose not to participate mentioned the length of the two sessions as a primary deterrent. Although shorter than the Baseline sessions, the actual average Session 1 interview took 89 minutes, the average Session 2 interview took 68 minutes, and the average nurse session (which usually occurred simultaneously to the Session 2) took 54 minutes. Visits that involved the deployment of all three environmental platforms (i.e., the substudy protocol) were on average 34 minutes longer than visits involving only one platform. Participants, however, may still have been remembering the longer Baseline Assessments when they declined to participate.

Unlike the Baseline data collection, there were no geographic disparities in participation or refusal rates at Follow-up. Again unlike the Baseline, the rate of participation varied substantially during the data collection period. In particular, two periods stand out. Households that were eligible for recontact between February 16 and March 30, 2013 (that is to say, completed their Baseline Assessments late September through the end of October) elected to participate only 80% of the time. On the other hand, the final 37 households recontacted after April 6, 2013 (that is, those who would have completed the Baseline Assessment in November or early December) all cooperated. The success with this latter group is particularly impressive since, given the approaching end of data collection; field interviewers did not have the full 2-month window to schedule a Follow-up Assessment with these households.

The conversion effort was far more successful during the Follow-up Assessment. Participants who initially refused elected to participate 34% of the time after a second field interviewer visited. Given the low rate of refusals and high rate of conversion, nonresponse was not a significant barrier to retaining participants through the Follow-up sessions.

Broken Appointments

Broken appointments continued to be an issue during the Follow-up Assessment, but far less so than during Baseline Assessment. Only 60% of participants honored the initial appointment they had made with the field interviewer. While 92% of these participants eventually did reschedule and complete the interview, this constant cycle of cancellation and rescheduling drew attention, time, and resources away from other work. Still, given the high proportion of participants who did eventually cooperate, this cycle did not prohibit retention.

3.5 Locating Medical Records

Feasibility Study Objective Met

Objective: CDC set a criterion of at least 25% of health records of participants located and reviewed.

Outcome: Of the 258 medical records that parents provided permission for access, we completed 142 at Baseline for a completion rate of 78%. We completed 48 at Follow-up for a completion rate of 63%.

Parents of children selected for medical record abstraction were asked to provide consent for medical record abstraction from up to three providers who had provided care to the participant since August 2003. Of these parents, 86% provided consent. From the Baseline Assessment visits, only 13% provided three providers; 34% provided two providers; and 53% provided a single provider. Each record yielded an average of 14 events (e.g., outpatient visits, hospitalizations, emergency department visits). ***Table 3-5a*** provides additional data on participant consent for abstraction based on exposure status, state of residence, and presence of signature health outcomes.

Data from ***Table 3-5b*** provides an overview of abstraction activity by state and indicates that CDC criterion to locate and review at least 25% of participant records was satisfied during Baseline Assessment data collection. ***Table 3-5c*** provides similar data for the Follow-up Assessment.

Table 3-5a. Medical Records Abstraction Consent Summary from Baseline Visits by State and Exposure Status

State	Strata	Participant Has At Least 1 Signature Health Outcome		Participant Has No Signature Health Outcomes			Total
		Parent Consented to Abstraction	Parent Refused Abstraction	Parent Consented to Abstraction	Parent Refused Abstraction	Participant Not Selected	
LA	Exposed	51	8	2	1	12	74
	Unexposed	13	5	1	0	5	24
LA Total		64	13	3	1	17	98
MS	Exposed	37	4	0	0	10	51
	Unexposed	19	2	0	0	11	32
MS Total		56	6	0	0	21	83
Total		120	19	3	1	38	181

Table 3-5b. Medical Records Accession and Completion Status for Baseline by State

	Duplicates	Cannot Locate		Records Were Destroyed	Access Was Refused	Completed Records Keyed	Total
		Provider	Record				
LA	1	2	17	16	2	74 (77%)	96
MS	2	4	12	11	0	68 (79%)	86
Total	3	6	29	27	2	142 (78%)	182

Table 3-5c. Medical Records Accession and Completion Status for Follow-up by State

	Duplicates	Cannot Locate		Records Were Destroyed	Access Was Refused	No New Data for Abstraction	Completed Records Keyed	Total
		Provider	Record					
LA	3	1	8	0	0	4	24 (60%)	40
MS	0	0	6	0	0	6	24 (67%)	36
Total	3	1	14	0	0	10	48 (63%)	76

During the Baseline, in response to request for access to records, two providers refused to provide records. Providers indicated that they had no records on 43 participants, and eight providers could not be located based on the information provided by the participant. Twenty-seven records were reported destroyed during the aftermath of the Hurricanes. From the Baseline Assessment, a total of 142 records were abstracted from 101 participants. From the Follow-up Assessment, 48 records were abstracted from 44 children. At the midpoint during

the Baseline Assessment abstraction period, the ATEN staff performed a quality review of 10% of 55 abstracted records; a 99.6% concordance was obtained.

3.6 Health Assessment Outcomes

Since health effects related to living in the Gulf Coast area following the Hurricanes were a primary interest of CHATS, questionnaire data identified those participants with an asthma diagnosis and other signature health outcomes (wheezing or extended dry cough without an asthma diagnosis, hay fever, allergies without an asthma diagnosis, rhinitis, eczema, itchy rash, and cancer). ***Table 3-6a*** provides this self-reported data for 179 participants at Baseline; ***Table 3-6b*** provides this self-reported data at Follow-up. The weighted rate of reported asthma is of particular note since national data reported a 9.0% prevalence, and data from the Louisiana Department of Health reported a 9.0% prevalence; Mississippi reported a statewide 10.2% prevalence.[10]

Table 3-6a. History of Asthma or Other Signature Health Outcomes (SHO) Reported by CHATS Participants at Baseline

Health Status	Number	Percent of Total N = 174
Asthmatic	52	29.9
Other SHO	82	47.1
No SHO	40	23.0

Table 3-6b. History of Asthma or Other Signature Health Outcomes (SHO) Reported by CHATS Participants at Follow-up

Health Status	Number	Percent of Total N = 155
Asthmatic	45	29.1
Other SHO	78	50.3
No SHO	32	20.6

Because atopic dermal and facial symptoms are associated with asthma and other allergic responses, nurses assessed CHATS participants for these features using the protocol established by the International Study of Asthma in Children (ISAAC).[11] A participant who exhibited at least one of the facial symptoms (example: red, swollen eyes, “allergic shiner”, evidence of nasal discharge) was classified as having facial symptoms (see ***Table 3-6c***). Similarly,

[10] CDC (2011). *Asthma’s Impact on the Nation*, State Data Profiles 2011. http://www.cdc.gov/asthma/stateprofiles.htm
[11] International Study of Asthma and Allergies in Childhood, Phase II Modules, Munster, Germany, 1998. http://isaac.auckland.ac.nz/phases/phasetwo/phasetwomodules.pdf (Accessed August 2013.)

a participant who exhibited at least one of the dermal symptoms (example: flexural dermatitis around the neck, elbows, or eyes) was classified as having dermal symptoms (see ***Table 3-6d).***

Table 3-6c. Presence of Facial Symptoms at Baseline and Follow-up

Facial Symptoms	Baseline		Follow-up	
	Number	Percent of Total	Number	Percent of Total
Facial Symptoms Present	46	26.4	21	13.6
No Facial Symptoms	123	70.7	131	84.5
No Report	5	2.8	3	1.9
Total	174	100	155	100

Table 3-6d. Presence of Dermal Symptoms at Baseline and Follow-up

Dermal Symptoms	Baseline		Follow-up	
	Number	Percent of Total	Number	Percent of Total
Dermal Symptoms Present	75	43.1	55	35.5
No Dermal Symptoms	94	54.0	97	62.6
No Report	5	2.9	3	1.9
Total	174	100	155	100

Pulmonary function testing included a measurement of the fractional exhaled nitric oxide (eNO using the NIOX MINO™ device (Aerocrine Inc., Morrisville, NC). Fractional eNO measurement is recommended in the diagnosis of eosinophilic airway inflammation. An eNO value that exceeds 35 parts per billion (ppb) in children is considered suggestive of asthma.[6] We measured all participants aged 5 years and older who self-reported an asthma diagnosis and/or respiratory symptoms suggestive of asthma. Only a random sample of participants without self-reported asthma and/or asthma symptoms were measured. Although the nurses demonstrated the procedure and a computer-assisted animated guide provided a further demonstration of the required inhalation/exhalation, younger participants had difficulty performing the maneuver. Of the 14 participants whose results of the eNO measurement in the Baseline Assessment period were noted as “did not understand,” 13 were aged 8 years or younger. Early in the study, the data collection instrument did not capture some successful attempts or maneuvers because of the nurses’ inability to follow the somewhat counter-intuitive program instructions. This problem resolved itself to a great extent as the study progressed and the staff became increasingly comfortable with the programming instructions. The NIOX MINO™ device was also temperature-sensitive. Thus, nurses could not take accurate measurements in homes in which the ambient temperature was hot or in which there was intense heat from cooking.

See ***Table 3-6e*** and ***Table 3-6f*** for findings from the eNO measurement at Baseline and Follow-up Assessments.

Table 3-6e. Descriptive Statistics of Exhaled Nitric Oxide Measurements by Reported Health Status at Baseline (ppb)

Descriptive Statistic	Asthmatic (N = 49)	Signature Health Outcomes	
		Other (N = 69)	No (N = 18)
Mean	22.5	15.9	14.4
Minimum	5.5	3.5	5.0
5th Percentile	6.0	5.3	5.0
25th Percentile	8.5	8.3	6.3
Median	17.5	11.0	9.3
Maximum	83.0	48.0	41.7
Percent of Population with NIOX Measurement	28.1%	39.7%	10.3%

Table 3-6f. Descriptive Statistics of Exhaled Nitric Oxide Measurements by Reported Health Status at Follow-up (ppb)

Descriptive Statistic	Asthmatic (N = 44)	Signature Health Outcomes	
		Other (N = 56)	No (N = 8)
Mean	17.2	17.5	15.5
Minimum	3.7	3.0	5.0
5th Percentile	3.7	5.0	5.0
25th Percentile	6.3	8.3	8.5
Median	13.5	13.5	10.0
Maximum	69	67.5	33.5
Percent of Population with NIOX Measurement	28.3%	36.1%	5.2%

Nurses conducted spirometry on all CHATS participants aged 5 years or older, regardless of asthma status, using the Easy-on PC device (ndd Medical Technologies, Andover, MA). The nurses also demonstrated this procedure, and a computer-assisted animation provided a visual instruction on the inhalation/exhalation maneuver. We used American Thoracic Society (ATS) standards[12] to assess test quality, and we used the Polgar standard[13], which is specific for pediatric populations, for interpretation. ***Table 3-6g*** provides outcomes on test quality based

[12] ATS/ETs Standardization of Lung Function Testing: Standardization of Spirometry (2005). *European Respiratory Journal, 26*:153-161

[13] Polgar, R. & Promadhat, V. (1971). *Pulmonary function testing in children: Techniques and standards*. Philadelphia: W.B. Saunders Co..

on the ATS standards. Unlike the eNO measurement, participants of all ages experienced difficulty performing the spirometry maneuver. Over one third of the Baseline participants aged 12 years and older were classified as "F" (no acceptable trial) by ATS standards. Although there was approximately a 15-minute interval between the eNO measurement and the spirometry, the difference in the maneuver requirements between the two may have caused confusion and also contributed to the difficulty. Of note is the increase in tests classified as the best score, "A"[14], from Baseline Assessment to Follow-up Assessment and a decrease in tests classified as the poorest score "F" (no acceptable trials) from Baseline Assessment to Follow-up. This may suggest a learning factor over the 6-month period. ***Table 3-6h*** and ***Table 3-6i*** includes spirometry outcomes for 159 Baseline and 153 Follow-up nurse visits, respectively.

Table 3-6g. Spirometry Quality Grades at Baseline and at Follow-up

Spirometry Quality Grade	Baseline		Follow-up	
	Number	Percent of Total Tested	Number	Percent of Total Tested
A	46	30.1	58	40.0
B	16	10.8	12	8.3
C	8	5.4	12	8.3
C2	4	2.7	5	3.3
D1	21	14.3	23	15.9
D2	20	13.5	12	8.3
F	33	22.3	23	15.9
Total Tested	148	100	145	100

Note: Tests were not obtained from 26 participants at Baseline and 8 participants at Follow-up.

Since 80% of the predicted value is the diagnostic reference point defined by the National Asthma Education and Prevention Program (NAEPP), that parameter is provided for CHATS participant data (***Table 3-6j*** for Baseline and ***Table 3-6k*** for Follow-up).

During the Baseline and Follow-up visits, nurses collected a urine sample from each child to assess for exposure to phthalates, VOCs, and cotinine. Creatinine was assessed to permit normalization of the measurements. Blood was collected at the Baseline visit for assessment of IgE and for a CBC. Protocols based on professional and regulatory standards guided the nurses in obtaining the specimens and preparing them for shipment/delivery to the laboratory. Results from these analyses are discussed in **Chapter 3.**

[14] An "A" score indicates at least 3 acceptable tests were performed by children 7 and older or 2 acceptable tests by children less than 7, and the difference between the best two Forced Expiratory Volume at 1 minute—FEV1—and Forced Vital Capacity—FVC—is $\leq$ 100 ml.

Table 3-6h. Means of Spirometry Measurements by Health Status at Baseline

Spirometric Measurement	Asthmatic (N = 46)	Signature Health Outcomes	
		Other (N = 76)	No (N = 39)
FVC (best trial) (liters)	2.64	2.52	3.01
FVC predicted value (liters)	2.47	2.45	2.70
FVC percent of predicted	105%	102%	107%
FEV1 (best trial) (liters)	2.08	2.03	2.30
FEV1 predicted value (liters)	2.30	2.29	2.53
FEV1 percent of predicted	90.%	89%	91%
FEV1:FVC Ratio (best trial)	0.79	0.83	0.80
FEV1:FVC Ratio predicted value	0.93	0.93	0.93
FEV1:FVC Ratio percent of predicted	85.60%	89%	86%
FEF2575 value	2.08	2.30	2.36
FEF2575 predicted value	2.90	2.88	3.05
FEF2575 (best trial)	71,68%	81%	79%
Percent of Population with Spirometry Measurement	26.4%	43.7%	22.4%

Abbreviations: FVC = Forced Vital Capacity; FEV1 = Forced Expiratory Volume at 1 minute; FEV1-FVC = Ratio of FEV1 to FVC; FEF2575 = Forced Expiratory Flow during the interval between 25-75% of FVC.

Table 3-6i Means of Spirometry Measurements by Health Status at Follow-up

Spirometric Measurement	Asthmatic (N = 43)	Signature Health Outcomes	
		Other (N = 74)	No (N = 32)
FVC (best trial) (liters)	2.75	2.65	2.87
FVC predicted value (liters)	2.57	2.59	2.61
FVC percent of predicted	105%	102%	106%
FEV1 (best trial) (liters)	2.22	2.18	2.42
FEV1 predicted value (liters)	2.41	2.43	2.44
FEV1 percent of predicted	92%	89%	97%
FEV1:FVC Ratio (best trial)	0.81	0.83	0.86
FEV1:FVC Ratio predicted value	0.93	0.93	0.93
FEV1:FVC Ratio percent of predicted	88%	89%	92%
FEF2575 value	2.25	2.29	2.66
FEF2575 predicted value	3.00	3.01	2.97
FEF2575 (best trial)	74.59	75.75	89.51
Percent of Population with Spirometry Measurement	27.74%	47.77%	20.65%

Abbreviations: FVC = Forced Vital Capacity; FEV1 = Forced Expiratory Volume at 1 minute; FEV1-FVC = Ratio of FEV1 to FVC; FEF2575 = Forced Expiratory Flow during the interval between 25-75% of FVC.

Table 3-6j. Number Attaining 80% of Predicted Value for Various Spirometry Measurements by Health Status at Baseline

Spirometry Measurement (liters)	Asthmatic (% of Total N = 52)	Signature Health Outcomes		Total (% of All Participants N = 174)
		Other (% of Total N = 82)	No (% of Total N = 40)	
FVC	39 (75.0%)	65 (79,3%)	31 (77.5%)	135 (77.6%)
FEV1	29 (55.8%)	51 (62.2%)	25 (62.5%)	105 (60.3%)
FEV1/FVC	31 (59.6%)	60 (79.3%)	26 (65.0%)	117 (65.4%)

Abbreviations: FVC = Forced Vital Capacity; FEV1 = Forced Expiratory Volume at 1 minute; FEV1/FVC = Ratio of FEV1 to FVC.

Table 3-6k. Number Attaining 80% of Predicted Value for Various Spirometry Measurements by Health Status at Follow-up

Spirometry Measurement (liters)	Asthmatic (% of Total N = 52)	Signature Health Outcomes		Total (% of All Participants N = 174)
		Other (% of Total N = 82)	No (% of Total N = 40)	
FVC	39 (86.7%)	70 (89,8%)	29 (90.6%)	138 (89.0%)
FEV1	32 (71.1%)	57 (73.0%)	25 (78.1%)	114 (73.5%)
FEV1/FVC	36 (80.0%)	63 (80.7%)	30 (93.8%)	129 (83.2%)

Abbreviations: FVC = Forced Vital Capacity; FEV1 = Forced Expiratory Volume at 1 minute; FEV1/FVC = Ratio of FEV1 to FVC.

The Asthma Control Test™ (ACT™) (QualityMetric, Lincoln, RI) and the Childhood Asthma Control Test (C-ACT) (Copyright GlaxoSmithKline, used with permission) were administered to those participants who self-reported an asthma diagnosis. While the ACT™ is designed for individuals aged 12 years and older, the C-ACT is designed for children aged 4 to 11 years and includes items requiring parental responses. Scores greater than 19 are associated with well-controlled asthma. Data from these instruments are provided in ***Table 3-6l*** for ACT and ***Table 3-6m*** for C-ACT.

Table 3-6l. Mean Scores for the ACT™

Baseline	Follow-up
21..79 (N = 25)	21.29 (N = 23)

Table 3-6m. Mean Scores for the C-ACT™

Baseline	Follow-up
21.89 (N = 24)	21.90 (N = 21)

Because of the influence of physical symptoms on psychological well-being and the psychological stressors typically experienced after a disaster, we measured health-related

quality of life in all CHATS participants. Nurses administered the PedsQL™ Pediatric Quality of Life Inventory (MAPI Research Trust, Lyon France), specifically the PedsQL™Short Form 15 Generic Core Scale, to participants without a self-reported asthma diagnosis. For those reporting an asthma diagnosis, nurses administered the PedsQL™ Short Form 22 Asthma Module. Both instruments consist of age-specific items for children and a parallel instrument their parents. Outcomes from Baseline and Follow-up are provided in ***Table 3-6n*** and ***Table 3-6o*** for the PedsQL and Asthma.

Table 3-6n. Mean Scores for the PedsQL™

	Baseline	Follow-up
Reported by Child	76.31 (N = 117)	80.39 (N = 106)
Reported by Parent	80.2 (N = 122)	82.13 (N = 109)

Table 3-6o. Mean Scores for the PedsQL™Asthma

	Baseline	Follow-up
Reported by Child (General QOL)	72.87(N = 49)	74.79 (N = 44)
Reported by Parent (General QOL)	77.18 (N = 52)	80.30 (N = 45)
Reported by Child (Treatment-related QOL)	82.42 (N = 492)	85.89 (N = 43)
Reported by Parent (Treatment-related QOL)	90.23 (N = 523)	91.25 (N = 44)

3.7 Evaluate Operational Issues

The Feasibility Study protocol for field data collection, which involved local field interviewers teaming with local registered nurses, worked well overall. As would be expected for a complex data collection effort like the CHATS study, there were numerous opportunities for equipment malfunction, as well as user error caused by inexperience. The overall success of the Feasibility Study's data collection is documented throughout this report. This section highlights the operational issues related to the various components of the Health Assessments, performed by the nurses, and the environmental assessments, performed by the field interviewers.

3.7.1 Health Assessment Compliance

The nurses conducted 170 Baseline Health Assessment visits and 152 Follow-up Health Assessment visits for a Follow-up rate of 89%. Participant refusal, which has been previously discussed, was the primary reason for lack of participation in the Follow-up visit. When

possible, the nurse who conducted the Baseline Assessment and who likely had developed a positive relationship with the participant was scheduled to conduct the Follow-up visit.

As shown in ***Table 3-7a***, overall, participants were cooperative with the data collection requirements. As described previously, many children experienced difficulties performing both of the pulmonary functions tests (PFTs), an observation that is also seen in clinical practice. While venipuncture and PFTs were limited to those aged 5 years and older, in general, the younger children were less cooperative in allowing the venipuncture and had more difficulty performing the eNO measurement.

Table 3-7a. Biospecimen Refusal at Baseline

Specimen Type	Total Samples Collected	Nurse Could Not Obtain Specimen	Refused FI during Visit 1	Refused Nurse during Visit 2	Age Ineligible	Health Condition that did not Allow for Specimen Collection
Urine	154	6	9	1	0	0
Blood	112	7	30	12*	7	2

*Includes 3 in which child cried and would not allow venipuncture to continue

3.7.2 Environmental Assessment Compliance

Table 3-7b describes a summary of overall completeness of environmental sample collection, comparing the number of samples successfully collected with that of the number assigned to cases. “Collection” is the combined deployment and retrieval of a sample. This summary is based on regular samples and does not include field QC and QA samples. We assessed the environmental sample collection quality by examining the information the field interviewer entered in the computer instrument during Sessions 1 and 2, and the environmental sample condition noted during sample receipt at RTI. We assigned one of three different environmental sample collection RQIs to individual samples based on those field and receipt conditions: 0 = No problems; 1 = Some issues potentially affecting results; and 2 = Unusable. We assigned an overall MicroPEM sample collection validity indicator at the time we reviewed the real-time data file that recorded various operation parameters. ***Tables 3-7c*** and ***3-7d*** show a summary of invalid samples at deployment and at retrieval stages during the two assessment phases.

Table 3-7b. Overall Percentage and Number of Environmental Samples that were Successfully Deployed, Retrieved, and Shipped (RQI = 0 or 1) in Each Sampling Platform during Two Study Phases (Number Valid/Number Total Planned Shown in Parentheses)

Sample Type	Baseline Study Phase				Follow-up Study Phase			
	Personal	Indoor	Outdoor	Central	Personal	Indoor	Outdoor	Central
Carbonyls	95.4% (146/153)	94.9% (75/79)	86% (43/50)	99% (95/96)	98.5% (134/136)	98.4% (121/123)	94.2% (98/104)	96.4% (80/83)
Hydrogen Sulfide	–	94% (47/50)	–	–	–	100% (9/9)	–	–
Nitrogen Dioxide	98% (150/153)	94.9% (74/78)	87.7% (43/49)	–	99.3% (135/136)	99.2% (122/123)	92.4% (97/104)	–
Volatile Organic Compounds	992.2% (141/153)	993.6% (73/78)	886% (43/50)	997.9% (94/96)	995.6% (130/136)	97.6% (120/123)	93.3% (97/104)	97.6% (81/83)
Floor Bulk Dust	–	89.1% (164/184)	–	–	–	92.7% (140/151)	–	–
MicroPEM Filter Sample	73% (112/153)	70.5% (55/78)	60% (30/50)	68.8% (66/96)	76.5% (104/136)	68.3% (84/123)	67.3% (70/104)	82% (68/83)
HOBO Temperature/ Relative Humidity Monitor	–	96.7% (176/182)	–	–	–	98.1% (152/155)	–	–

Table 3-7c. Percentage and Number of Samples Determined to be Invalid (RQI = 2) during Deployment of Each Sampling Platform during the Two Study Phases (Number Invalid/Number Total Planned Shown in Parentheses)

Sample Type	Baseline Study Phase				Follow-up Study Phase			
	Personal	Indoor	Outdoor	Central	Personal	Indoor	Outdoor	Central
Carbonyls	1.3% (2/153)	3.8% (3/79)	14.0% (7/50)	0.0% (0/96)	0.7% (1/136)	0.8% (1/123)	4.8% (5/104)	2.4% (2/83)
Hydrogen Sulfide	–	6.0% (3/50)	–	–	–	0.0% (0/9)	–	–
Nitrogen Dioxide	1.3% (2/153)	5.1% (4/78)	12.0% (6/50)	–	0.0% (0/136)	0.8% (1/123)	6.7% (7/104)	–
Volatile Organic Compounds	2.0% (3/153)	5.1% (4/78)	14.0% (7/50)	0.0% (0/96)	0.0% (0/136)	0.8% (1/123)	6.7% (7/104)	0.0% (0/83)
Floor Bulk Dust	–	10.9% (20/184)	–	–	–	7.3% (11/151)	–	–
MicroPEM Filter Sample	24.8% (38/153)	17.9% (14/78)	32.0% (16/50)	15.6% (15/96)	19.9% (27/136)	30.9% (38/123)	28.8% (30/104)	13.3% (11/83)
HOBO Temperature/ Relative Humidity Monitor	–	1.6% (3/182)	–	–	–	1.9% (3/155)	–	–

Table 3-7d. Percent and Number of Samples Determined to be Invalid (RQI = 2) during Retrieval of Each Sampling Platform during the Two Study Phases (Number Invalid/Number Total Planned Shown in Parentheses).

Sample Type	Baseline Study Phase				Follow-up Study Phase			
	Personal	Indoor	Outdoor	Central	Personal	Indoor	Outdoor	Central
Carbonyls	3.3% (5/151)	1.3% (1/76)	0.0% (0/43)	1.0% (1/96)	0.7% (1/135)	0.8% (1/122)	1.0% (1/99)	1.2% (1/81)
Hydrogen Sulfide	–	0.0% (0/47)	–	–	–	0.0% (0/9)	–	–
Nitrogen Dioxide	0.7% (1/151)	0.0% (0/74)	0.0% (0/43)	–	4.4% (6/136)	0.0% (0/122)	1.0% (1/98)	–
Volatile Organic Compounds	6.0% (9/150)	1.4% (1/74)	0.0% (0/43)	2.1% (2/96)	4.4% (6/136)	1.6% (2/122)	1.0% (1/98)	2.4% (2/83)
Floor Bulk Dust	–	0.0% (0/164)	–	–	–	0.0% (0/140)	–	–
MicroPEM Filter Sample	2.6% (3/115)	14.1% (9/64)	11.8% (4/34)	18.5% (15/81)	4.6% (5/109)	1.2% (1/85)	5.4% (4/74)	5.6% (4/72)
HOBO Temperature/ Relative Humidity Monitor	–	1.7% (3/179)	–	–	–	0.0% (0/152)	–	–

Table 3-7e describes various issues that resulted in unsuccessful sample collection; field interviewers identified these issues in the field or RTI observed them when samplers were returned. We found a high percentage of invalid samples due to MicroPEMs across all platforms; floor bulk dust; and passive gas samplers on indoor-outdoor platforms. Field interviewer errors or battery pack malfunctions caused the invalid MicroPEM samples. A few field interviewers continually did not follow the proper start procedure for the MicroPEM. As a result, the MicroPEM did not properly record QC records necessary to validate the sample in the internal data file. Late in the Follow-up phase, RTI noticed that corrosion of the battery pack on outdoor MicroPEMs caused a short circuit that caused the unit to stop operating. Participant refusal to allow collection of an indoor dust sample caused the higher percentage of invalid samples. Site access problems prevented passive gas samples from being deployed at some residences. These residences did not have a balcony or patio for the outdoor platform or did not have a suitable location for the indoor platform.

Except in a few instances, study participants did not tamper with the personal, indoor, or outdoor monitoring platforms. Field interviewers noted four instances of tampering with or destruction of the PEM platform. In three instances participants removed the instruments from the PEM platform and replaced them in the wrong location. In another instance, the family dog destroyed a PEM platform.

Table 3-7e. Various Issues that Prevented Successful Environmental Sample Collection

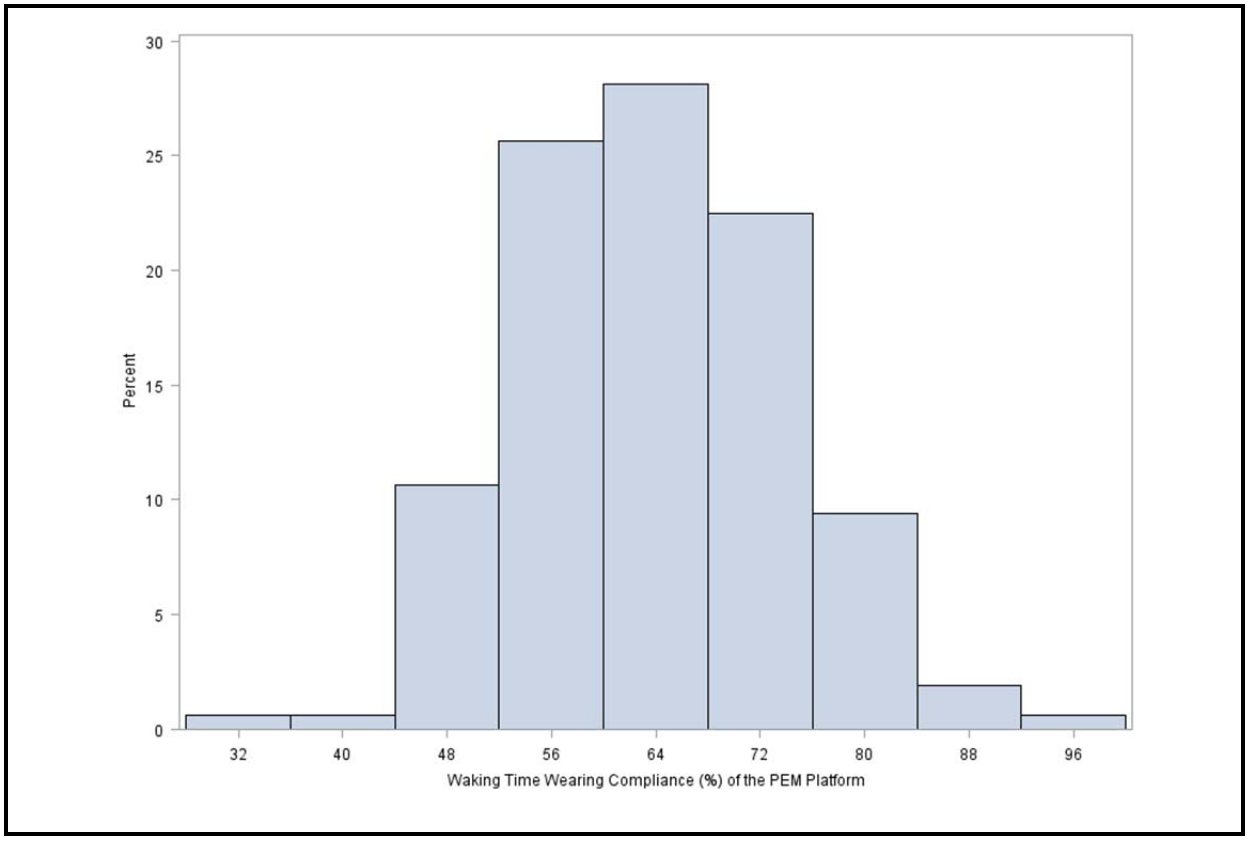
Sampler Type	Issues	% (Number of Samplers)
AL	▪ Manufacturer defect (slide bar fused shut) ▪ Refusal/site restriction ▪ Delayed retrieval due to weather (>9 days)	1.1% (9) 1.9% (16) 0.4% (3)
HS	▪ Delayed retrieval due to weather (>9 days) ▪ Refusal/site restriction	1.7% (1) 1.7% (1)
NX	▪ Delayed retrieval due to weather (>9 days) ▪ Major damage to the sampler ▪ Refusal/site restriction	0.5% (3) 0.2% (1) 2.3% (15)
VC	▪ Delayed retrieval due to weather (>9 days) ▪ Membrane torn ▪ Manufacturer defect (sample inoperable) ▪ Refusal/site restriction ▪ Sample lost	0.4% (3) 3.8% (31) 0.2% (2) 1.7% (14) 0.2% (2)
DF	▪ Delayed retrieval due to weather (>9 days) ▪ Refusal/site restriction	0.3% (1) 10.4% (35)
UP	▪ Delayed retrieval due to weather (>9 days) ▪ Instrument issue (battery pack, etc.) ▪ Field Interviewer's operational error ▪ Early shutdown ▪ Refusal/site restriction	0.4% (3) 7.1% (58) 12.0% (99) 0.9% (7) 2.2% (18)
TR	▪ Delayed retrieval due to weather (>9 days) ▪ Lost sampler ▪ Refusal/site restriction	0.3% (1) 0.6% (2) 1.8% (6)

Figure 3-7f shows the distribution of "waking-time wearing compliance" for the personal platform that children aged 7 years or older wore. Waking-time wearing compliance is the percentage of time the child followed the protocol for wearing the personal platform, excluding time sleeping.[15] We used the accelerometer data collected by the MicroPEM to calculate the waking-time wearing compliance. More than 85% of the children wore the personal platform more than 50% of the time they were awake. Previous studies identified a waking compliance between 40% to 60% to be representative of a cohort's exposure.[16,17]

[15] Lawless, P.A., Thornburg, J., Rodes, C.E., & Williams, R.W. (2012). Personal exposure monitoring wearing protocol compliance: An initial assessment of quantitative measurement. *Journal of Exposure Science and Environmental Epidemiology,* 22:274-280.

[16] Brook, R., Shin, H., Bard, R., Burnett, R., Vette, A., Croghan, C., Thornburg, J., Rodes, C., & Williams, R. (2011). Exploration of the rapid effects of personal fine particulate matter exposure on arterial hemodynamics and vascular function during the same day. *Environmental Health Perspectives, 119*:688-694.

Figure 3-7f. Distribution of the Waking-Time Wearing Compliance of the PEM Platform during Baseline and Follow-up



Note: Waking-time wearing compliance is the percent of waking time that the MicroPEM was worn.

Table 3-7g shows the trends in personal platform waking-wearing compliance among three different health status groups by state during the two assessment phases. The qualitative trends show minimal difference in compliance based on the child's health status. Waking wearing compliance appeared to decrease during the Follow-up Assessment phase.

Table 3-7g. Average Wearing Compliance (% Waking Time) of Personal Exposure Monitor by Study Phase, State, and Health Group

Health Status Groups	Baseline, %		Follow-up, %	
	LA	MS	LA	MS
Doctor-diagnosed asthma	64.0	67.8	56.9	65.4
Other health outcomes	64.7	65.4	61.4	64.4
No outcomes	68.1	62.9	61.7	62.2

[17] Delfino, R.J., Quintana, P.J.E., Floro, J., Gastañaga, V.M., Samimi, B.S., Kleinman, M.T. et al. (2004). Association of FEV_1 in asthmatic children with personal and microenvironmental exposure to airborne particulate material. *Environmental Health Perspectives, 112*:932–941.

3.8 Evaluate Selection Bias

3.8.1 Exposed Sample

The data in ***Table 2-1c*** are informative as to the presence of selection bias, some of it by design, some as a result of implementation. The sampling probabilities range considerably from a low of 0.0136 (East Baton Rouge, Louisiana) to a maximum value of 0.2324 (George, Mississippi). This variation is largely due to intentional over- and undersampling in the various strata. Within each of the nine counties/parishes, the selection probability is consistently lower for the private substratum than for the group substratum. Much of the variation is due to a desire to have a minimum sample size within each substratum. Consequently, small substrata tend to have large selection probabilities (i.e., George, Mississippi; Livingston, Louisiana; and St. Helena, Louisiana).

RTI calculated the "address design weights" only for responding households, and, thus, they incorporate nonresponse bias as well as selection bias. The design weights range from a minimum of 1 (St. Helena, Louisiana) to a maximum of 73.53 (East Baton Rouge, Louisiana)—a range that is not unusual for household surveys. The pattern of design weights generally follows that of the sampling probabilities, suggesting that the nonresponse bias is not a major factor. As with the selection probabilities, the design weights are uniformly higher for the private substrata.

3.8.2 Unexposed Sample

The data in ***Table 2-1d*** can be used to imply the presence of selection bias. The selection probabilities range from a low of 0.0024 (CBG#280470033021) to a high of 0.0551 (CBG#220710017323), and the design weights for participants range from a low of 18.14 (CBG#220710017323) to a high of 467.67 (CBG#280470033021). As with the exposed sample, the range of the two indicators is similar (maximum divided by minimum is about 25 in both cases), suggesting that nonresponse bias is not much of an issue. Much of the selection bias was introduced as a result of the design, which called for matching each exposed sample point with a corresponding unexposed point, all in the same CBG and without regard to the size of the CBG. This factor is probably the major determinant of the selection bias.

For both the exposed and unexposed sample, the final weight is designed to compensate for the biases discussed above.

3.8.3 Participation Rate by Various Characteristics

Table 3-8a summarizes the data presented in ***Sections 3-3*** and ***3-4*** about participation rates at various stages of CHATS. Although the participation rate for screening was higher among unexposed households, the participation rates for Baseline and Follow-up were higher among the exposed households.

Table 3-8a. Participation Rates for Various Stages of CHATS by Exposure Status

Exposure Status	Screening Participation Rate, %	Session 1 Participation Rate, %	
		Baseline	Follow-up
Exposed	65	67	91
Unexposed	82	57	89

Sections 3-3 and ***3-4*** also discuss the limited amount of data available to address the potential selection factors of whether participation in the extensive litigation activities surrounding the Hurricanes affected participation and whether having asthma in the family, not necessarily the participant child, affected participation. Responses were available from the screening interview for 25 households with eligible exposed children who elected not to participate. (Of these, seven refused to answer the questions regarding litigation and seven refused to answer questions regarding asthma among children in the household.) Responses were also available from the Baseline Assessment interview for 181 participants. Too few participants responded to the screener for us to make statistical conclusions. However, the percentage of participants currently part of a lawsuit was smaller among nonparticipants than among participants (1 out of 25 (5%) and 32 out of 181 (18%), respectively). Having at least one child in the household diagnosed with asthma (not necessarily the participant child) may have motivated some participation (11% and 40% of families had at least one child with asthma, respectively). However, the number of nonparticipants with responses was small, so the degree of selection bias is not conclusive.

3.9 Evaluate Information Bias

Information bias (due to recall bias or other factors) is inherent in self-reporting of any data. This tendency may be heightened in CHATS with its focus on respiratory symptoms. An additional factor is the time interval since the Hurricanes and the potential difficulty in recall time of initial diagnoses and onset of conditions/symptoms. The medical record abstraction data provide validation of the data obtained from participants' responses to the Health Assessment questionnaire.

The presence of an asthma diagnosis was available from two data sources: the questionnaire (Q) and medical record abstraction (A). The questionnaire asked, "Has a provider ever told you that your child had asthma?" The medical record abstraction documented a diagnosis of asthma by the provider. Generally, the questionnaire data and medical abstraction data agreed 73% of the time (20% Q = Yes/A = Yes and 53% Q = No/A = No) and disagreed 27% of the time (18% Q = Yes/A = No and 9% Q =No/A = Yes). (See ***Table 3-9a.)***

Table 3-9a. Comparison of Asthma Diagnosis as Reported by the Participant and by the Medical Records

		Medical Record Abstraction – Asthma		
		Yes	**No**	**Total**
Questionnaire – Asthma	Yes	28 (20%)	25 (18%)	53 (38%)
	No	13 (9%)	74 (53%)	87 (62%)
	Total	41 (29%)	99 (71%)	140 (100%)

For children who responded "Yes" to the asthma question on the questionnaire, 53% had a diagnosis of asthma by their provider and 47% did not. For children who responded "No" to the asthma question on the questionnaire, 15% had a diagnosis of asthma by their health care provider and 85% did not. (See ***Table 3-9b.)***

Table 3-9b. Verification of Participant Reported Asthma Diagnosis by Medical Records

		Medical Record Abstraction - Asthma		
		Yes	**No**	**Total**
Questionnaire – Asthma	Yes	28 (53%)	25 (47%)	53 (100%)
	No	13 (15%)	74 (85%)	87 (100%)

Another area of recall bias that can be evaluated in the CHATS study is the experience of living in the FEMA-provided THUs. Because the FEMA list of applicants contained almost complete information on SSNs, we could determine the number of applicants who were issued multiple trailers. Overall, 8% of the more than 100,000 applicants were issued multiple trailers, often different types of THUs. The 118 exposed participants in the Baseline with information on residence in multiple THUs (1 exposed participant refused to answer this question) were matched to the FEMA list and 9 (7.6%) were found to have been issued multiple THUs (see ***Table 3-9c***). However, when asked whether they had lived in multiple trailers, 33 participants (27.7%) reported that the child lived in multiple trailers. The degree of information bias, however, is difficult to assess because the children may have lived part-time in several different THUs.

Table 3-9c. Comparison of Multiple THU Residence as Reported on the FEMA Applicant List and Self-reported by the Participant

		Report of Multiple THU Residence by Participant		
		Yes	No	Total
Report of Multiple THUs Issued to Participant Household in FEMA List	Yes	29 (25%)	80 (68%)	110 (93%)
	No	4 (3%)	5 (4%)	9 (8%)
	Total	33 (30%)	85 (72%)	118 (100%)

3.10 Evaluate Quality of Data

To ensure that RTI collected high-quality data for the CHATS Feasibility Study, we developed and implemented numerous protocols throughout the data collection as well as analysis. This section describes the protocols implemented as part of the QA steps, as well as the outcomes for the Feasibility Study. Beginning with the QC checks for the field interviewers, ***Section 3.10.1*** describes the reviews of the audio files, verification phone calls, and field observations. Overall, the results of the QA measures implemented for the adherence to protocols for the field interviewers and RNs was excellent. There were no significant deviations from protocol identified through the audio file review, verification calls, or field observations. In ***Section 3.10.2***, we describe the QA steps included for the laboratory activities, including biological and environmental samples collected.

3.10.1 Quality of Interview Data

Methods

The quality and authenticity of the interview data being collected was a top priority for CHATS project management. To ensure that the questionnaires were administered following standardized protocol, that all information collected reflected the intended answers of each participant, and that no participant rights were violated during interactions with the CHATS field interviewers, RTI employed three methods: computer-audio-recorded interview (CARI) reviews, verification calls, and field observations.

CARI Review

Reviewing the CARI files was the primary method RTI used to ensure that field interviewers did not deviate from protocol during administration of the questionnaires. At the beginning of the first session of both Baseline and Follow-up, the participants were asked if their interview could be recorded as a measure of QC. Most consented, at which point a microphone in the laptop began recording the administration of the questionnaire. Rather than record the entire interview, field interviewers recorded excerpts while the instrument was on

certain key screens in the questionnaire. These excerpts included standard questions to the participant and particularly focused on those screens where the field interviewer requested the participant's consent or issued an incentive. When the field interviewer transmitted the interview data back to RTI, these audio files were available to RTI monitors. When a case was selected for review, the monitor listened to all the audio selections for that interview to ensure that all required text was read and the answers entered reflected the participant's responses. The monitor also checked an audit trail, which was a list of all the keystrokes made during the interview, as well as time stamps indicating when each keystroke was made. Using a combination of the audio file and audit trail, the monitor was able to largely recreate how the field interviewer proceeded through the questionnaire and could detect any discrepancies.

Internal RTI systems randomly selected cases for review. Additional field interviewers' work was manually selected for review so that a sample of each field interviewer's work was reviewed. Furthermore, additional cases were selected during two time periods. When each field interviewer first began working, more cases were selected to ensure the field interviewer understood the protocol. More cases were also selected following the announcement of contests at the conclusion of both Baseline and Follow-up to ensure that additional incentives had not altered the field interviewer's adherence to those protocols.

During Baseline, 52 of the Session 1 interviews were selected for review, which accounted for 30% of the completed work. Additionally, 27 of the Session 2 interviews were selected, or 16% of the completed work. In total, one of the two questionnaires was reviewed in 45% of the completed Baseline participant visits.

During the Follow-up Assessments, 31 of the Session 1 interviews were selected for review, or 20% of the completed work. Additionally, 12 of the Session 2 interviews were selected or 8% of the work. In total, one of the two questionnaires was reviewed in 28% of the visits completed during Follow-up.

Each week, the monitor compiled a report of the reviews to be shared with the field supervisors. In this report the monitors listed any concerns they had with the field interviewer's work they had reviewed, as well as procedures that were handled particularly well. Although errors were uncovered throughout the data collection period, they were relatively small and had no apparent impact on the data. These small errors included minor deviations from the verbatim script, failure to enunciate properly, and off-topic side conversations with the participant. The field supervisor was responsible for sharing this feedback and retraining the field interviewer. As data collection proceeded, the frequency of these small errors diminished. In 10 cases the monitor discovered a strange pattern of entries or odd timing. In these cases, the monitor and field supervisor asked the field interviewer for more information. In each case,

an adequate explanation or a technical error was uncovered. In only one case was there sufficiently poor handling of a protocol on the field interviewer's part to warrant a more formal warning and retraining. In no situations did the monitor uncover any issue that called the authenticity of the interview into question.

Verification Calls

A sample of completed cases that had not received a CARI review was instead selected to receive calls from a verifier. These scripted calls lasted, on average, between 4 and 7 minutes. The verifier contacted the parents or guardians who had participated in the interview and asked them to confirm the presence of both the field interviewer and the nurse, the proper handling of the environmental equipment, and the receipt of the incentive money. These cases were generally selected at random; however, additional cases were selected to ensure a sample of each field interviewer's work was included. Additional cases were also selected toward the conclusion of both Baseline and Follow-up, when there had been more pressure to expedite the work. Once assigned, the verifier was instructed to call all known phone numbers for the participant and to make a minimum of attempts on 5 different days before moving onto the next case.

During Baseline, 24 cases, or 14% of all completed work, were verified via this method. An additional 11 cases had been selected but were not able to be verified because the participant could not be reached. During Follow-up, 17 cases, or 11% of all completed work, were verified. An additional 3 cases had been selected but were not verified because the participant could not be reached. During either verification effort was any case discovered where the participant was reached but could not recall the field interviewer and nurse or the incentives. No participants reported any concerning behavior. Many of the participants contacted took a moment to comment on the professionalism of both the field interviewer and nurse.

Field Observations

CHATS management staff made eight trips to the sampled area to observe field interviewer interactions with participants and potential participants. The purpose of these trips was to gauge field interviewer behavior, assess the effectiveness of the instruments, and obtain a better sense for participant concerns with the study. During Baseline, four field interviewers were observed conducting either the Session 1 or 2 interview in a participant's home. Additionally, seven field interviewers were observed while conducting screenings. During Follow-up, five field interviewers were observed conducting either the Session 1 or 2 interviews. Over the course of these observations, observers echoed many concerns regarding the field interviewers' case management skills and efficiency, but observed and reported

virtually no errors as field interviewers interacted with the participants or navigated the instruments.

To monitor the accuracy of the Health Assessment, the nurse supervisor from LSU accompanied each nurse on a minimum of two Health Assessment visits for QC purposes. She evaluated how well the nurse performed the various Health Assessment activities and provided feedback directly to the nurse. These visits were particularly important to resolve issues with the NIOX device early on in the Feasibility Study. In addition, RTI staff monitored the Health Assessment data to identify within case inconsistencies and to ensure protocol adherence. Examples of measures that were monitored included successful eNO collections, spirometry quality grades, and that participants with a diagnosis of asthma were administered the ACT or C-ACT.

Authenticity Concerns

Cumulatively, 144 of the cases completed received a QC check from one of the above listed methods during either Baseline or Follow-up. No suspicions of falsification were uncovered. A significant dissuading influence on the intentional inclusion of fraudulent data is the Session 2 interview. During the administration of this questionnaire, both the field interviewer and nurse should be present. During the first month of data collection, a field interviewer attempted to falsify a case. The assigned nurse instantly recognized the problem during the Session 2 interview and reported it. The field interviewer was immediately dismissed. By design, falsifying the CHATS interview required a significant conspiratorial effort, which is likely the study's strongest safeguard against this behavior.

3.10.2 Quality Assurance (QA) Activities of Laboratory Data

As part of the QA activities, RTI submitted a Quality Assurance and Surveillance Plan (QASP) with the proposal. We prepared and submitted subsequent revisions of the QASP to CDC in November 2010 and September 2011. The QASP was monitored and activities conducted in accordance to expectations. Also, standard operating procedures (SOPs) were prepared for laboratory analyses and other routine tasks (***Appendix B***). The QA staff and project management reviewed the SOPs and revised them when necessary. In addition to regular visits of QA staff to the RTI laboratories, the QA staff visited the LSU Interim Hospital laboratory in November 2012 to observe project analyses.

Data Review by Laboratory Directors

Laboratory directors reviewed laboratory results before submitting them to project QA staff. Laboratory directors assigned the laboratory RQIs as part of their review. A laboratory RQI

of 0 indicated that no problems occurred during the analysis. A laboratory RQI of 1 indicated that data were of less than optimal quality, but could be reported. A laboratory RQI of 2 indicated that the data were considered unreliable because of problems in the laboratory that could not be mitigated.

Quality Control (QC) and Quality Assurance (QA) Samples

Field duplicates and field blanks of environmental samples of gaseous pollutants (i.e., passive badges for carbonyls, VOCs, NO_2 and H_2S) were deployed in addition to the regular samples. In total, at least 3.5% of regular samples had associated duplicates or field blanks for each metric. We used these samples to assess the field interviewer's sampling protocol compliance, consistency of sampling device handling, and any contaminations to the sampling device due to the handling. Additional laboratory QC samples included lab blanks, lab spike controls, and ongoing assessment of calibration stability through repeated analysis, approximately every 10 samples, of solutions of known concentration (check standards) at one or more concentrations.

QA samples were analyzed independently (i.e., by a second laboratory) to assess accuracy of analyses. For this second review, we split samples and shipped them to a secondary lab, as was the case with the urinary VOC and phthalate metabolites, or we participated in proficiency testing programs (also commonly referred to as "round robin" analyses) where a a third party prepared a sample and sent it to many laboratories, which reported results back the proficiency testing provider. The QA/QC activities performed as part of CHATS are described or presented in the following sections.

LSU Interim Hospital Clinical Chemistry Core Laboratory

All CBC, IgE, and urinary creatinine and cotinine data were generated in the LSU Interim Public Hospital's Core Clinical Lab. All testing in the laboratory was subject to two QA mechanisms:

- Internal QC was conducted daily, covering at least two and sometimes three levels. The only exception was if zero patient samples were run for a specific test on a particular day, which was mainly a precision check, but also secondarily covered "accuracy."
- External QC was conducted quarterly via five College of American Pathologists (CAP) challenge samples per test, with comparison of lab results to peers using same analyzers or sometimes as a whole, to check accuracy.

Comparison of Urine Metabolite Results between LSU and RTI

LSU Interim Hospital sent 10 aliquots of Baseline urine samples and 18 aliquots of Follow-up urine samples to RTI for determination of phthalate and VOC metabolites. The data produced by each of the two laboratories for those samples are discussed below.

Phthalate Metabolites

Table 3-10a lists the phthalates and their metabolites***.*** Monocyclohexyl phthalate (MCHP), monooctyl phthalate (MOP), and monononyl phthalate (MNP) were all found at low concentrations, if at all. Otherwise, RTI analyses generally yielded analyte concentrations consistent with values generated by the LSU Laboratory. ***Figure 3-10a*** illustrates the good agreement between results at the two laboratories for four of the five analytes. (Results of the comparison of 2-ethylhexyl phthalate and its metabolites are discussed below.) There was one outlier (sample UR0573, monobenzyl phthlate [MBP] findings of 95.6 [LSU Interim Hospital] versus 32.9 [RTI]) that had closely eluting peaks with similar mass spectral peaks that may have contributed to measurement error by one or both labs, which was excluded from statistical summaries. Orthogonal regression data were generated using SAS JMP 5.0.1 (***see Table 3-10b***). The two outlier data points cited above were excluded from the analysis. Correlation for monomethyl phthlate (MMP) was weak, and is reflected in the wide confidence limits (CL) around the slope. Agreement (based on slope, where a slope of 1.0 is ideal) showed a tendency for LSU Interim Hospital to determine concentrations higher than RTI. The minimum detectable levels (MDLs) for the two labs were 0.88 ng/mL (LSU) and 1.4 ng/mL (RTI), so these samples may have been pushing the methodology.

Table 3-10a. Phthalates and Metabolites

Parent	Metabolite	Acronym
Dimethyl phthalate	Monomethyl phthalate	MMP
Diethyl phthalate	Monoethyl phthalate	MEP
Dibutyl and	Monobutyl phthalate	MBP
Butyl benzyl phthalate		
Dibutyl phthalate	Mono-3-carboxypropyl phthalate	MCPP
Butyl benzyl phthalate	Monobenzyl phthalate	MBzP
Dicyclohexyl phthalate	Monocyclohexyl phthalate	MCHP
Bis-2-ethylhexyl phthalate	Mono-2-ethylhexyl phthalate	MEHP
	Mono-2-ethyl-5-hydroxyhexyl phthalate	MEHHP
	Mono-2-ethyl-5-oxohexyl phthalate	MEOHP
	Mono-2-carboxymethylhexyl phthalate	MCMHP
Dioctyl phthalate	Monooctyl phthalate	MOP
Dinonyl phthalate	Monononyl phthalate	MNP

Figure 3-10a. Comparison of Phthalate Metabolite Data Generated by LSU Interim Hospital and RTI for Frequently Measured Analytes (Other than DEHP Metabolites)

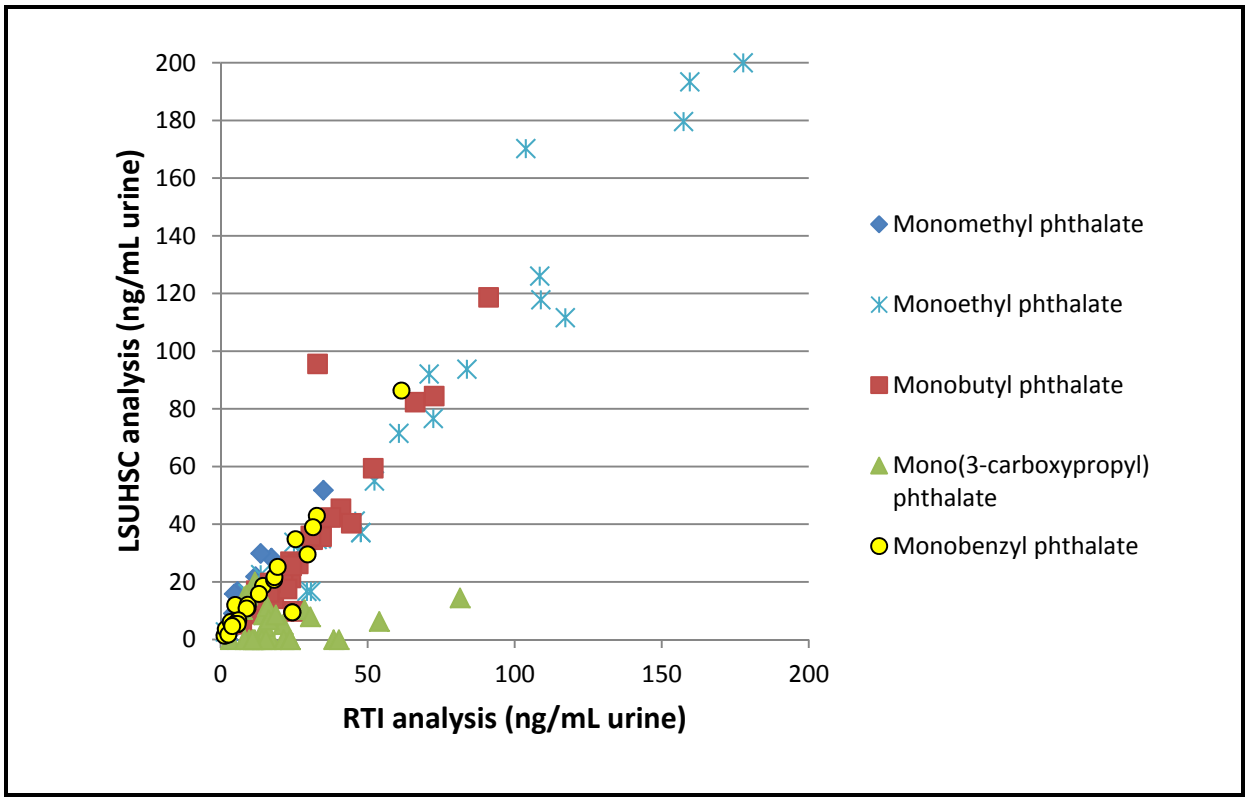


Table 3-10b. Regression Data for RTI versus LSU Interim Hospital Phthalate Monoester Analyses of QA Samples

	MMP	MEP	MBP	MCPP	MBzP
R	0.941	0.986	0.976	0.052	0.940
Slope	1.73	0.91	1.32	0.03	1.33
Lower slope CL	1.51	0.86	1.21	-0.17	1.16
Upper slope CL	2.00	0.95	1.44	0.22	1.54

CL = confidence limits, MMP = monomethyl phthalate, MEP = monoethyl phthalate, MBP = monobutyl phthalate, MCPP = mono(3-carboxypropyl) phthalate, MBzP = monobenzyl phthalate.

Table 3-10c presents a comparison of the distribution of values from the two laboratories for five monoalkylphthalates with comparison range of values from National Health and Nutrition Examination Survey (NHANES) 2003-4. The NHANES distribution was quite skewed, so we use percentiles to gauge the ranges for NHANES and LSU Interim Hospital samples. Because we had only a small number of QA samples at RTI, we used the full range of those. For MMP, MBP, and MBzP, both labs' results were in line with NHANES data. For MMP and MCPP, both labs' results were higher compared to NHANES.

Table 3-10c. Distribution of Phthalate Monoester Concentrations from RTI and LSU Interim Hospital Compared to NHANES Data

	Number of Samples	MMP	MEP	MBP	MCPP	MBzP
RTI	n = 28	2.3 – 35	1.7 - 820	7.2- 91.1	3.3 – 81.4	1.3 – 61.5
LSU*	n = 195	4.2 - 13.6	27.6 – 109	13.6 – 45.7	5.9 – 15.1	6.2 – 32.8
NHANES 2003-4*	n = 2648	0.7 - 4.3	53.3 - 371	12.3 - 50.4	1.8 - 6.2	5.26 - 27.5

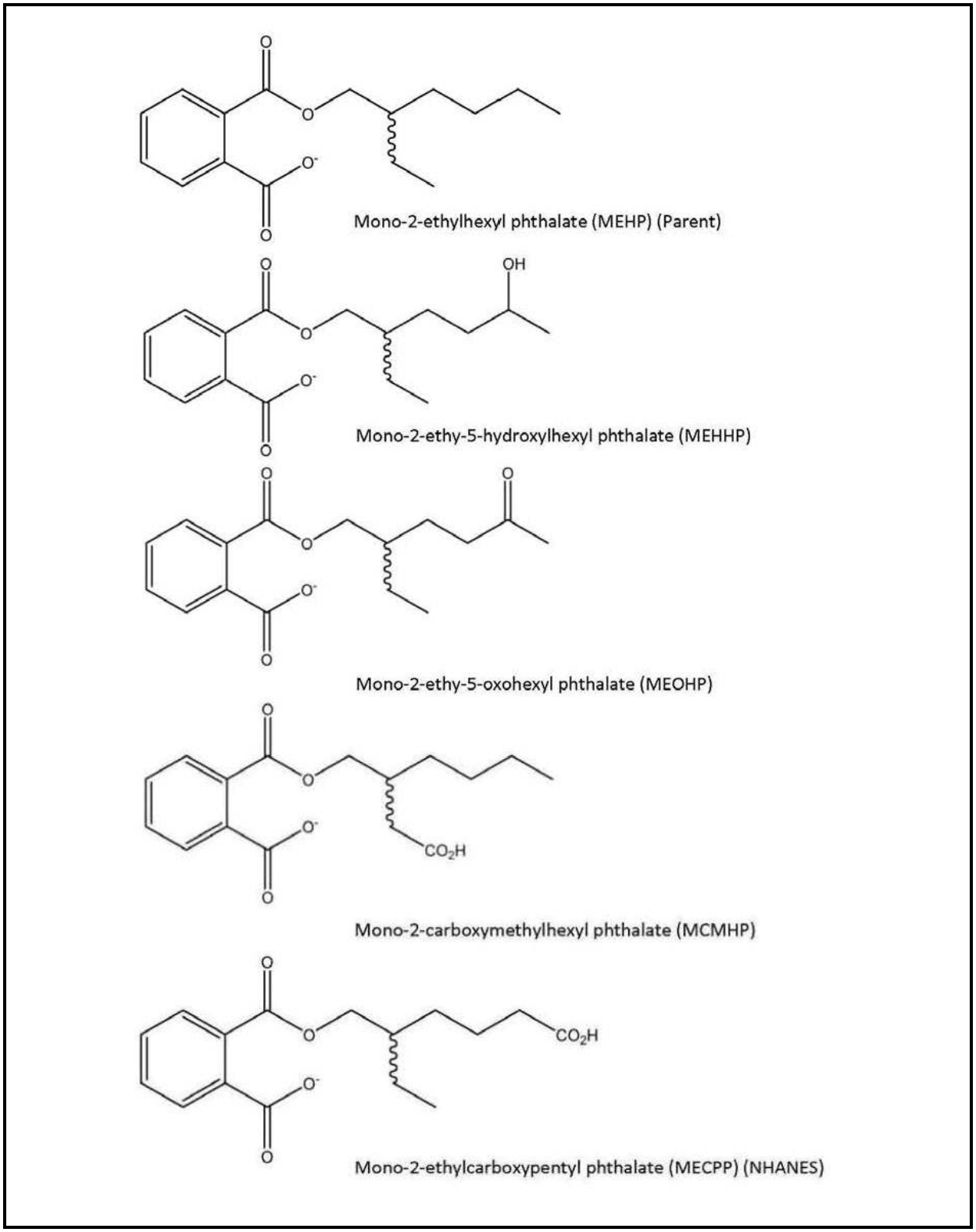
*25 – 75 percentiles

Abbreviations: MMP = monomethyl phthalate; MEP = monoethyl phthylate; MBP = monobutyl phthalate; MCPP = mono(3-carboxypropyl) phthalate; MBzP = monobenzyl phthalate; NHANES = National Health and Nutrition Examination Survey

MCPP is a secondary metabolite of dibutyl phthalate. NHANES data show that while secondary metabolites of the shorter-chain dimethyl and diethyl phthalates are not important, the secondary metabolites are about of equal importance starting at dibutyl phthalate (DBP) and are more important as markers for di933333(2-ethylhexyl) phthalate (DEHP). The range of concentrations determined by RTI for monobutyl phthalate (MBP) and MCPP were fairly similar, consistent with the NHANES data. The range of concentrations determined by LSU Interim Hospital was much lower for MCPP than for MBP, so the LSU Interim Hospital method may have been subject to interferences. However, the good correlation between the two laboratories for DBP suggests that DBP data should be adequate for assessing exposure to DBP and butyl benzyl phthalate.

The lack of good correlation for some metabolites of DEHP requires some discussion. ***Figure 3-10b*** shows the different structures for DEHP and its metabolites. The primary metabolite ("primary" refers to the initial metabolite in the metabolic pathway, not the predominant metabolite), MEHP, results from simple hydrolysis of one of the two 2-ethylhexyl side chains of DEHP. The secondary metabolites are produced by oxidation at one of several sites. The 5- position of the hexyl backbone is progressively oxidized, first to the hydroxy compound (MEHHP), then to the carbonyl (or oxo, MEOHHP) compound. The terminal methyl groups are oxidized all the way to the carboxylate. NHANES and CHATS used different carboxylated standards. The standard used for CHATS was 2-carboxymethyl) hexyl metabolite (MCMHP) and NHANES reported data for the 2-ethyl-5-carboxypentyl (MECPP) metabolite. The carboxylated standard for CHATS was purchased from Cambridge at the start of the study.

Figure 3-10b. Structure of Di(2-ethylhexyl) Phthalate (DEHP) and Various Metabolites



The correlation between RTI and LSU Interim Hospital was poor to nonexistent for all metabolites of DEHP except MEOHP. We suspect that low correlations are most likely associated with analyte stability derived from storage and/or sample handling during analysis or subtle differences in sample preparation (hydrolysis and clean-up). There was one outlier for MEOHP (sample 0573, same sample that was an outlier for MCCP). When that sample was dropped (n = 9), the correlation was good (r = 0.98) with fairly good agreement (slope = 1.34 [CI 1.13 – 1.60]), not far from what we found for metabolites of other dialkyl phthalates. We conclude that MEOHP may be used as the most reliable marker of exposure to DEHP. Data in ***Table 3-10d*** suggest that the median values of two of the DEHP metabolites, MEHP and MEOHP, are comparable between NHANES 2003-4 and the LSU Interim Hospital Baseline.

Table 310d. Median Values for Di(2-ethylhexyl) Phthalate (DEHP) Metabolites (ng/mL)

	NHANES 2003-4	CHATS LSU Substudy
MEHP	2.2	3.05
MEHHP	23.2	14.2
MEOHP	16.1	10.2
MECPP/MCMHP*	35.9	6.2

*MECPP from NHANES; MCMHP from CHATS

Abbreviations: MEHP = mono(2-ethylhexyl)phthalate; MEHHP = mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP = mono(2-ethyl-5-oxohexyl)phthalate (MEOHP); MECPP = mono-2-ethylcarboxypentyl phthlate; MCMHP = mono-2-carboxymethylhexyl phthlate.

MEHP is hardly the most abundant marker (only a few percent of the total in NHANES). The mismatch between NHANES and CHATS for the MECPP and MCMHP data may be an analytical issue or it may reflect a real difference in the relative abundance of the two metabolites. The data suggest, however, that both should be included in any method attempting to get a complete accounting of the dose. Of primary importance to data analysis, is the fact that in NHANES, the secondary metabolites are fairly strongly correlated with each other, and this correlation is mirrored in the LSU data (***Table 3-10e***). Based on those strong correlations, MEOHP concentrations can be regarded as a reliable marker of DEHP exposure. (An intriguing possibility is that large deviation of other DEHP metabolite concentrations from expected values, based on MEOHP concentration, might flag particular samples for either quality problems, or participants of interest for further evaluation.)

Table 3-10e.Correlations between Di(2-ethylhexyl) Phthalate (DEHP) Metabolites (r) by Laboratory

	NHANES 2003-4 Correlations			LSU Interim Hospital Substudy Correlations		
	MEHHP	MEOHP	MECPP	MEHHP	MEOHP	MCMHP
MEHP	0.689	0.700	0.663	0.607	0.637	0.504
MEHHP		0.969	0.938		0.970	0.911
MEOHP			0.950			0.914

Note: The values for sample UR0714, MCMHP = 24899 ng/mL dropped from analyses.

VOC Metabolites

For VOCs, not only can one parent compound yield multiple metabolites, but a single metabolite may be a marker of exposure to multiple parent compounds. Refer to ***Table 3-10f*** for the crosswalk between metabolites and parent compounds. ***Table 3-10g*** provides summary statistics for the distributions of these analytes in the 10 CHATS specimens that were analyzed at both LSU Interim Hospital and RTI and for a number of smokers and nonsmokers analyzed by CDC.[1] Many of the metabolites were consistently undetected or found only at very low concentrations in the study samples by both laboratories, so meaningful quantitative statistical comparisons are impractical for those compounds. These included 1,2-DCVMA and 2,2-DVCMA (trichloroethylene); all three isomers of DPMA (xylenes); HEMA (vinyl chloride, acrylonitrile, and ethylene oxide); MHBMA (1,3-butadiene); PHEMA (styrene); and PMA (benzene). In addition, LSU was unable to resolve HA (benzene) from a large matrix interferent. However, it is important to note that during method installations at both laboratories, the comparisons of shared solvent-based standards showed that all of the analytes were detected by both laboratories. In those installation analyses, the same trends for all target analytes (LSU value being slightly higher) were observed and are consistent with the QA comparisons seen in the study samples for the most frequently measured metabolites.

Table 3-10f. VOCs and Metabolites

Parent	Metabolite	Acronym
Acrolein	N-Acetyl-S-(2-carboxyethyl)-L-cysteine	CEMA
Acrylamide	N-Acetyl-S-(2-hydroxy-3-propionamide)-L-cysteine*	GAMA
Acrylonitrile	N-Acetyl-S-(2-cyanoethyl)-L-cysteine	CYMA
Benzene	N-Acetyl-S-(phenyl)-L-cysteine	PMA
	trans, trans -Muconic acid	MU
1,3-Butadiene	N-Acetyl-S- (3,4-dihydroxybutyl)-L-cysteine*	DHBMA
	N-Acetyl-S-(1-hydroxymethyl-2-propenyl)-L-cysteine + N-Acetyl-S-(2-hydroxy-3-butenyl)-L-cysteine	MHBMA
Crotonaldehyde	N-Acetyl-3-hydroxypropyl-1-methyl)-L-cysteine*	HPMMA
p-Dichlorobenzene	Not analyzed	
Methyl ethyl ketone	Not analyzed	
Methyl t-butyl ether	Not analyzed	
Naphthalene	Not analyzed	
n-Octane	Not analyzed	
α-Pinene	Not analyzed	
Styrene	N-Acetyl-S-(1-phenyl-2-hydroxyethyl-L-cysteine + N-Acetyl-S-(2-phenyl-2-hydroxyethyl)-L-cysteine	PHEMA
	Phenylglycolic acid (Mandelic acid)	MA
	Phenylglyoxylic acid (Benzoylformic acid)	PGA
Trichloroethylene	N-Acetyl-S-(1,2-dichlorovinyl)-L-cysteine	1,2-DCVMA
	N-Acetyl-S-(2,2-dichlorovinyl)-L-cysteine	2,2-DCVMA
Toluene	N-Acetyl-S-(benzyl)-L-cysteine	BMA
	Hippuric acid	HA
Vinyl chloride	N-Acetyl-S- (2-hydroxyethyl)-L-cysteine	HEMA
m&p-Xylenes	N-Acetyl-S-(2,4-dimethylphenyl)-L-cysteine	2,4-DPMA
	N-Acetyl-S-(2,5-dimethylphenyl)-L-cysteine	2,5-DPMA
	N-Acetyl-S-(3,4-dimethylphenyl)-L-cysteine	3,4-DPMA
	2-methylhippuric acid	2-MHA
	3-methylhippuric acid	3-MHA
	4-methylhippuric acid	4-MHA

Table 3-10g. Statistical Comparison of VOC Metabolite Data from LSU Interim Hospital, RTI Analytes with Significant Measurable Quantities from Both Laboratories

	2MHA (Xylenes)	3/4-MHA (m/p-Xylene)	BMA (Toluene)	CEMA (Acrolein)	CYMA (Acrylonitrile)	DHBMA (Butadiene)	GAMA (Acrylamide)	HPMMA (Crotonaldehyde)	MA (Styrene)	MU (Benzene)	PGA (Styrene)
Correlation (r)	0.788	0.875	0.913	0.722	0.889	0.909	0.762	0.896	0.661	0.123	0.947
Slope	0.796	1.27	1.21	1.26	0.977	0.832	0.822	1.29	1.334	0.416	0.584
Min	6.18	48.3	2.52	0	0.645	83.8	1.17	53.0	57.4	29.1	4.30
Max	90.6	1759	59.5	456	14.8	1392	88.4	797	556	1126	1001

For illustrative purposes, data for the other 12 target metabolites are broken out into four plots in ***Figure 3-10c***, spanning three orders of magnitude in concentration (in ng analyte/mL urine). Quantitative comparisons are provided in ***Table 3-10g*** for all 12 target metabolites. We found no profound disagreements in magnitude between the two laboratories. It is important to keep in mind in reviewing these data that the VOC metabolite method is new, and the standard reference materials used are available only as the neat materials, not as certified reference standard solutions. Thus, each of the two laboratories had to prepare standard solutions independently (both target analyte and labeled internal standard). The standards were provided by the vendor, in many cases in milligram quantities, and were extremely hygroscopic. The potential differences between calibration standards alone presented opportunities for disagreement between the two laboratories.

To put the data from RTI and LSU Interim Hospital into context, ***Table 3-10h*** presents the sample ranges in comparison to the data presented by CDC at the International Society for Exposure Science (Baltimore, October 2011), where the method was first described. Here again, we found few disagreements in magnitude, although it is worth noting that CYMA (acrylonitrile metabolite) concentrations from the QA samples appear to reflect a population of nonsmokers.

Proficiency Testing Data

Formaldehyde

Analytical laboratory activities for CHATS began in the summer of 2011 with the conduct of aldehyde (carbonyl) sampler stability studies. ***Figure 3-10d*** presents a control chart for formaldehyde quarterly proficiency testing data spanning the period June 2011–March 2013, as part of a program administered by the American Industrial Hygiene Association (AIHA). Four samples were required to be analyzed during each test event; results are shown here as the ratio of the RTI result to the expected (“true”) value, with the optimum result of 1.00 shown by the dashed red line, and the shaded area representing the ±15% acceptance criterion for check standards. The RTI laboratory was assigned a performance rating of 1, the highest level, throughout this period.

Figure 3-10c. Comparison of VOC Metabolite Data Generated by LSU Interim Hospital and RTI

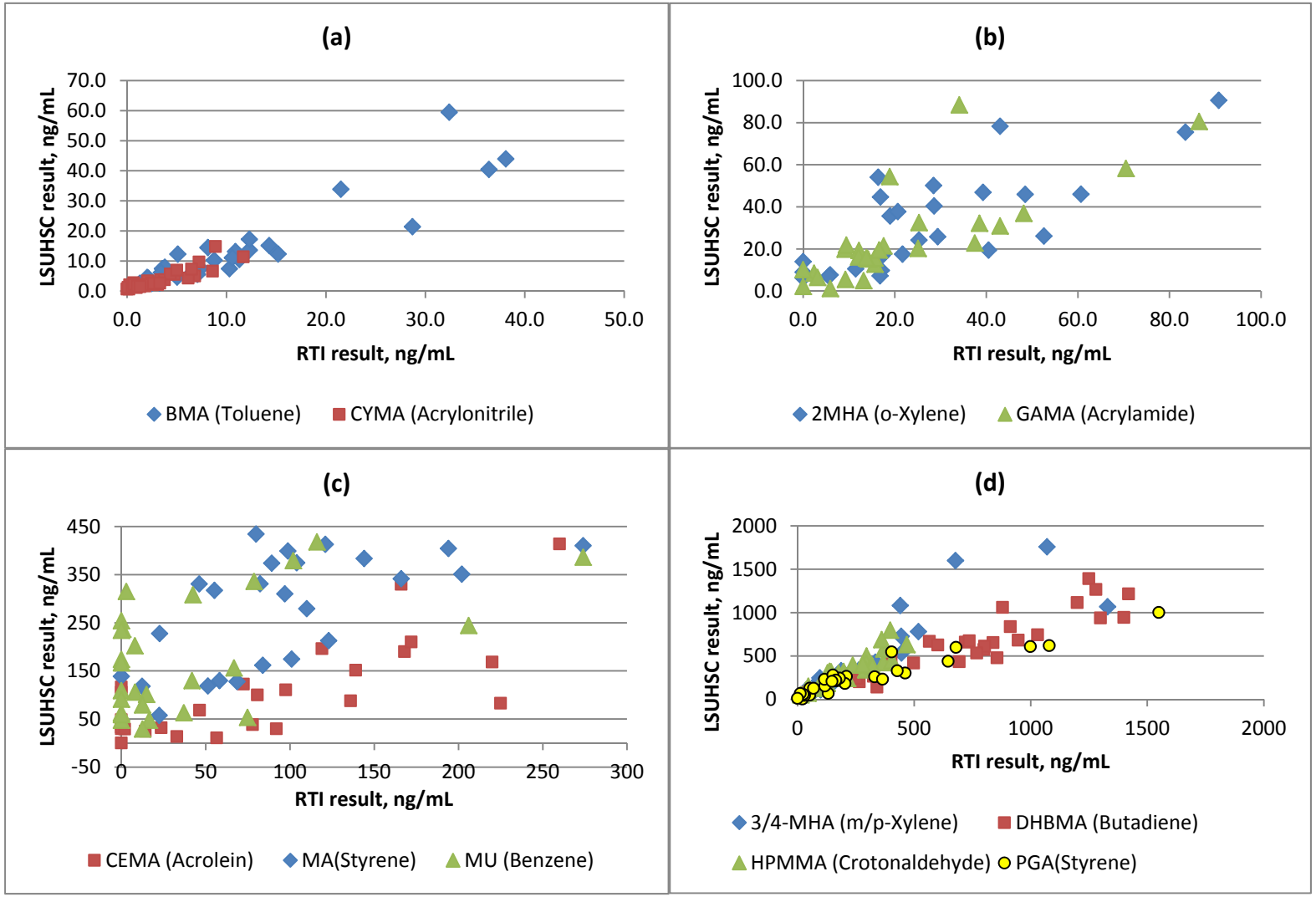
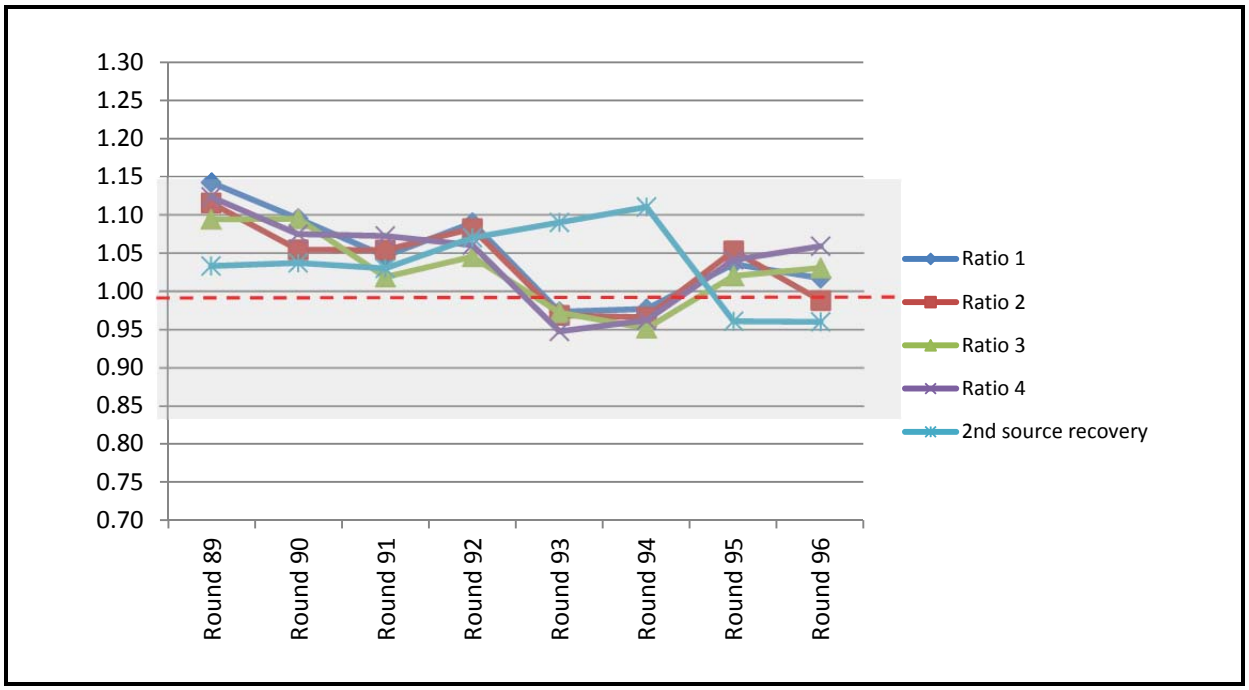


Table 3-10h. Comparison of Centers for Disease Control and Prevention[1] and CHATS Quality Assurance Sample Distributions by Laboratory

Analyte	Smokers*	Nonsmokers*	RTI	LSU Interim Hospital
Number of Specimens	347	1203	28	28
BMA	16.0 ± 29.0	15.0 ± 32.0	1.28 - 38.1	2.52 - 59.5
CEMA	305 ± 294	128 ± 119	1.96 - 260	< LOD - 456
CYMA	187 ± 181	4.60 ± 35.0	< LOD - 11.7	0.65 - 14.8
1, 2-DCVMA	< LOD	< LOD	<LOD - 63.2	< LOD - 0.78
2, 2-DCVMA	< LOD	< LOD	< LOD	< LOD - 0.44
DHBMA	440 ± 311	331 ± 279	47.0 - 1420	83.8 - 1392
DPMA	< LOD	< LOD	< LOD - 0.243	< LOD - 1.68
GAMA	57.0 ± 57.0	28.0 ± 36.0	< LOD - 86.5	1.17 -88.4
HEMA	1.90 ± 3.70	0.66 ± 1.16	< LOD - 11.6	< LOD - 7.50
HPMMA	1992 ± 2009	429 ± 478	13.8 - 469	53.0 - 797
MA	420 ± 357	198 ± 226	< LOD - 321	57.4 - 566
2-MHA	144 ± 265	71.0 ± 277	< LOD - 90.8	6.18 - 90.6
3/4-MHA	1020 ± 1379	579 ± 3692	17.9 - 1330	48.3 - 1759
MHBMA-1	< LOD	< LOD	< LOD - 0.81 (all 3 analytes)	< LOD - 17.3 (all 3 analytes)
MHBMA-2	1.80 ± 2.10	< LOD		
MHBMA-3	36.0 ± 34.0	6.40 ± 10.0		
MU	473 ± 410	358 ± 291	< LOD - 274	29.1 - 1126
PGA	330 ± 425	169 ± 224	< LOD - 1550	4.30 - 1001
PHEMA	< LOD	< LOD	< LOD - 4.2	< LOD - 4.72
PMA	0.92 ± 2.11	0.50 ± 0.40	< LOD - 2.55	< LOD - 4.28

[1] "Urinary VOC Metabolites as Biomarkers of Exposure to Volatile Organics". K. Udeni Alwis, Benjamin C. Blount, Hannah L. Barks, April N. Sheppard and David L. Ashley. Poster presented at the annual meeting of the International Society for Exposure Science, October 23-27, 2011, Baltimore, Maryland.

Figure 3-10d. Control Chart for Formaldehyde Proficiency Testing Data



Note: Red dashed line represents 100% of actual value; gray area represents target range of 85% - 115%.

VOCs (BTX compounds)

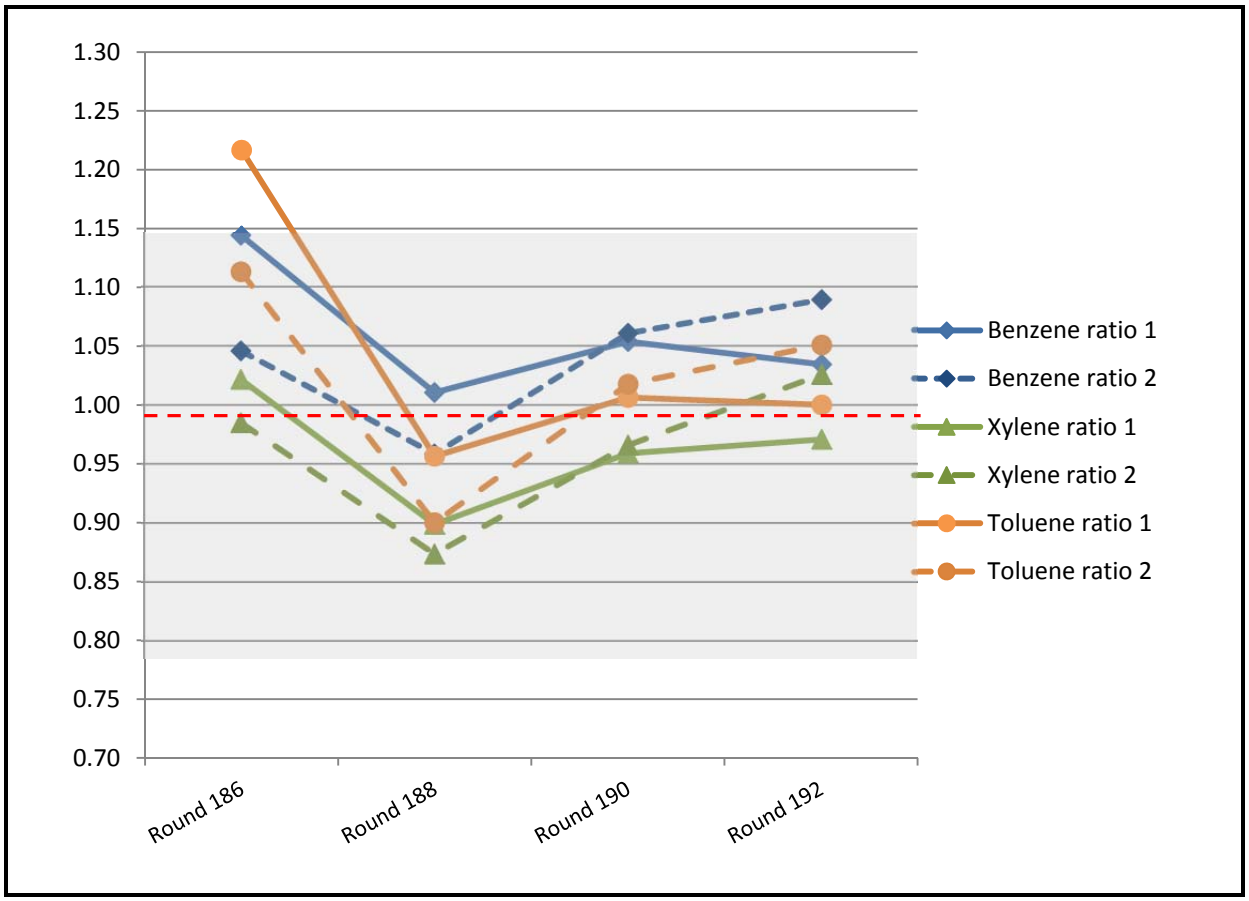
VOC proficiency testing using BTX compounds (benzene, toluene, xylenes) was conducted on a semi-annual basis, so fewer results are available over the period when CHATS-related laboratory operations were active. ***Figure 3-10e*** is an analogous control chart for VOCs proficiency testing, also administered by AIHA. The red dashed line represents the optimum ratio of 1.00, and the shaded area represents the ±15% acceptance criterion for check standards. For BTX proficiency testing, two rounds were conducted at each test period.

Stability Study Results

Carbonyl Badge Storage Stability Pilot Test

The manner in which the passive sampling devices were sent to the field interviewers required that materials be stored up to 3 weeks under field conditions prior to use for a participant sampling. The manufacturer (SKC) indicated that the background increases if badges are not kept cold and that an increase in background can impact limit of detection and might create variable bias. In addition, the ability of the badges to collect carbonyls might also be affected. Consequently, we performed a pilot test to assess the impact of storage.

Figure 3-10e. Control Chart for BTX Proficiency Testing Data



A test group of badges was stored in the trunk of a car in Research Triangle Park, North Carolina, in August. Nine badges were stored for 7 days, 9 badges for 14 days, and 9 badges were stored for 21 days. After the indicated storage period, all badges were then stored at -20°C until deployment. A control group of badges was stored in an environmental chamber (25°C). All badges (54 total) were then deployed for 7 days near highway I-40 (Research Triangle Park). Retrieved samples were stored at -20°C until analysis. An additional 6 badges were never deployed and stored at -20°C until analysis (method blanks). Badges were then analyzed according to the relevant CHATS extraction/analysis protocol. Statistical analysis was conducted using SAS v. 9.3 with univariate statistics and percent measurable across replicates. We also tested our hypothesis (H0: neither storage time nor storage condition explain any of the variability in the measurement) using the general linear model of the form: Analyte Concentration = Storage Condition + Storage Time.

The backgrounds for carbonyl badges stored for up to 3 weeks at both ambient temperatures and in a car trunk increased, relative to badges stored frozen, with the most marked increases observed for those badges stored in the car (***Figure 3-10e***). In general, we

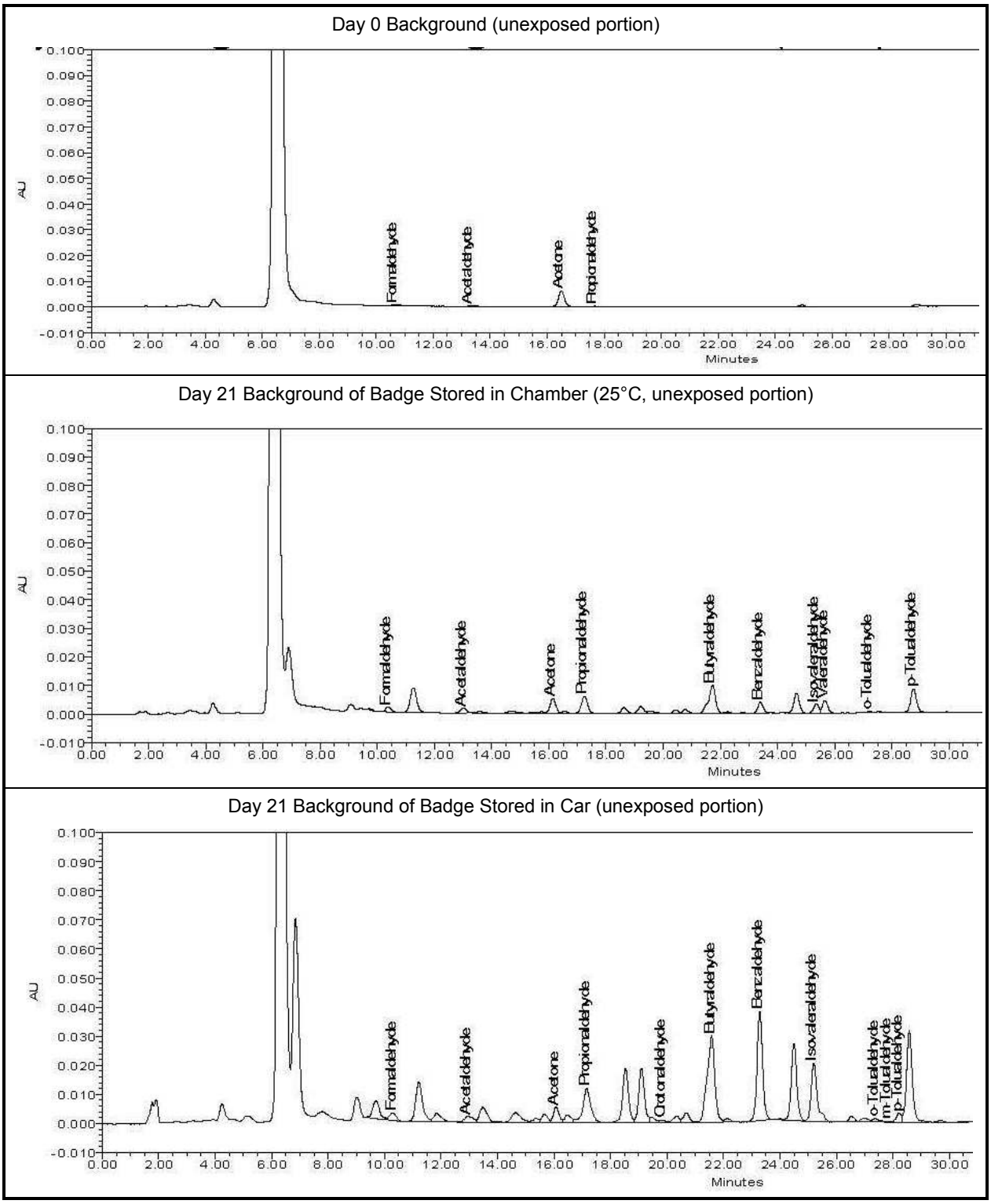
measured statistically significant increases as a function of storage time for most chemicals except formaldehyde (***Figure 3-10f***). Formaldehyde showed an initial increase, but then showed no further increase with either storage location or increased time. The increase in background is expected to adversely impact the limit of detection for all target analytes; however, this change should be consistent for formaldehyde. The 7-day deployment of stored badges showed consistent measurements for formaldehyde and propionaldehyde; the measurement variances (RSD) were not impacted by storage (***Figure 3-10g***). The passive badges as used in CHATS will provide formaldehyde measurements that are free from variable bias and that show consistent limits of detection.

Formaldehyde Extract Storage Stability Pilot Test

There is conflicting information on storage stability of DNPH sorbent cartridge extracts. The vendor product insert indicates that extracts should be analyzed within 3 days, while EPA Method TO-11a indicates 2 weeks, with the added provision that if samples are to be stored for a longer period of time, they should be extracted with a larger volume of solvent. The CHATS protocol called for samples to be analyzed within the 3-day period specified by the vendor.

During the course of the study, however, instrument issues occasionally forced samples (extracts) to exceed the specified holding time. To determine the impact of holding time on analyte measurement, we selected archived, analyzed samples (all were recapped and stored at $4^{o}C$) from batches analyzed at approximately 2-week intervals and analyzed both the front and back pad extracts. Two samples were taken from each batch and chosen to approximate a representative range of analyte concentrations. ***Figure 3-10h*** plots the ratio of the reanalyzed value to the original value against sample holding time; a ratio of 1.0 (dashed red line) is optimal. The data show that only formaldehyde yielded reasonably consistent data over these time periods. On this basis, samples that briefly exceeded the prescribed 3-day holding time were assigned laboratory RQIs of 1, unless other factors dictated a RQI of 2. For samples stored for more than 1 week and up to 12 weeks (45 samples, about 5% of the total), formaldehyde was assigned an RQI of 1, and all other analytes were assigned an RQI of 2.

Figure 3-10f. Chromatogram Showing Carbonyl DNPH Derivatives from Unexposed Badge Portions



Note: Red dashed line represents 100% of actual value; gray area represents target range of 85% - 115%.

Figure 3-10g. Concentrations of Selected DNPH Derivatives in Extracts (ng/mL) from Unexposed Badge Portions

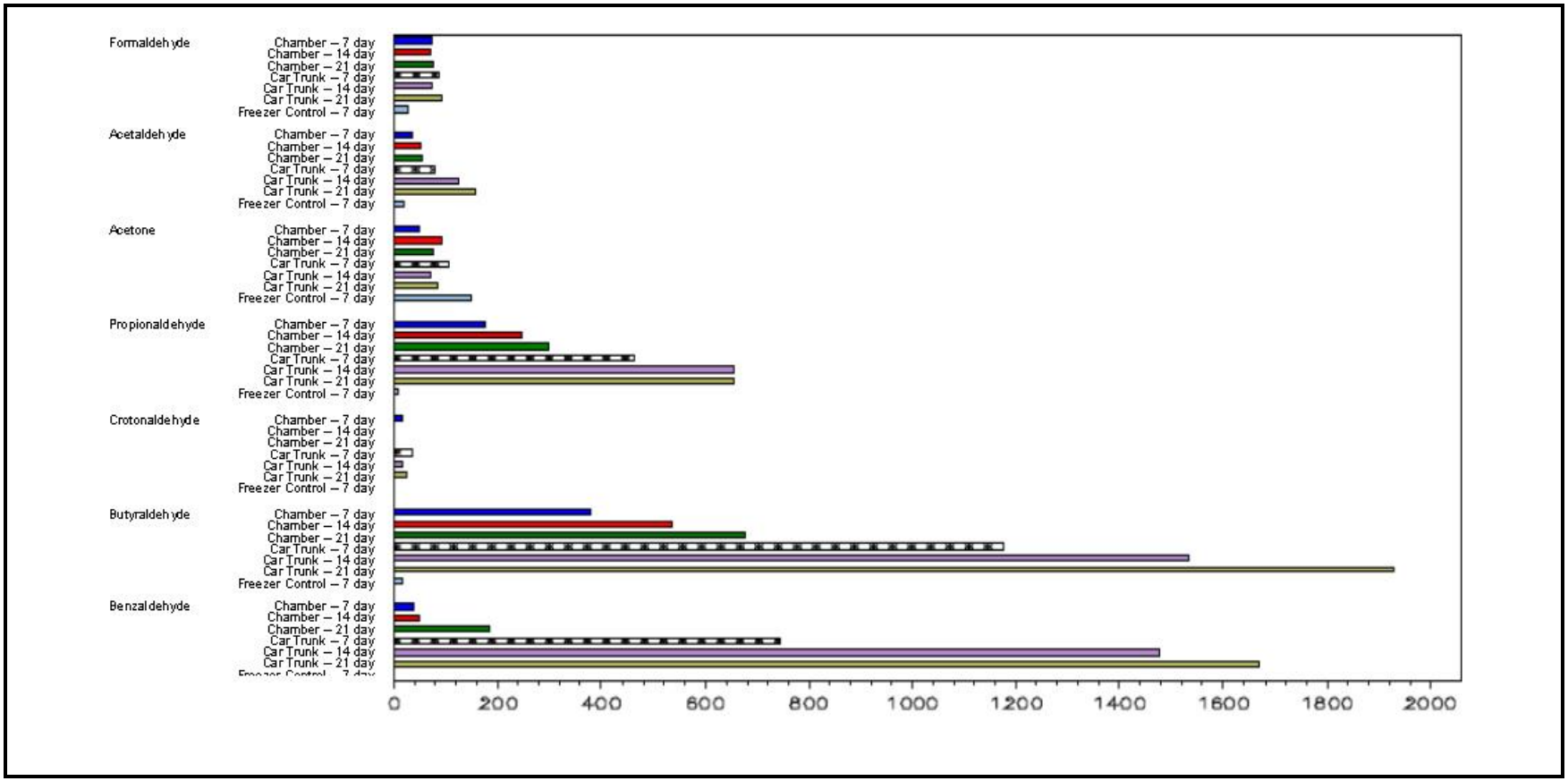
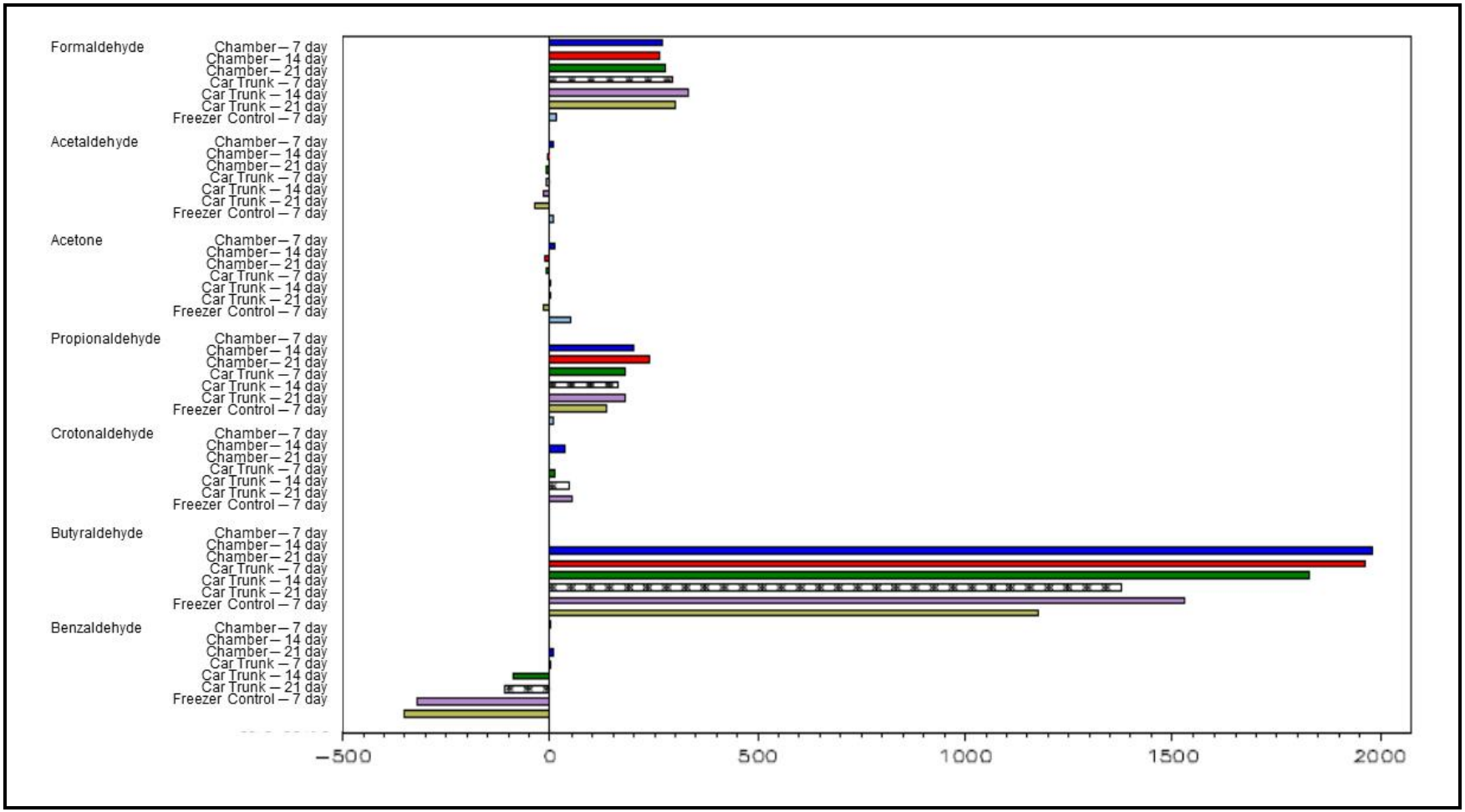


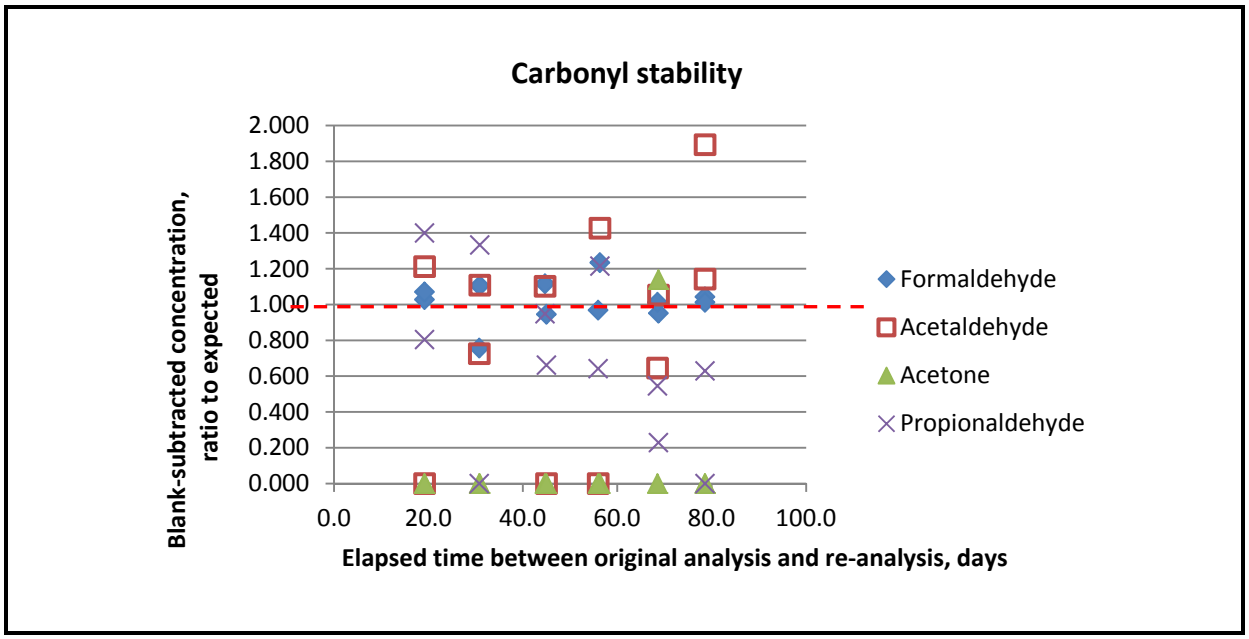
Figure 3-10h. Carbonyls Measured in Extracts (Background-Corrected, ng/mL) from Badges Exposed for 7 Days Near a Roadway Following Storage



VOC Extract Storage Stability Pilot Test

We conducted this evaluation in the same manner as described for carbonyl extracts. ***Figure 3-10i*** shows the ratio of reanalyzed, method-blank-subtracted controls to the original method blank-subtracted values. The batches shown were originally extracted and analyzed between July and December 2012, and were reanalyzed in May 2013. Vinyl chloride and butadiene control concentrations deteriorated in most samples throughout the holding period. (Curiously, the older samples appear to have survived better.) This result is of little consequence because (a) both these analytes went largely undetected across all study samples, regardless of holding time; and (b) both these analytes were problematic with regard to passing continuing calibration checks, and would have been assigned an RQI of 2 regardless of stability. All other analytes agreed within 25% of the initial measurement. Thus, archived extracts can be used if the additional uncertainty is acceptable for the purpose at hand.

Figure 3-10i. Stability Testing of Archived Carbonyl Samples

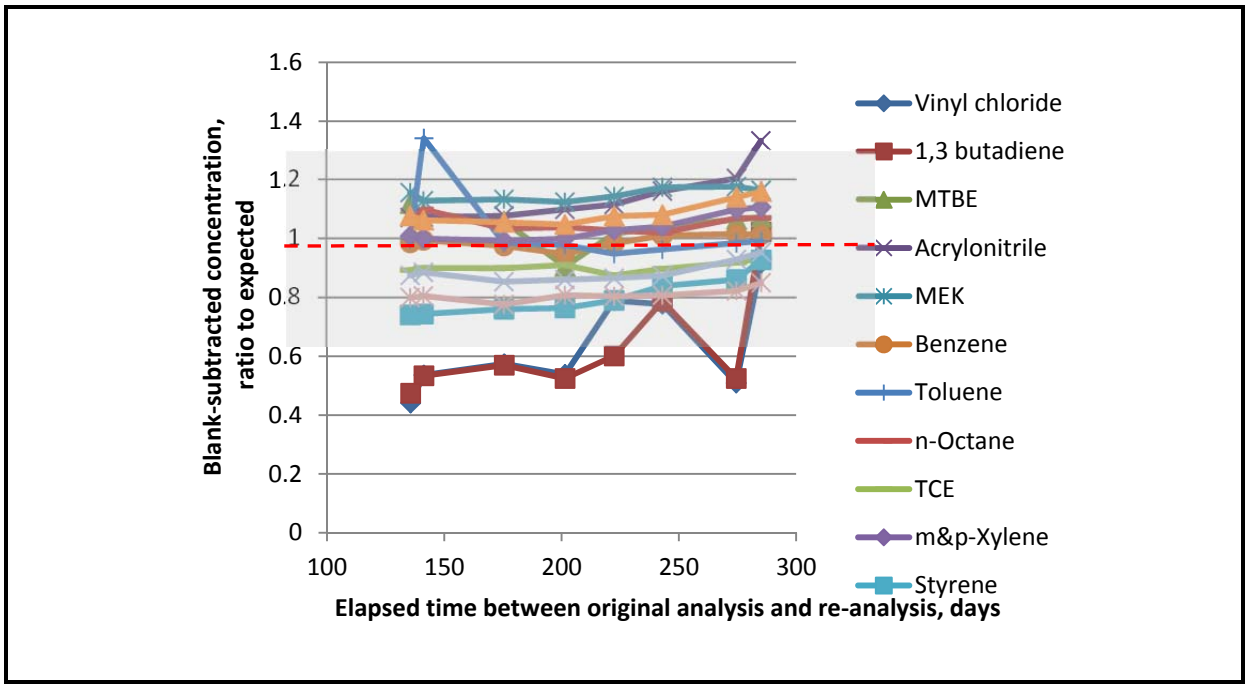


Note: Red dashed line represents 100% of initial value (no change).

Dust Storage and Impacts on Measurements

To determine the impact of shipping/storage on dust samples, the laboratory collected and sieved representative dust samples according to the sieving SOP for CHATS. The dusts were then aliquoted and placed in HEPA collection socks that were then placed in several different environments. These storage conditions were meant to either be worst-case (wet HEPA sock and dust, high relative humidity) dust stored under ambient conditions, and dusts stored with a desiccant. After a 7-day incubation under each condition, the aliquots were processed and analyzed following SOPs for endotoxin, glucan, and allergen assays (see ***Figure 3-10j***.)

Figure 3-10j. Stability Testing of Archived VOC QC Samples



(MTBE=methyl t-butyl ether; MEK = methyl ethyl ketone; TCE = trichloroethylene; p-DCB = p-dichlorobenzene)
Note: Red dashed line represents 100% of initial measurement. Gray area represents 70% to 130% of initial measurement.

While the results (***Table 3-10i***) vary depending on the dust sample, endotoxin and glucan values were considerably lower in samples stored at higher relative humidity for 7 days, compared to the desiccation and room storage samples. Endotoxin levels decreased by between 20% and 77%, while glucan levels decreased between 40% and 55% in two of three instances. For the most part, allergens were unaffected with one exception. These data were valuable in indicating that a robust collection/shipping protocol had to be implemented and proper storage conditions used i to preserve the integrity of the dust samples. Storage study results also pointed to the need for dust samples to be received in the RTI Laboratory in a timely fashion.

Field Duplicates

During the course of CHATS, duplicate (colocated) samples were collected for many of the media, including carbonyl badges, hydrogen sulfide sorbent tubes, NO_2 badges, and VOC badges. Samples and colocated samples were unknown to the laboratories and were revealed at the end of the study. For each case, the percentage difference between the two samples was calculated as the absolute value of the difference of the two measures divided by the mean of the two measures and then multiplied by 100. For all tables, we computed descriptive statistics only when both sample and duplicate measurements were above the laboratory-reported method detection limit, exclusive of cases where FINAL_RQI = 2.

Table 3-10i. Assessment of Dust Samples Stored under Several Conditions

Sample/ Storage Condition	Endotoxin (EU/mg Dust)	1,3-B-D-glucan (pg/mg Dust)	ng/g Dust			Bla g1 (U/g Dust)
			Asp f1	Der p1	Der f1	
Sample 1						
Day 0	90.82	1.26E+04	N/A	BDL	BDL	450.34
Room storage (Day 7)	216.50	3.84E+03	N/A	BDL	BDL	BDL
Dessicator (Day 7)	436.74	5.20E+03	N/A	BDL	BDL	257.07
High humidity (Day 7)	47.67	5.55E+03	N/A	BDL	BDL	BDL
Sample 2						
Day 0	55.61	1.04E+04	N/A	268.84	BDL	BDL
Room storage (Day 7)	90.32	2.93E+03	N/A	252.08	BDL	BDL
Dessicator (Day 7)	129.66	2.93E+03	N/A	252.52	BDL	BDL
High humidity (Day 7)	43.94	7.56E+03	N/A	BDL	BDL	BDL
Sample 3						
Day 0	146.70	5.83E+03	N/A	BDL	BDL	BDL
Room storage (Day 7)	N/A	N/A	N/A	BDL	BDL	BDL
Dessicator (Day 7)	197.54	6.74E+03	N/A	BDL	BDL	BDL
High humidity (Day 7)	33.26	6.05E+03	N/A	BDL	BDL	BDL

Table 3-10j provides descriptive statistics of the duplicates for the carbonyl badges. Formaldehyde gave the best precision; for 27 sample pairs, the mean percentage difference was less than 10% and is acceptable. Acetaldehyde and propanal had differences less than 20%. Acetone is a common contaminant in both the laboratory and the home. Since each measurement is obtained by subtracting the mass of the DNPH derivative from the unexposed portion of the badge from the exposed portion of the badge, the percentage difference becomes the difference of two differences, and the uncertainty is increased. While accuracy for acetone is arguable, data from the study will permit differentiation of +/- 50%. The acrolein measure is unreliable because this DNPH derivative is unstable on the badge.

Two pairs of duplicate hydrogen sulfide sorbent tubes were analyzed during the Follow-up Assessment. The results are shown in ***Table 3-10k***. ***Table 3-10l*** provides statistics on the duplicate pairs of NO_2 badges. Colocated samplers for hydrogen sulfide and nitrogen dioxide showed mean percentage differences of 10% or less, which is acceptable.

Table 3-10j. Descriptive Statistics for Percent Difference between Duplicate Carbonyl Badges

Chemical Analyte	Study Phase	No. Duplicate Pairs	Mean Percent Diff.	SD	Min. Percent Diff.	25th Percentile	Med. Percent Diff.	75th Percentile	Max. Percent Diff.
Crotonaldehyde	Baseline	1	3.5		3.5	3.5	3.5	3.5	3.5
	Follow-up	2	78.7	62.9	34.3	34.3	78.7	123.2	123.2
Acetone	Baseline	1	29.5		29.5	29.5	29.5	29.5	29.5
	Follow-up	4	47.4	74.3	2.9	6.6	14.1	88.2	158.5
Acetaldehyde	Baseline	3	13.1	14.0	2.2	2.2	8.3	28.9	28.9
	Follow-up	16	18.8	31.0	0.2	2.9	10.0	17.1	125.9
Formaldehyde	Baseline	5	9.1	6.5	0.5	5.1	9.8	13.7	16.7
	Follow-up	22	9.6	8.0	0.5	4.5	7.6	12.4	35.8
Propanal	Baseline	3	17.5	14.2	4.9	4.9	14.8	32.9	32.9
	Follow-up	20	19.7	17.4	0.4	7.4	11.1	34.3	59.2

Abbreviations: SD = Standard deviation.

Table 3-10k. Descriptive Statistics for Percent Difference between Duplicate Hydrogen Sulfide Sorbent Tubes

Chemical Analyte	Study Phase	No. Duplicate Pairs	Mean Percent Diff.	SD	Min. Percent Diff.	25th Percentile	Med. Percent Diff.	75th Percentile	Max. Percent Diff.
Hydrogen sulfide	Follow-up	2	9.9	0.0	9.9	9.9	9.9	9.9	9.9

Abbreviations: SD = Standard deviation.

Table 3-10l. Descriptive Statistics for Percent Difference between NO_2 Badges

Chemical Analyte	Study Phase	No. Duplicate Pairs	Mean Percent Diff.	SD	Min. Percent Diff.	25th Percentile	Med. Percent Diff.	75th Percentile	Max. Percent Diff.
Nitrogen dioxide	Baseline	4	6.6	6.7	0.6	1.0	5.6	12.1	14.5
	Follow-up	23	10.0	8.2	0.1	2.4	8.2	13.9	30.3

Abbreviations: SD = Standard deviation.

Table 3-10m provides statistics on the duplicate pairs of VOC badges. For the VOCs, the mean percent differences for all analytes were less than 15%, and this is considered acceptable.

Table 3-10m. Descriptive Statistics for Percent Difference between VOC Badges

Chemical Analyte	Study Phase	No. Duplicate Pairs	Mean Percent Diff.	SD	Min. Percent Diff.	25th Percentile	Med. Percent Diff.	75th Percentile	Max. Percent Diff.
Acrylonitrile	Follow-up	3	10.7	12.0	1.7	1.7	6.1	24.4	24.4
Benzene	Follow-up	18	6.5	7.0	0.5	1.6	3.5	10.1	27.2
	Baseline	1	5.6	—	5.6	5.6	5.6	5.6	5.6
Methyl ethyl ketone	Follow-up	14	7.3	5.4	1.2	3.4	5.1	11.3	19.1
	Baseline	3	14.2	4.4	9.1	9.1	16.1	17.4	17.4
Naphthalene	Follow-up	12	7.5	5.0	1.1	2.9	7.3	11.9	14.9
	Baseline	2	11.6	16.1	0.2	0.2	11.6	22.9	22.9
Styrene	Follow-up	9	2.6	2.3	0.4	0.9	2.4	2.7	7.0
	Baseline	4	3.5	6.1	0.1	0.2	0.7	6.9	12.6
Toluene	Follow-up	22	6.4	9.2	0.1	1.5	2.6	7.2	36.3
	Baseline	6	10.5	10.0	2.4	2.9	6.5	16.9	27.5
Trichloroethylene	Follow-up	15	2.5	2.0	0.8	1.0	2.3	2.9	7.1
α-Pinene	Follow-up	22	5.4	10.9	0.0	0.6	2.6	5.6	51.9
	Baseline	6	4.9	5.2	0.0	0.4	3.2	11.0	11.6
m,p-Xylenes	Follow-up	22	7.1	9.6	0.1	0.6	2.3	8.3	30.3
	Baseline	4	11.2	4.4	4.9	8.4	12.4	14.1	15.2
n-Octane	Follow-up	13	10.3	7.3	2.6	4.5	9.1	13.2	25.0
	Baseline	2	9.3	8.2	3.5	3.5	9.3	15.1	15.1
p-Dichloro-benzene	Follow-up	15	4.8	6.5	0.0	0.9	2.4	7.1	26.2
	Baseline	1	2.2	—	2.2	2.2	2.2	2.2	2.2

Abbreviations: SD = Standard deviation.

Field Blanks

In a manner analogous to the deployment of field duplicates, some samplers were sent to the field where they were removed from the packaging and exposed for only the short amount of time required to repackage them for return shipment to RTI, according to the SOPs. These data, therefore, represent potential contaminations associated only with shipping and handling of the samplers. In all cases, the mass of target measured in the extract was divided by the nominal sampling time (volume) so that it could be converted to a concentration that can be compared to the measured concentrations. In all cases, no corrections of field samples for field blanks were made.

Descriptive summaries of the field blank data are shown ***Table 3-10n*** for the carbonyl badge field blanks, ***Table 3-10o*** for the NO_2 badges, and ***Table 3-10p*** for the VOC badges. Most

analytes were measureable at low concentrations in the field blanks. Benzene, toluene, and octane were measured at the highest concentrations. Although most of the blanks were low and fairly consistent, toluene gave some high values that suggest that some contamination of the VOC badges occurred during sample handling and shipment.

Table 3-10n. Descriptive Statistics for Carbonyl Badge Field Blanks

Chemical Analyte	Study Phase	No. Blanks	Mean Blank	SD	Min. Blank	25th Percentile	Med. Blank	75th Percentile	Max. Blank
Acetone	Baseline	9	4.7	5.4	1.2	1.6	2.0	5.8	18.3
	Follow-up	15	2.9	8.9	0.0	0.2	0.3	0.8	34.6
Acetaldehyde	Baseline	10	0.8	0.9	0.0	0.1	0.4	1.9	2.3
	Follow-up	19	0.2	0.4	0.0	0.1	0.1	0.2	2.0
Formaldehyde	Baseline	9	0.8	0.9	0.0	0.1	0.3	1.4	2.4
	Follow-up	16	0.2	0.5	0.0	0.1	0.1	0.2	1.9
Propanal	Baseline	7	1.3	1.4	0.0	0.1	0.9	3.1	3.2
	Follow-up	6	0.4	0.5	0.1	0.1	0.2	0.4	1.3

Abbreviations: SD = Standard deviation.

Table 3-10o. Descriptive Statistics for NO_2 Badge Blanks

Chemical Analyte	Study Phase	No. Blanks	Mean Blank	SD	Min. Blank	25th Percentile	Med. Blank	75th Percentile	Max. Blank
Nitrogen dioxide	Baseline	5	2.8	0.6	1.9	2.7	2.8	3.1	3.6
	Follow-up	19	1.3	0.4	0.9	1.0	1.2	1.5	2.3

Abbreviations: SD = Standard deviation.

Table 3-10p. Descriptive Statistics for VOC Badge Blanks

Chemical Analyte	Study Phase	No. Blanks	Mean Blank	SD	Min. Blank	25th Percentile	Med. Blank	75th Percentile	Max. Blank
Benzene	Baseline	3	1.8	0.8	1.0	1.0	1.8	2.5	2.5
	Follow-up	2	0.7	0.2	0.5	0.5	0.7	0.8	0.8
Methyl ethyl ketone	Follow-up	2	0.5	0.1	0.4	0.4	0.5	0.6	0.6
Naphthalene	Follow-up	2	0.2	0.1	0.2	0.2	0.2	0.3	0.3
Styrene	Baseline	3	0.6	0.9	0.0	0.0	0.1	1.6	1.6
	Follow-up	1	0.1		0.1	0.1	0.1	0.1	0.1
Toluene	Baseline	5	48.1	63.9	1.3	1.7	2.5	105.7	129.2
	Follow-up	2	3.4	1.1	2.6	2.6	3.4	4.2	4.2
a-Pinene	Baseline	6	1.0	0.3	0.3	0.9	1.0	1.1	1.3
	Follow-up	1	2.8		2.8	2.8	2.8	2.8	2.8
m,p-Xylenes	Baseline	3	0.4	0.2	0.1	0.1	0.4	0.6	0.6
	Follow-up	1	1.2		1.2	1.2	1.2	1.2	1.2
n-Octane	Baseline	4	3.8	0.7	3.0	3.3	3.7	4.2	4.7
	Follow-up	1	5.2		5.2	5.2	5.2	5.2	5.2
p-Dichloro-benzene	Baseline	2	0.1	0.1	0.0	0.0	0.1	0.1	0.1

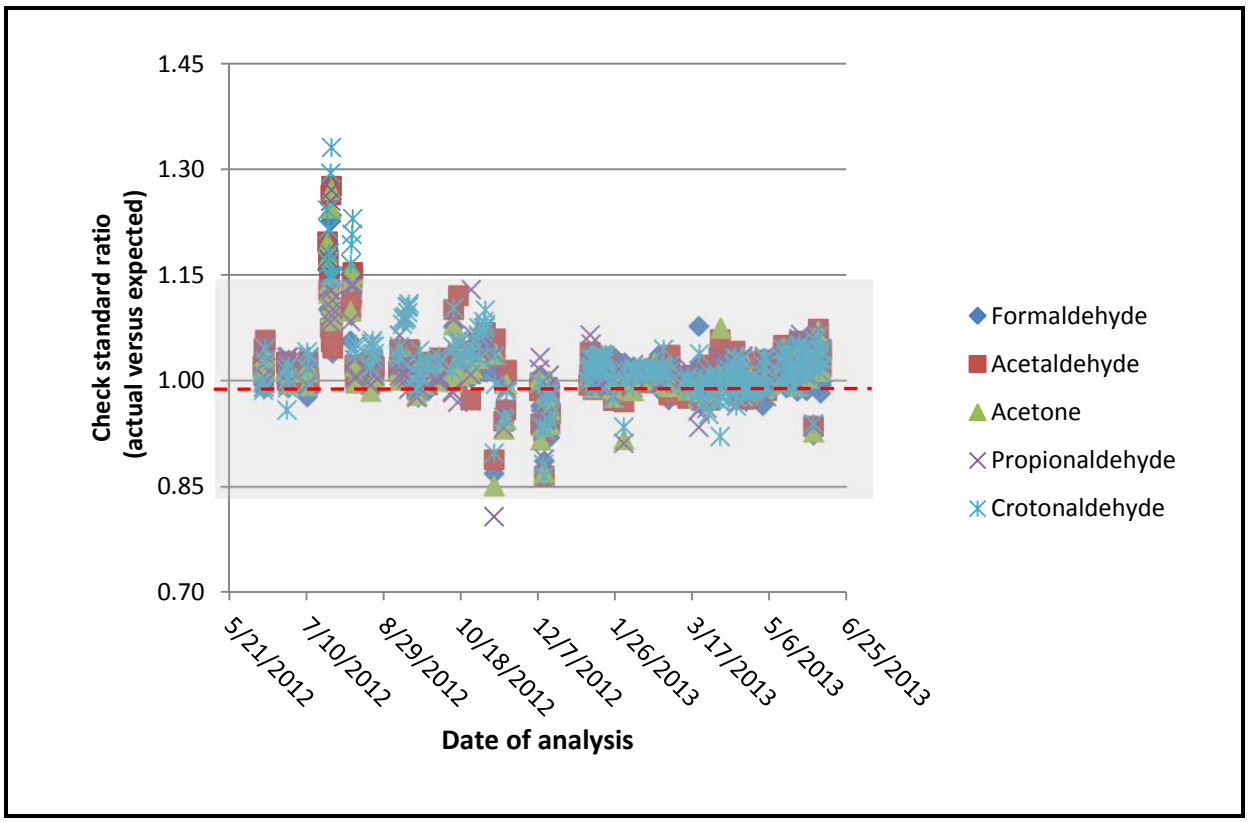
Abbreviations: SD = Standard deviation.

Carbonyls (Aldehydes)

All extraction batches included a method blank and a method control. All instrument batches comprised a single extraction batch and included bracketing continuing calibration checks, a second source calibration check, solvent blanks, and duplicate injections of one sample.

Figure 3-10j shows the control chart for all calibration check standards over the course of the study. There were several significant instances (July to August 2012; November to December 2012; May 2013) where check standards fell outside the mandatory 85–115% (shaded area; red dashed line is optimum 100%), and/or check standard recoveries were falling outside the long-term trend; each of these was followed by corrective actions, recalibration and redetermination of MDLs, and reanalysis of samples, if necessary. One batch of samples (July 2012) failed QC for all analytes but was inadvertently not reanalyzed; as a result, analytical data from this batch were mostly assigned RQI = 2.

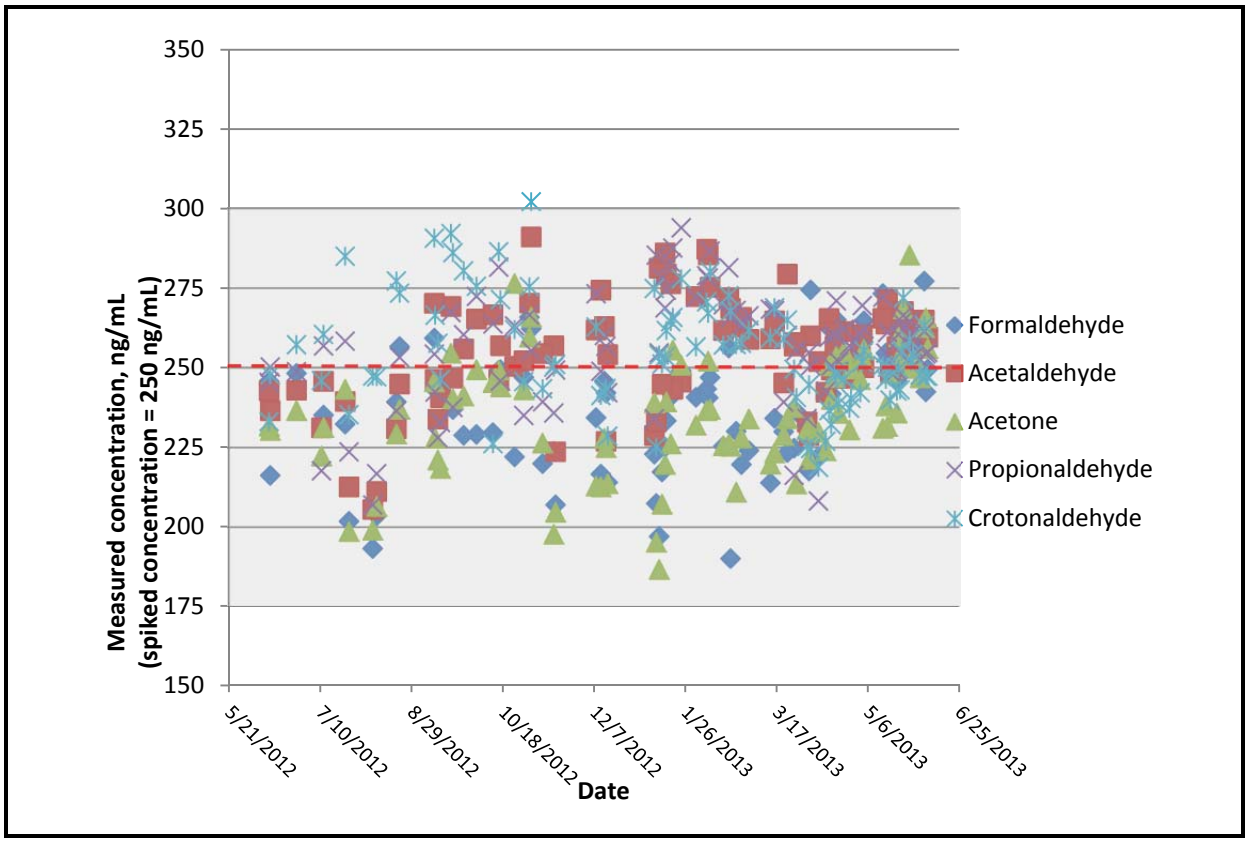
Figure 3-10j. Carbonyls Continuing Calibration Check Standard Control Chart



Notes: Red dashed line represents expected value. Gray area represents 85% - 115% acceptance range.

Figure 3-10k shows the control chart for blank-subtracted method controls. Red dashed lines indicate the optimum 100% control recovery, and the shaded area spans the acceptance limits (70–130%) for control recovery. All analytes in all batches met control recovery objectives.

Figure 3-10k. Carbonyls Method Controls, Control Chart



Notes: Red dashed line represents expected value. Gray area represents 70% - 130% acceptance range.

Method duplicates could not be obtained because the entire sample was extracted. We used injection duplicates to assess chromatographic (instrument) precision. Precision from duplicates (as the relative standard deviation) was estimated as shown in ***Eq. 3-1.***

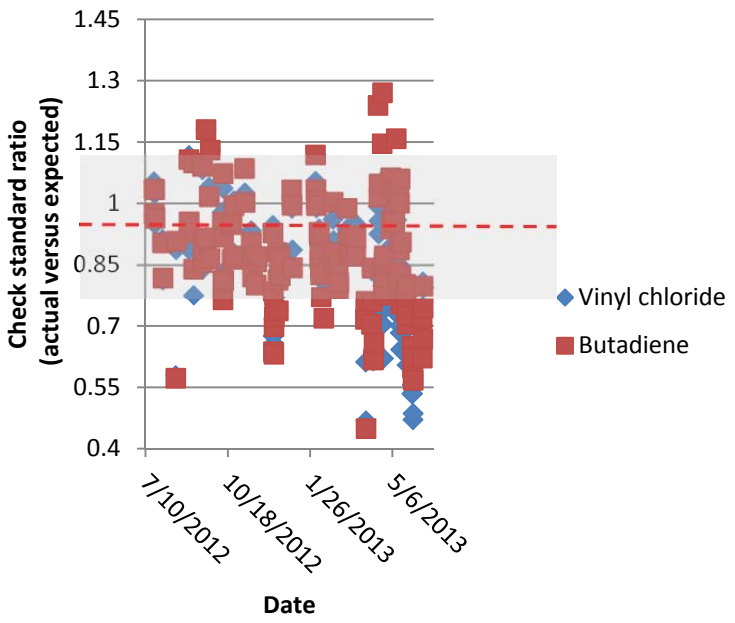
$$\boldsymbol{Eq.3-1}\ RSD = \frac{\sqrt{\frac{\sum(x_{i1}-x_{i2})^2}{2n}}}{\overline{x}} \times 100.$$

Precision for each analyte was formaldehyde, 2.2% (n = 99); acetaldehyde, 2.6% (100); acetone, 3.5% (100); propionaldehyde, 5.6% (94, with one outlier dropped); and crotonaldehyde, 17.5% (22, with one outlier dropped). The imprecision in the crotonaldehyde data is expected due to the relative instability of this compound.

VOCs

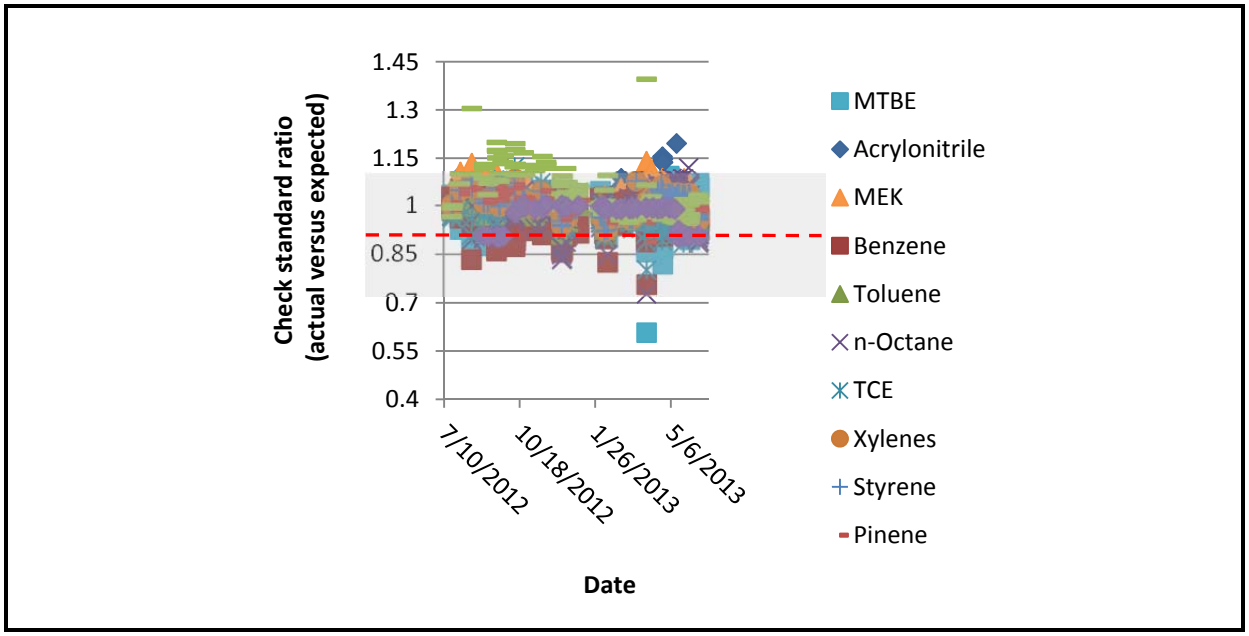
All extraction batches included a method blank and a method control; the method blank was subtracted from the method control, as well as from all samples in that batch. Instrument batches generally comprised one extraction batch (promoted samples from different batches were analyzed together). Samples were bracketed by continuing calibration check standards at two levels (2 or 5 ppm and 25ppm), and each analytical batch included a second source calibration check standard. Due to the high volatility of both the analytes and the solvent, we did not attempt duplicate injections. Control charting is presented for the low-check standards, as most of the samples were near or below the low-check concentration. ***Figure 3-10l*** shows the control chart for the two analytes with boiling points below room temperature. Although these compounds yielded largely unreliable data, the consequences were relatively insignificant, as they were usually not detected in study samples. ***Figure 3-10m*** presents the calibration check control chart for the remaining compounds. Alpha-pinene presented some issues early in the study that were ultimately resolved through routine maintenance and recalibration. Otherwise, there were only sporadic incidences of calibration check failures.

Figure 3-10l. VOCs Continuing Calibration Check Standard Control Chart – Low-Boiling Analytes



Notes: Red dashed line represents expected value. Gray area represents acceptance range.

Figure 3-10m. VOCs Continuing Calibration Check Standard Control Chart – Other Analytes (MTBE = methyl t-butyl ether; MEK = methyl ethyl ketone; TCE = trichloroethylene; p-DCB = p-dichlorobenzene)



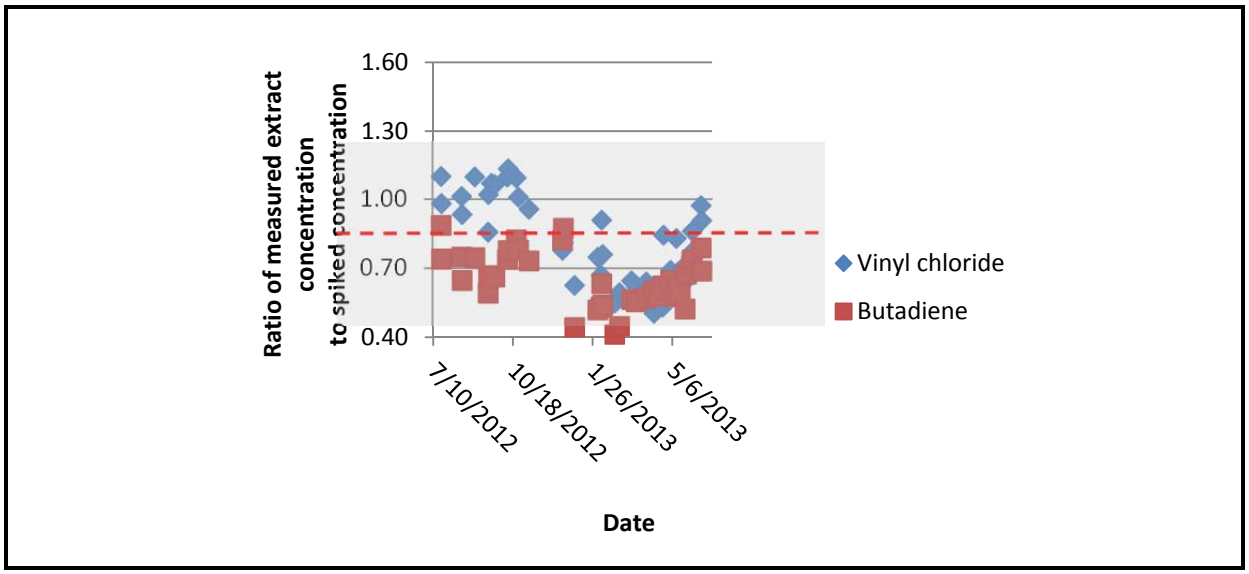
Notes: Red dashed line represents expected value. Gray area represents acceptance range.

Similar data for method controls (with batch-associated method blanks subtracted) are presented in ***Figures 3-10n*** and ***3-10o***. Again, vinyl chloride and butadiene were problematic analytes, whereas the other analytes consistently fell within the target 70%–130% range.

Phthalates

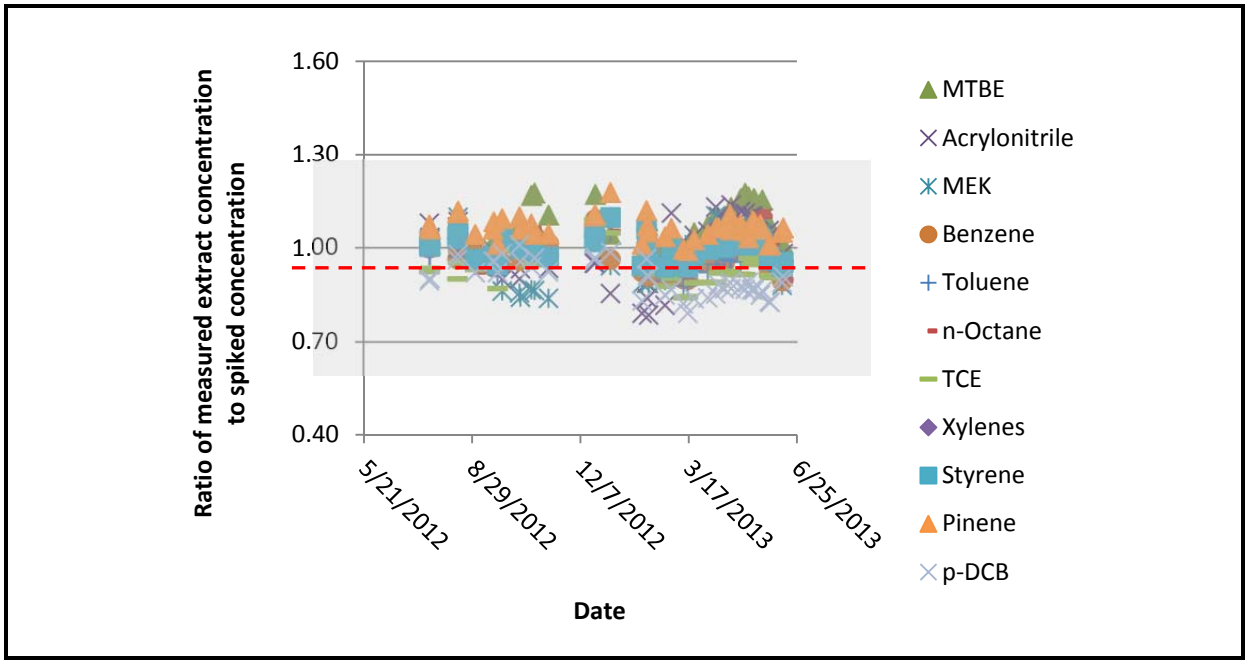
All extraction batches included a method blank and a method control, as well as one sample extracted in duplicate. All instrument batches comprised a single extraction batch and included bracketing with continuing calibration check standards, a second source check standard, solvent controls, and an injection duplicate. Instrument performance for the original Batch A and Batch B analyses (20 samples each) was poor. The extracts from those batches were archived and reanalyzed following commissioning of a new instrument. (We did not perform a stability experiment because all but one of the target analytes had an isotopically labeled analog as a specific internal standard, so that both analyte and internal standard should be affected proportionately.) ***Figure 3-10n*** presents the control chart for calibration checks on the new instrument. Benzyl butyl phthalate frequently failed check standard criteria, so a significant amount of data for that compound was not usable. We ascribe that to the use of a nonanalogous labeled internal standard. Other analytes were generally well-behaved.

Figure 3-10n. VOCs Method Controls, Low-Boiling Analytes, Control Chart



Notes: Red dashed line represents expected value. Gray area represents acceptance range.

Figure 3-10o. VOCs Method Controls, Other Analytes, Control Chart

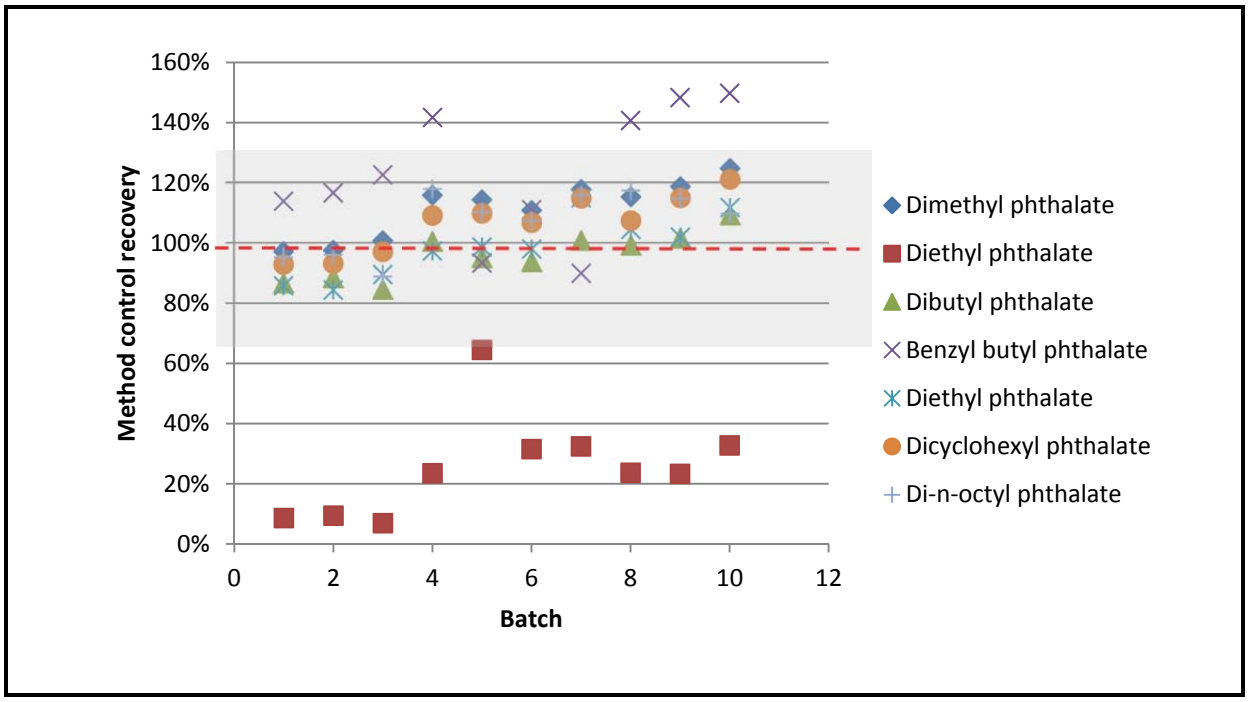


(MTBE = methyl t-butyl ether; MEK = methyl ethyl ketone; TCE = trichloroethylene; p-DCB = p-dichlorobenzene)

Notes: Red dashed line represents expected value. Gray area represents acceptance range.

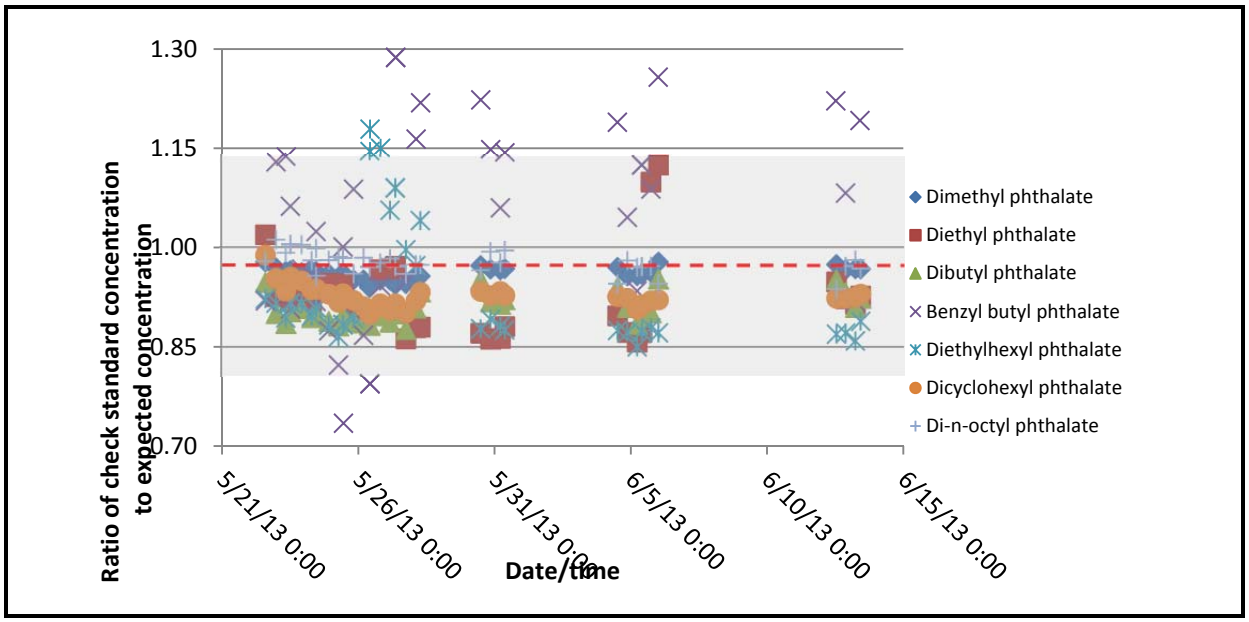
Method controls were within acceptable ranges for five of the seven analytes. As shown in ***Figure 3-10p,*** recoveries of benzyl butyl phthalate exceeded the 70%–130% recovery target range several times, but check standards (as seen in ***Figure 3-10q***) also ran high, so it is likely that the apparently high recoveries were an artifact of instrument performance. Recoveries of diethyl phthalate from method controls, on the other hand, were well below the target range, and we could not find any evidence of instrumental effects—the chromatograms were clean, and check standards were acceptable. The result is particularly difficult to explain because the isotopically labeled internal standard, diethyl phthalate-d_4, was added to the method control sample at the same time as the unlabeled spike and was subject to the same methodological influences.

Figure 3-10p. Dust Phthalates Method Controls, Control Chart



Notes: Red dashed line represents expected value. Gray area represents acceptance range.

Figure 3-10q. Dust Phthalate Continuing Calibration Check Standard, Control Chart



Notes: Red dashed line represents expected value. Gray area represents acceptance range.

Method precision was evaluated from duplicates as described above and is shown in ***Table 3-10q***.

Table 3-10q. Dust Phthalates Precision (Calculated as Relative Standard Deviation Eq. 3-1; n = 9 pairs)

Duplicate	Phthalate, %						
	Dimethyl	Diethyl	Dibutyl	Benzyl Butyl	Diethyl	Dicyclohexyl	Di-n-octyl
Injection	0.4	1.9	0.3	0.4	0.8	8.1	1.7†
Method	6.5	5.0	5.2	2.1	3.1	4.2 (74.2)*	n/a**

†Only two pairs gave measurable amounts
*Value in parentheses includes one outlier pair
**Only one measurable pair

Dust Phthalates Precision. Injection precision was excellent; method precision (extraction and injection of duplicate samples) was generally acceptable. Di-n-octyl phthalate (DNOP) data were disappointing, however, because of substantial interfering peaks. Di-isononyl phthalate (DINP) had originally been intended to be included in the method but is a mixture of a number of isomers that do not chromatographically resolve, resulting in poor peak shapes and uncertain quantitation. DNOP co-elutes with a portion of DINP, and DINP actually occurred more frequently in the samples, interfering with clean quantitation of DNOP.

Corrective Actions for Carbonyl and VOC Analyses

During carbonyls analysis, we experienced two episodes of failing check standards that were traced to failure of the injection system. In the first instance, the autosampler was replaced with a back-up unit and then recalibrated, which restored method performance. In the second instance, the analysis was moved to a backup HPLC system, which was then recalibrated and new method detection limits were determined for the new system. At other times, indications of check standard drift were corrected via instrument recalibration.

In the early stages of VOC analysis (prior to the start of study sample analysis), we observed that the retention time of naphthalene was much later than the retention time of the internal standard being used to quantitate it. Normal practice is to have the internal standard elute as closely in time as possible to the analyte. As a result, we incorporated naphthalene-d_8 into the method to serve as the internal standard for naphthalene, and redetermined method detection limits accordingly.

Microbiology (Endotoxin, Glucan, Allergens)

Dust and MicroPEM™ samples received by RTI's Microbiology laboratory were processed according to established protocols and methods specifically developed for CHATS during a pilot study. Samples were stored at optimal conditions determined during the pilot study until processed. Raw data for the seven biological components analyzed:

- endotoxin
- β-D-glucan (synonyms: "glucan", "1,3-β-diglucan", "β-glucans")
- Asp f1 (Aspergillus fumigatus allergen)
- Bla g1 (Cockroach Blatella germanica allergen)
- Der f1 (Dust mite Dermatophagoides farinae allergen)
- Der p1 (Dust mite Dermatophagoides pteronyssinus allergen
- Fel d1 (Cat dander allergen)

These data were generated by an automated plate reader according to preset data reduction parameters. Results obtained were subject to two levels of internal laboratory QC prior they were sent to the project QA manager.

We conducted initial data QC immediately following the completion of an assay. Each endotoxin and glucan assay had positive and negative controls, as well as an internal standard curve. Once an assay was completed, the originator of the data examined both controls to validate the assay and ensure that the correlation coefficient (R^2) of the linear regression line for the standard curve was within the acceptable range ($R^2 \geq 0.980$). All samples, standards, and

controls were assayed in duplicates, and the coefficient of variance (%CV) was calculated for each by the automated plate reader. Samples within the acceptable range (%CV ≤ 30%) were queued for data analysis. Remaining samples were analyzed but assigned a laboratory RQI value of 2. As part of the laboratory QC process, field blanks as well as lab duplicate samples were analyzed.

Following each allergen assay, controls were examined for validation and the internal standard curve was plotted on a log/linear scale and a linear regression curve fitted to the data. The R^2 values for the linear regression are checked to ensure that the values fit within an acceptable minimal value ($R^2 \geq 0.94$). The equation for the linear regression is then used to calculate allergen concentrations in each sample.

Once the initial quality checks were performed and the analyte concentrations calculated for each sample in the assay, the data were stored in the project folder on the shared network. A secondary reviewer in the Microbiology Laboratory (the group supervisor or someone else who was trained in performing the assays as indicated in the SOPs) conducted the data review. This secondary reviewer checked all aspects of the data analysis, including the standards, to ensure that calculations were performed correctly for each analyte being assayed. Following secondary QC, the data files were sent to the project QA manager for final approval. Any queries raised by the project QA manager were addressed by the originator as well as the secondary reviewer.

Table 3-10r provides a summary of the QC data.

Table 3-10r. Quality Control Data for the Standard Curves for Each Analyte and the Limits of Detection

Analyte	Number of Assays	Median R^2	Mean R^2	R^2 Standard Deviation	Limit of Detection
Endotoxin	24	0.999	0.9960833	0.0077174	0.005 EU/mL
1,3 –β-di-glucan	26	0.998	0.9937308	0.0083213	3.125 pg/mL
Asp f1	17	0.9594	0.9589353	0.0109327	0.08 ng/mL
Bla g1	17	0.9603	0.9595941	0.0051977	0.002 U/mL
Der f1	18	0.9577	0.9565056	0.0116261	0.49 ng/mL
Der p1	18	0.95165	0.9505278	0.0140511	0.49 ng/mL
Fel d1	19	0.9571	0.9605222	0.0114107	0.19 ng/mL

Laboratory blanks were always below detection limits, and spiked samples showed recovery greater than 73%, which is a reflection of the difficulty involved with analyzing a nonhomogeneous sample such as dust.

MicroPEM™ Filter Samples

Individual MicroPEM™ filters were gravimetrically and optically analyzed for PM_{10} mass and ETS, respectively. We preweighed and pre-optically analyzed all filters prior to sampler deployment. We performed gravimetric and optical analysis of collected samples on batches of at least 25 filters after equilibration for 24 hours at 23°C and 35% relative humidity. To ensure accuracy and precision of the gravimetric analysis, a standard weight of 100 mg and a reference filter were analyzed before every pre- and postweighing session (see ***Table 3-10s***). These standard weight and reference filter measurements were plotted and compared with 3-standard deviations to identify any extreme values after every weighing session. In addition, we performed at least one repeat measurement for one of the filters in the session and compared those measurements to identify any weight difference greater than 3 micrograms. ***Table 3-10t*** presents a summary of the findings. For ETS analysis, a standard filter was analyzed for the optical transmissivity at every pre- and postanalysis session (see ***Table 3-10u*** for the coefficients of variation by wavelength). We compared each measurement with previous measurements using 3-standard deviations to identify any outstanding values. The Baseline optical transmissivity was measured on each sampling filter for all seven wavelengths. Each wavelength transmissivity was compared with previous measurements from unused filters of the same manufacturing lot using 3-standard deviation. Only a few laboratory QC measures for PM_{10} mass or optical transmissivity showed values slightly outside the threshold ranges. Those QC samples were reanalyzed and compared with the previous data (for the standards and reference filters) or the initial measurement (for replicate analysis). The resulting values were within the acceptable ranges.

Table 3-10s. Summary of Quality Control Measures for Integrated PM Mass on MicroPEM™ Filters

QC Measures	Meas. Unit	Number of Measure-ment	Min.	25th Pctl	Median	75th Pctl	Max.	Mean	Std. Dev.	Coefficient of Variance (%)
Standard weight	mg	121	99.992	99.994	99.995	99.996	99.999	99.995	0.001	0.001
Reference filter weight1 (4/12/2012-7/23/2012)	mg	30	43.086	43.088	43.089	43.090	43.093	43.089	0.001	0.003
Reference filter weight2 (7/24/2012-4/24/2013)	mg	73	42.225	42.227	42.228	42.230	42.650	42.235	0.049	0.117
Reference filter weight3 (4/12/2013-5/31/2013)	mg	9	44.172	44.173	44.173	44.173	44.173	44.173	0.001	0.001

Table 3-10t. A Summary of Coefficient of Variance (%) of Replicate Analysis of PM Mass on MicroPEM™ Filters

No. Measurements	Mean	Standard Deviation	Minimum	25th Percentile	Median	75th Percentile	Maximum
57	0.0021	0.0019	0.0000	0.0008	0.0013	0.0031	0.0081

Table 3-10u. Coefficient of Variance (%) of Quality Assurance Measures for Optical Density of Each Light Source Wavelength Assessed for MicroPEM™ Filters

Wavelength (nm)	No. Meas.	940	660	620	587	565	460	430
Standard filter	41	5.1	5.1	4.7	5.1	5.2	6.4	6.0
Reference filter1 (Lot T03369)	39	4.4	7.4	8.2	8.3	8.5	8.5	8.3
Reference filter2 (Lot T30289)	12	8.9	10.1	10.6	10.7	10.9	11.5	11.9

We did not plan to deploy MicroPEM filter field blanks with any field QC samples because of space limitations in the shipping boxes. However, we handled returned, unused MicroPEM filters due to cancelled appointments in the same way the regular samples and shipped between the field and RTI were handled. This handling meets the requirements of typical field blanks. Therefore, those unused filters were analyzed for both mass and ETS as field blanks. ***Table 3-10v*** provides descriptive summaries of the mass and ETS data from field blank samples that had detectable measurements. Out of 37 samples, 23 samples had PM mass under the detection limit (1 µg), and most of the field blank sample data (29 out of 37) showed weight changes 3 µg or less. Seven samples had weight changes between 3 and 9 µg, and one sample had almost 29 µg. This field blank with large weight change was included in the same participant box with two other field blanks that were analyzed on the same dates and showed much smaller weight changes (less than 6 µg), indicating this particular sample had a high level of contamination. ETS values of 35 field blank samples were under the lower limit of detection. The detectable measurements of two samples were lower than 3 µg.

Table 3-10v. Descriptive Statistics for PM_{10} Mass and ETS of MicroPEM Filter Field Blanks

Analyte	No. Blanks	No. Blanks Above Detection Limit	Mean Blank	Std. Dev.	Min. Blank	25th Pctl.	Median Blank	75th Pctl.	Max. Blank
PM_{10} mass	37	14	5.63	6.88	2.32	2.65	3.38	5.22	28.88
ETS	37	2	1.97	0.86	1.36	1.36	1.97	2.58	2.58

Passive NO_2

At the beginning of each day that passive NO_2 samples were analyzed, our analyst performed a complete calibration of the ion chromatograph (IC) for nitrite ion. The analyst compared the regression parameters for the calibration curve to those obtained in the past. If significant differences were observed, the analysis was stopped, the problem identified and corrected, and the IC recalibrated. Before any CHATS samples were analyzed, two QC samples (RTI-prepared) and a QA sample (prepared from NIST-traceable solutions purchased from a commercial supplier) were analyzed. The analyst calculated the nitrite ion recoveries based on the known nitrite concentrations of the QA/QC samples. The recoveries had to be within 90% and 110% to proceed with the analysis of the CHATS samples. During the analysis session, a spiked pad extract, calibration check standard (QC sample), and a QA sample were analyzed at least every 20 samples (or daily). Recoveries of the spiked extracts are plotted in ***Figure 3-10r***. Recoveries for the QA/QC samples are plotted in ***Figures 3-10s*** to ***3-10w.*** Additionally, at least 5% of the sample extracts are analyzed in duplicate for the determination of analytical precision. Duplicates are plotted in ***Figure 3-10x***. Method Blanks are presented in ***Figure 3-10y***. Laboratory Control Samples, all within the 90–110% recovery as required, are presented in ***Figure 3-10z***.

Figure 3-10r. Spiked Extract Recoveries

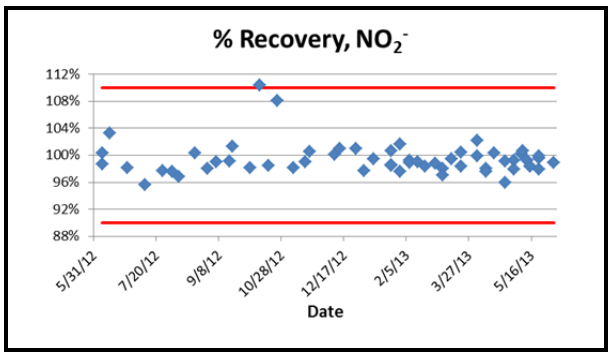


Figure 3-10s. QA-CPI_Low for NO_2

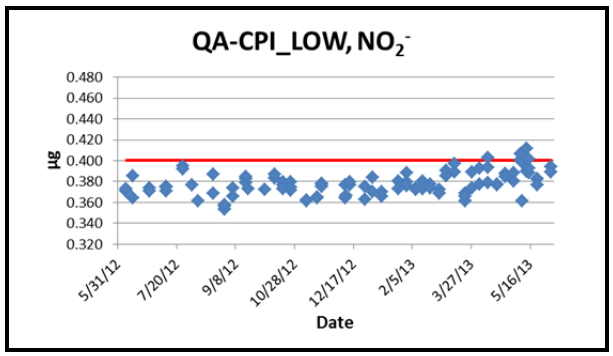


Figure 3-10t. QA-CPI_Med-Hi for NO_2

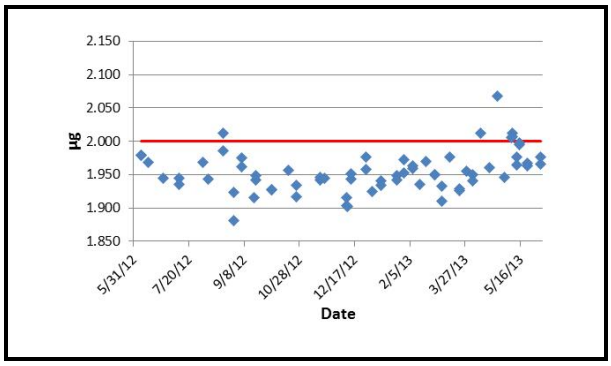


Figure 3-10u. QC-HIGH for NO_2

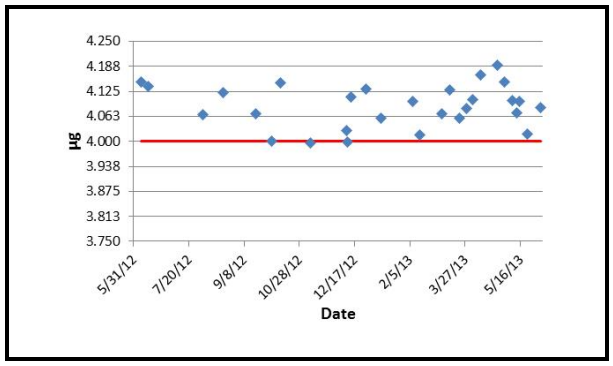


Figure 3-10v. QC-Low for NO_2

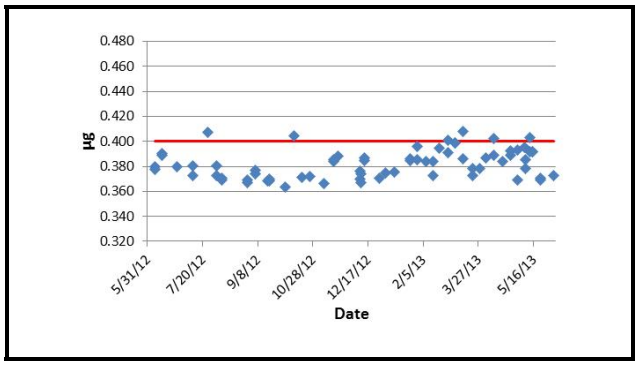


Figure 3-10w. QC-MED for NO_2

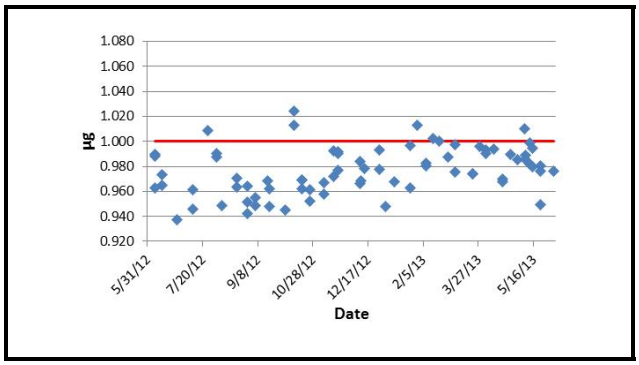


Figure 3-10x. Duplicate Sample Analysis

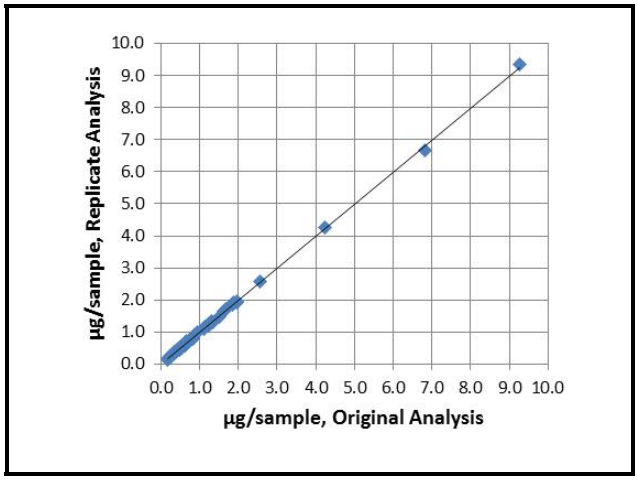


Figure 3-10y. Method Blanks

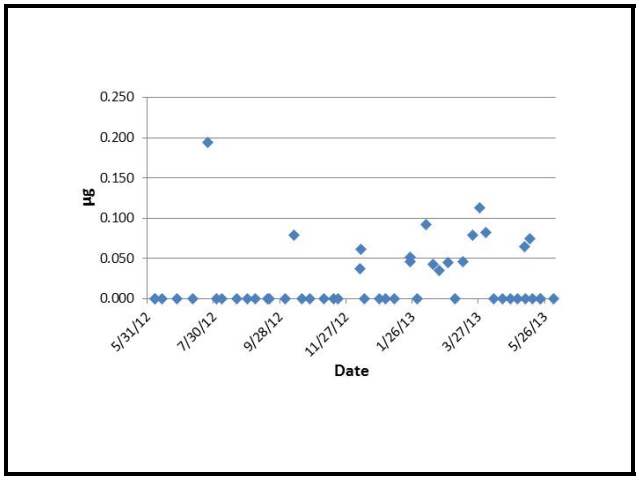
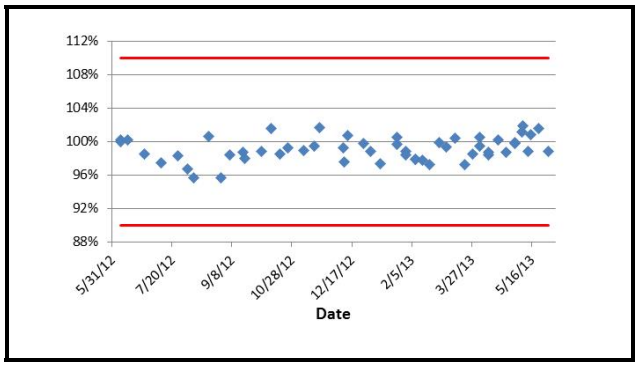


Figure 3-10z. % Recovery NO_2 Lab Control Samples



Real-Time Data

MicroPEM real-time data: Individual data files were processed with RTI's MicroPEM docking station software and validated with the MicroPEM file validation program for data

collection completeness and MicroPEM performance. We assigned separate RQIs to each MicroPEM QC parameter (all unit types) based on the acceptable range identified from previous testing. We combined this information to assign MicroPEM filter collection validity, and later combined it with the MicroPEM filter analytical validity to create the final MicroPEM mass and ETS validity. For personal units, real-time nephelometer and accelerometer data were processed to calculate average PM_{10} exposure level (µg/m^3) and percent of time the participant wore the unit during the sampling period (e.g., wearing compliance) , respectively. About 28% (227/805) of the MicroPEM files that were either improperly started by field interviewers or encountered hardware issues were irregularly formatted and sometimes had inaccurate information recorded for one or more QC parameters. Irregularly formatted files had random occurrences of data with a single time stamp on two separate lines of the output file. Inaccurate pressure sensor (used to calculate flow) or battery voltage QC parameters were also sometimes recorded. The validation program could not validate these files, and they had to be manually examined by cross-comparison with the main dataset that included all information collected during field activities for data collection completeness. We compared analytical mass data with the overall sampling duration recorded in the file to assess if the MicroPEM properly operated for the entire period. If we suspected that any MicroPEM had run at a lower flow rate than the acceptable range (within ±10% of 0.5 liter per minute) for more than 20% of operating time, we assigned a laboratory RQI = 2 value, and subsequently, the filter sample became invalid.

Table 3-10w shows the summary statistics of averaged real-time personal PM_{10} exposures from data files of valid measurements. The reasons for invalid MicroPEMs are described in ***Section 3.4***. ***Figure 3-10aa*** shows the distribution of averaged real-time personal PM_{10} exposures. About 25% of children among the participants, whose MicroPEM PM_{10} measurements are valid, were exposed to 50 µg/m^3 or greater, which is the World Health Organization guideline for 24-hour mean.

Table 3-10w. Descriptive Statistics for Averaged Real-Time PM_{10} Exposures Measured by Personal MicroPEMs

Measure-ment	Sampling Location	Study Phase	Meas. Unit	No. files	Mean	Std. Dev.	Min.	25th Pctl.	Median	75th Pctl.	Max.
PM_{10}	Personal	Baseline	µg/m3	91	38.4	28.2	4.6	18.5	28.7	52.1	128.7
PM_{10}	Personal	Follow-up	µg/m3	69	37.7	23.3	7.2	17.8	30.5	52.0	122.8

HOBO real-time data: Individual data files were examined for the data collection completeness and exceptional values. We assigned separate laboratory RQIs to each temperature and relative humidity data item.

Figure 3-10aa. Distribution of Averaged Real-Time PM_{10} Concentrations Measured by Personal MicroPEM

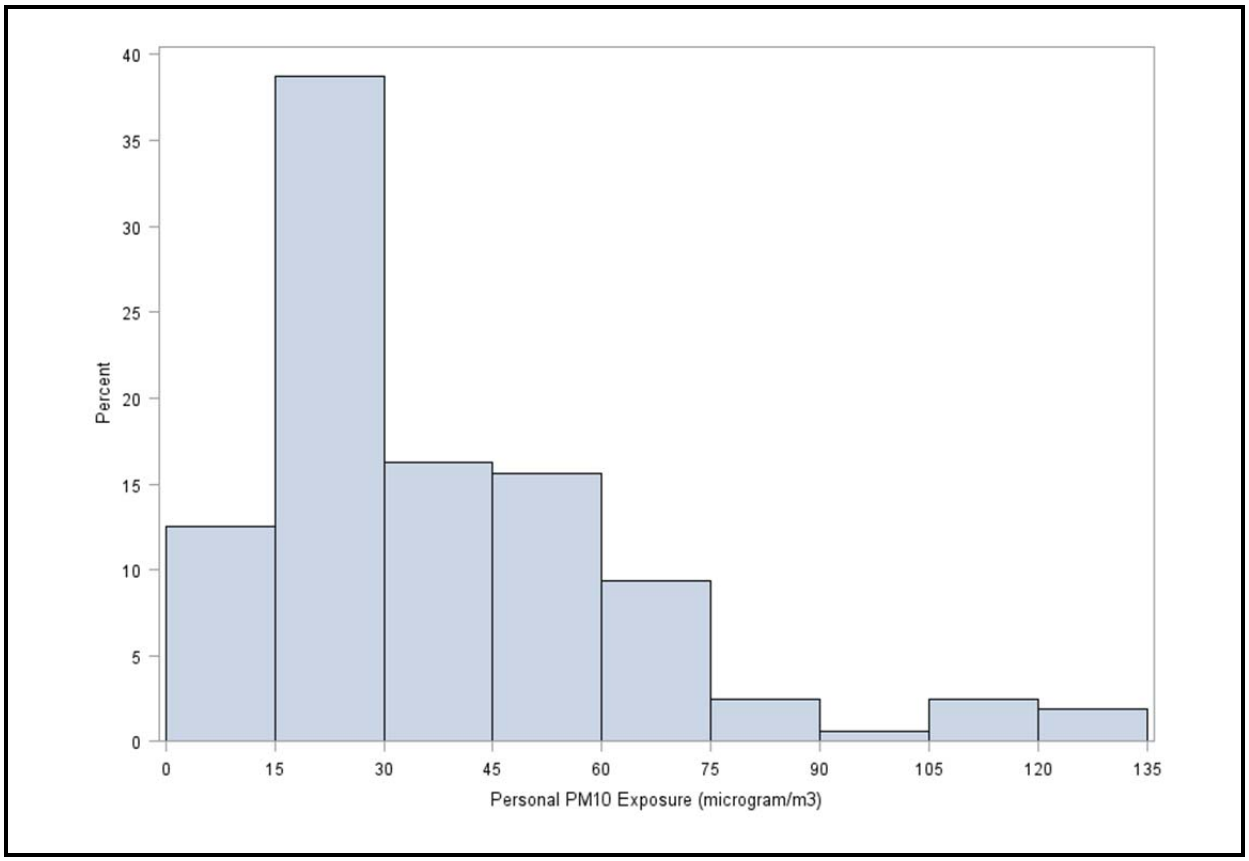


Table 3-10x provides descriptive statistics of averaged real-time measurements of temperature and humidity measured indoors. The distributions of temperature and relative humidity were not much different between two study phases.

Table 3-10x. Descriptive Statistics for Averaged Real-Time Indoor Temperature and Relative Humidity from HOBO

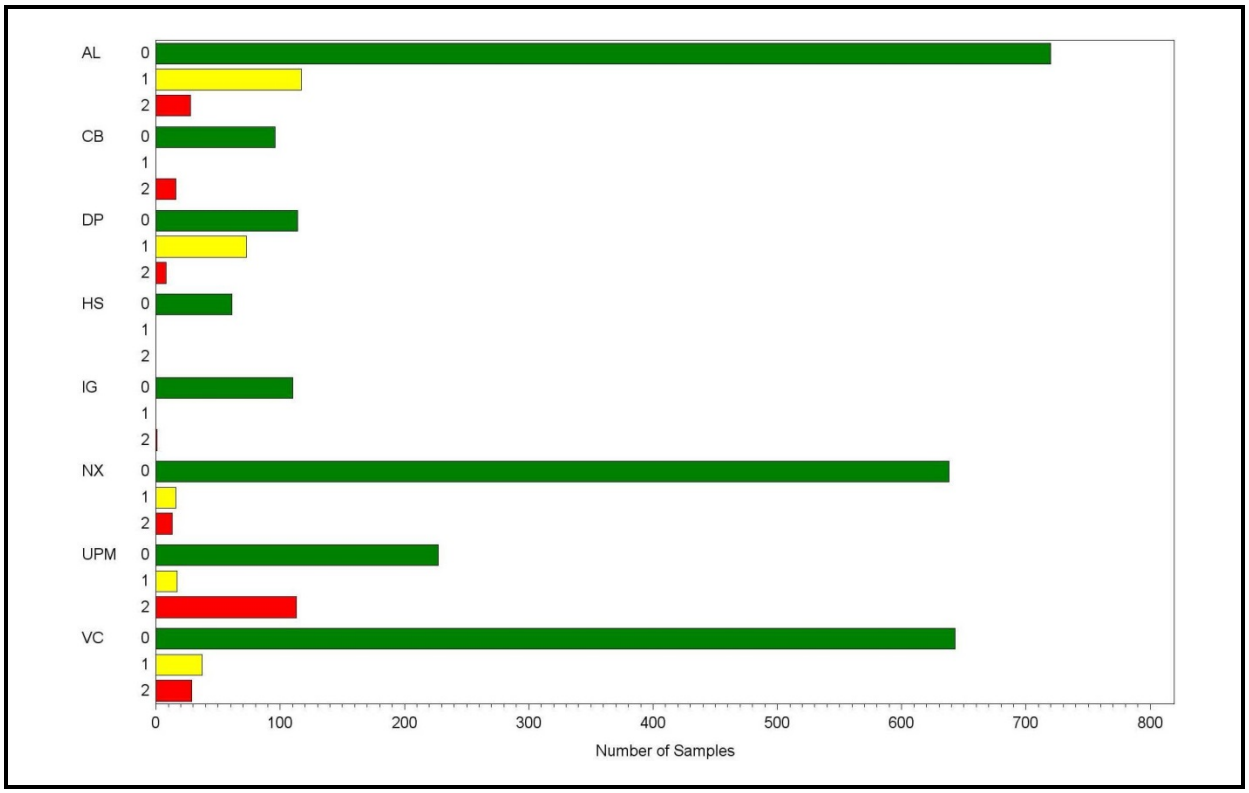
Measurement	Sampling Location	Study Phase	Meas. Unit	No. files	Mean	Std. Dev.	Min.	25th Pctl.	Median	75th Pctl.	Max.
Temperature	Indoor	Baseline	°F	168	74.4	3.5	67.0	71.9	74.3	76.0	89.4
	Indoor	Follow-up	°F	152	72.9	2.9	63.2	71.1	73.2	74.9	79.7
Relative Humidity	Indoor	Baseline	%	168	53.6	7.0	26.6	49.2	52.5	57.7	76.8
	Indoor	Follow-up	%	152	54.8	7.3	35.7	49.5	55.0	59.8	73.0

3.10.3 Record Quality Indicators

The uncertainty of each laboratory measurement is categorized through the assignment of an RQI that captures both the integrity of the collected sample/specimen and the quality of the analytical measurement. Two variables were created for this purpose: FIELD_RQI to contain the quality information for the sample collection and shipment processes and ARQI to contain the complementary information for the sample workup and analysis processes. As described in ***Section 2.8.3***, values of 0, 1, or 2 were assigned to each variable to indicate useable, somewhat questionable, or unusable data records, respectively. Due to differences in both sample collection and analysis procedures among sample types, field or laboratory RQIs were available only for selected data modules.

The distributions of these RQIs, or quality 'flags', for the collection and shipment processes, shown in ***Figure 3-10bb***, illustrate that most of the collected samples were of high integrity. Problems with ambient aldehyde, dust phthalate, ambient NO_2, and ambient VOC collections were typically related to physical damage to the passive sampler, itself, or holding times in excessive of analysis SOP limits.

Figure 3-10bb. Distribution of Field Record Quality Indicators by Sample Type

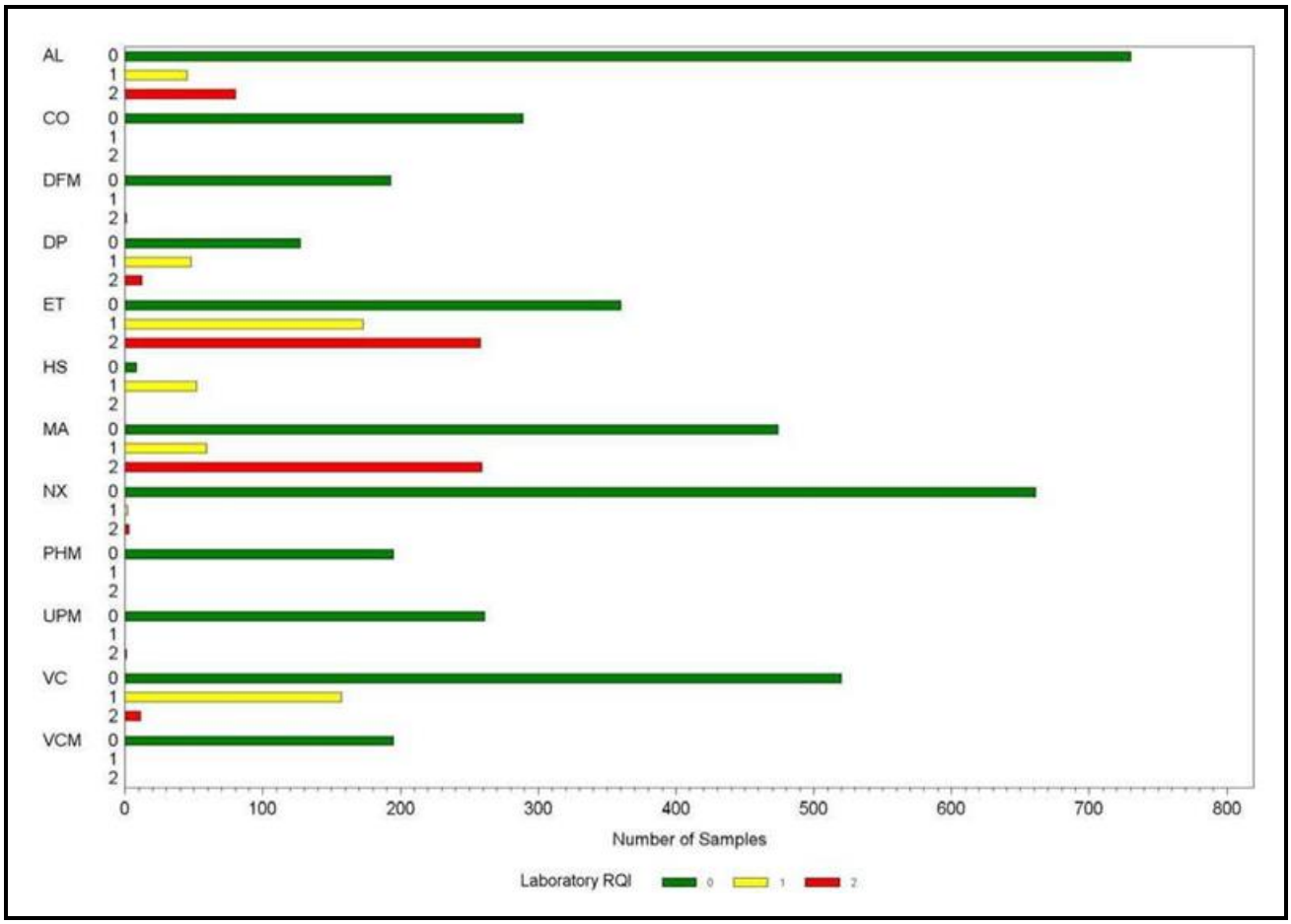


Abbreviations: AL = aldehyde badge, CB = blood clinical, DP = dust phthalates, HS = H_2S diffusive tube, IG = blood IgEs, NX = NO_2 badge, VC = VOC badge.

RQIs that laboratory managers assigned to individual analyte results are presented in ***Figure 3-10cc*** as the median value across all analytes for a given data module. In general,

median RQIs were zero, with aldehydes, ETS, and mass as notable exceptions. Several aldehydes presented analytical problems and there were performance difficulties with the MicroPEM samplers in the early phases of the study.

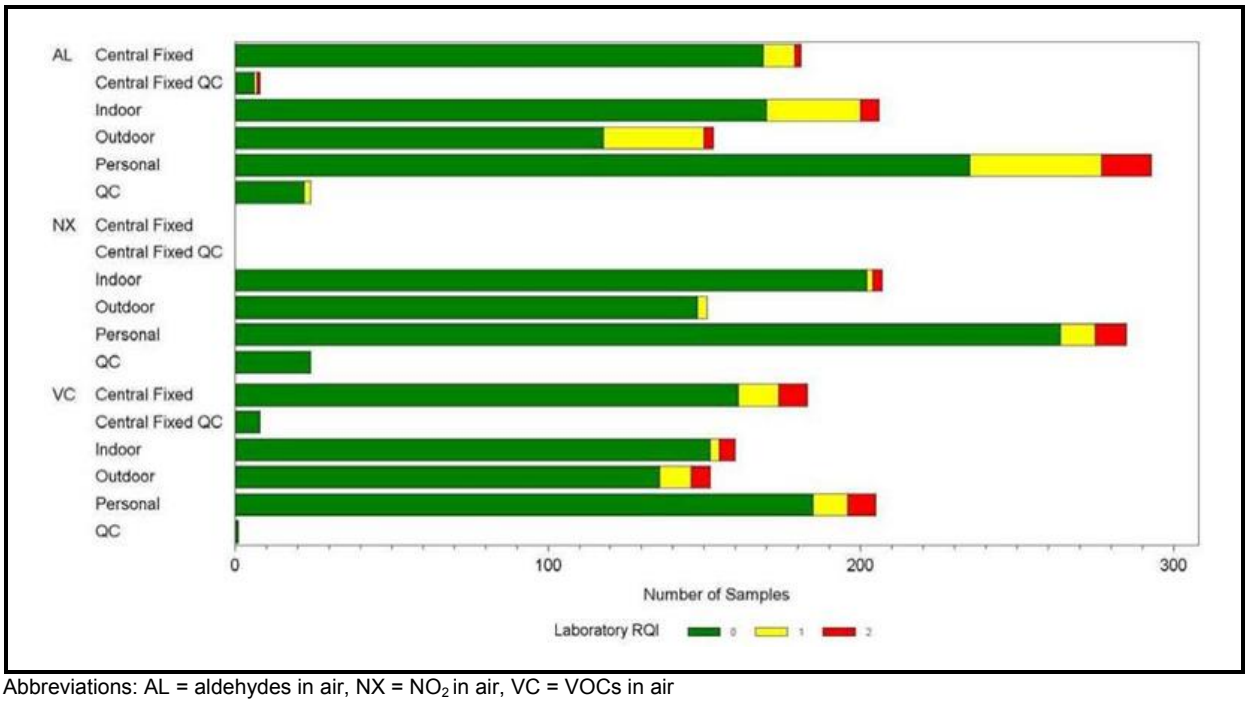
Figure 3-10cc. Distribution of Median Laboratory Record Quality Indicators by Sample Type



Abbreviations: AL = aldehydes in air, CO = urine cotinine, DFM = vacuum dust microbiologicals, DP = Phthalates in dust, ET = MicroPEM ETS, HS = air H_2S, MA = MicroPEM PM_{10} mass, NX = air NO_2, PHM = urine phthalate metabolites, UPM = Microbiologicals from MicroPEM filters, VC = VOCs in air, VCM = urine VOC metabolites

Our assessment of differences in sampler/sample integrity as a function of collection location (***Figure 3-10dd***) suggests a slight increase in unacceptable samples (RQI = 2) for samplers from personal platforms is likely attributable to the increased risk of physical damage to the sampler when an individual wore it.

Figure 3-10dd. Effect of Sample Collection Location on Field Record Quality Indicators by Sample Type



Abbreviations: AL = aldehydes in air, NX = NO_2 in air, VC = VOCs in air

RQIs for individual analytes, within a data module, are shown in ***Figures 3-10ee*** through ***3-10pp***. An RQI of 0 indicates that everything was acceptable, from the field to the reporting of data. Analytes that are truly "not detected" in an assay shown to perform properly, that is, the "not detected" was not a function of assay performance, were also assigned an RQI of 0. In general, RQIs of 1 were reported for successful measurements except for those that were below the lowest calibration point; this does not indicate a problem with the analysis, but rather reflects the uncertainty in a value extrapolated outside the range of the calibration curve. RQIs of 2 were typically reported when the QC check standard was outside the performance range specified in the relevant analysis SOP. This latter case was particularly evident for air aldehydes (***Figure 3-10ee***), dust phthalates (***Figure 3-10ff***), and air VOCs (***Figure 3-10gg***). Difficulties with endotoxin and glucan analyses (***Figures 3-10hh*** and ***3-10ii***) were caused by a lack of analytical precision for these two species. The high proportion of RQI = 0 for the allergens from the PM filters, which included many nondetects, reflects that a nondetect from a well-performing method was a reliable measurement. The appreciable proportions of RQIs equal to 1 or 2 for the MicroPEM results (***Figures 3-10jj*** and ***3-10kk***) are largely attributable to hardware problems with the sampling devices that prevented accurate computation of the volume of air sampled. All results for trans, trans-Muconic acid (***Figure 3-10ll***) were censored (RQI = 2) because of unresolvable chromatographic problems with this analyte. ***Figure 3-10mm*** shows that most of the H_2S measurements were detected but below the lowest calibration point. Urinary cotinine presented no difficulties (***Figure 3-10nn***). ***Figure 3-10oo*** shows that nearly all of the NO_2 analyses produce an RQI of 0. The RQIs for

urinary phthalate metabolites ***(Figure 3-10pp)*** are, on average, quite good. In most cases, RQI = 1 resulted from the measured value falling below the lower limit of quantitation.

Figure 3-10ee. Distribution of Laboratory Record Quality Indicators by Analyte for Air Aldehydes

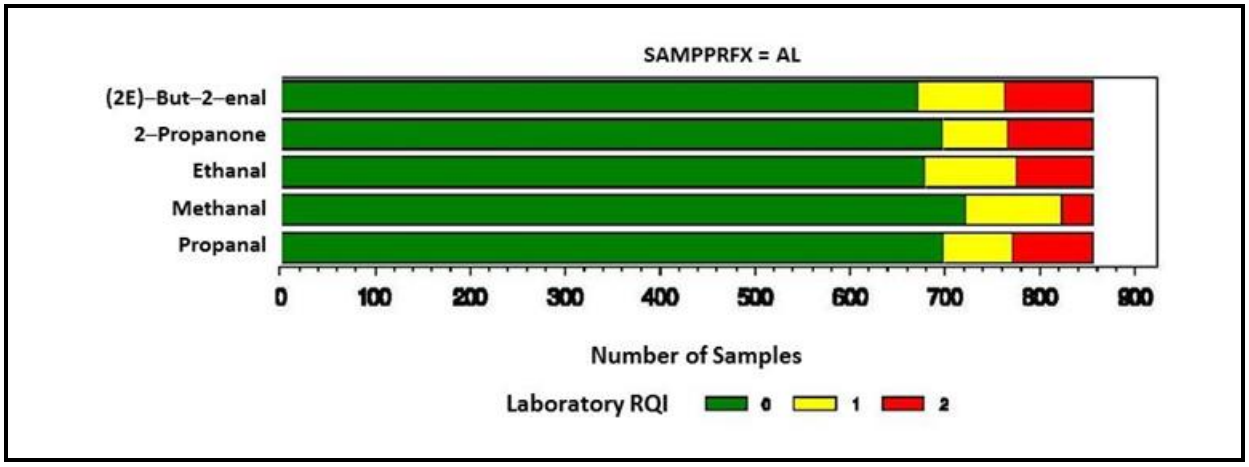


Figure 3-10ff. Distribution of Laboratory Record Quality Indicators by Analyte for Dust Phthalates

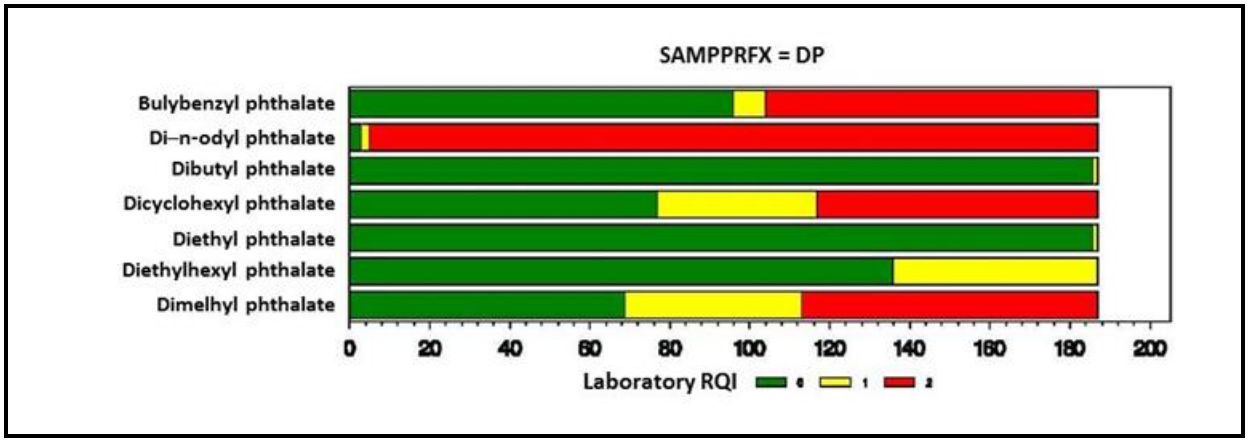


Figure 3-10gg. Distribution of Laboratory Record Quality Indicators by Analyte for Air VOCs

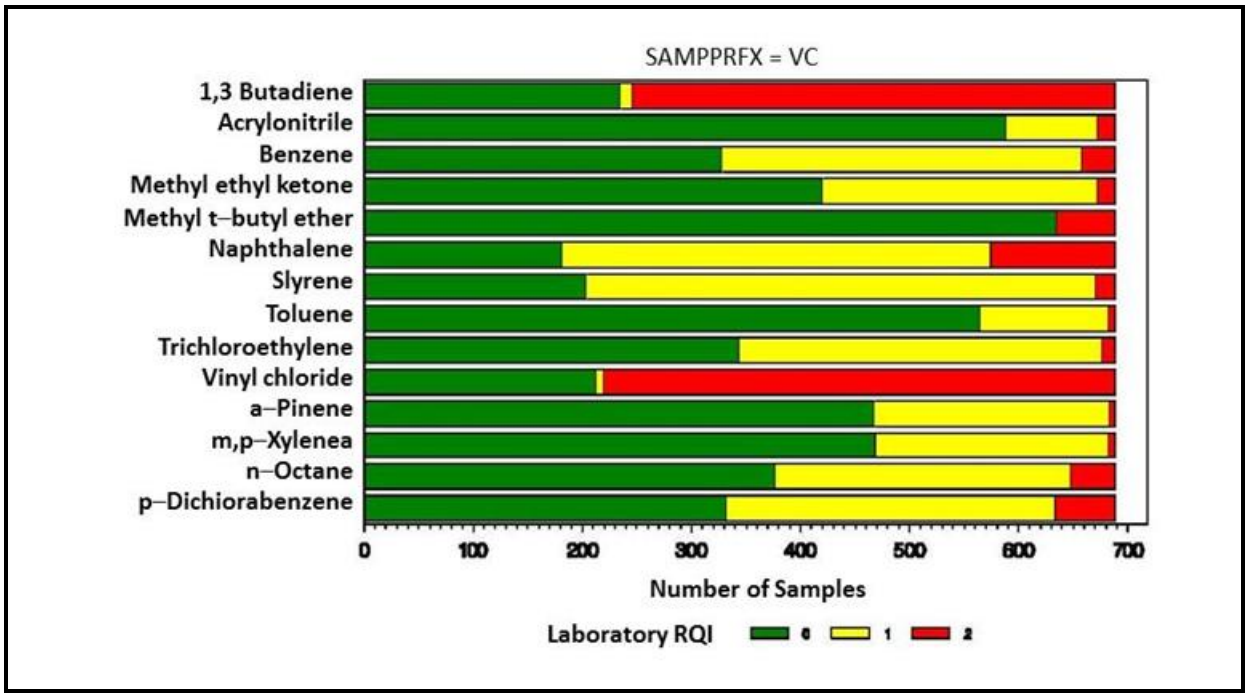


Figure 3-10hh. Distribution of Laboratory Record Quality Indicators by Analyte for Dust Microbiologicals

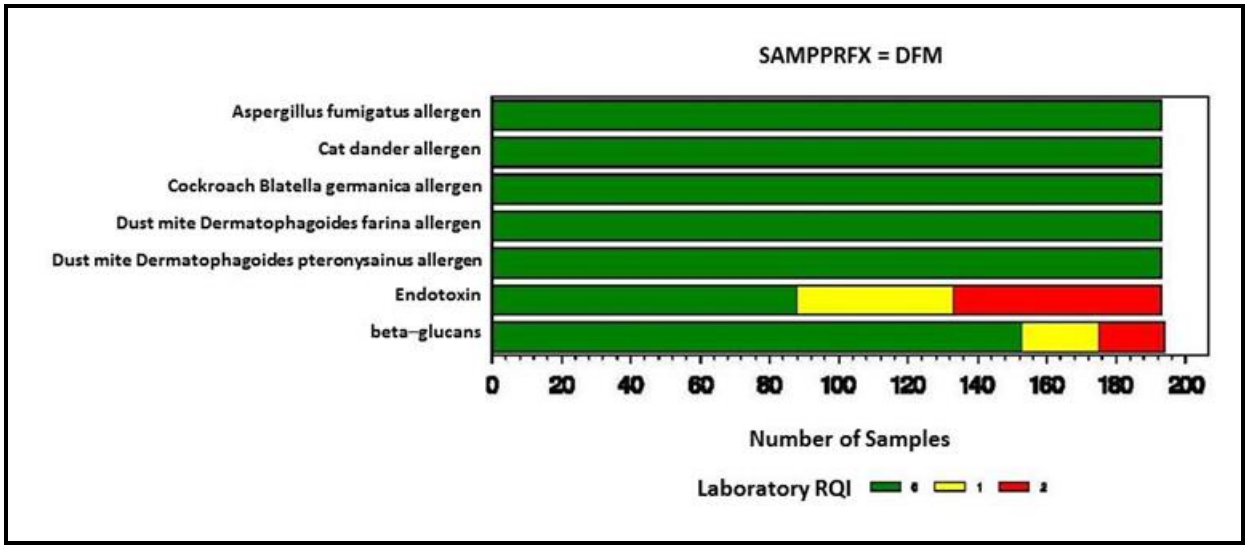


Figure 3-10ii. Distribution of Laboratory Record Quality Indicators by Analyte for Air µPEM Dust Microbiologicals

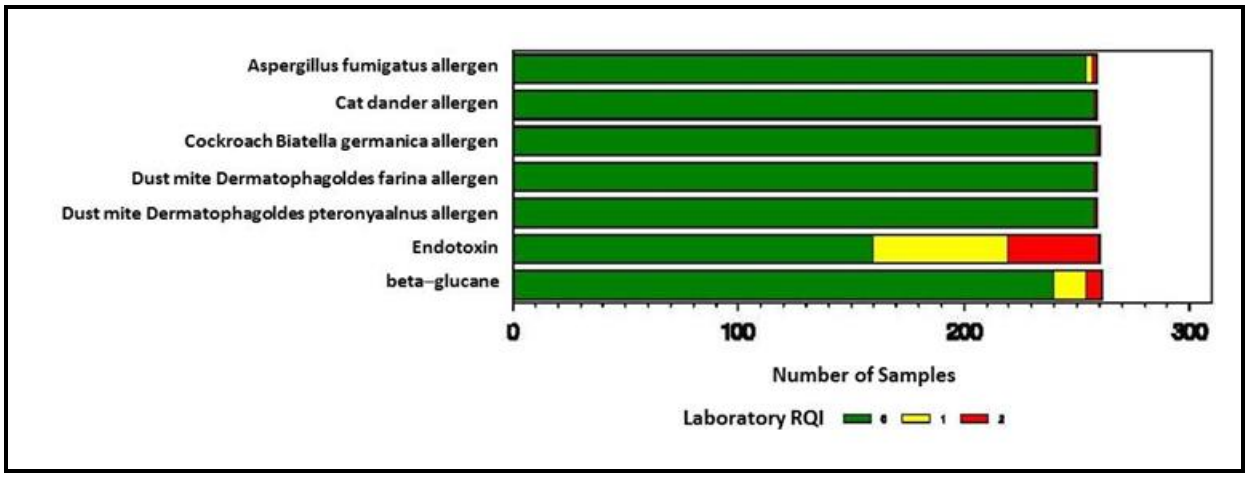


Figure 3-10jj. Distribution of Laboratory Record Quality Indicators for Air µPEM Environmental Tobacco Smoke

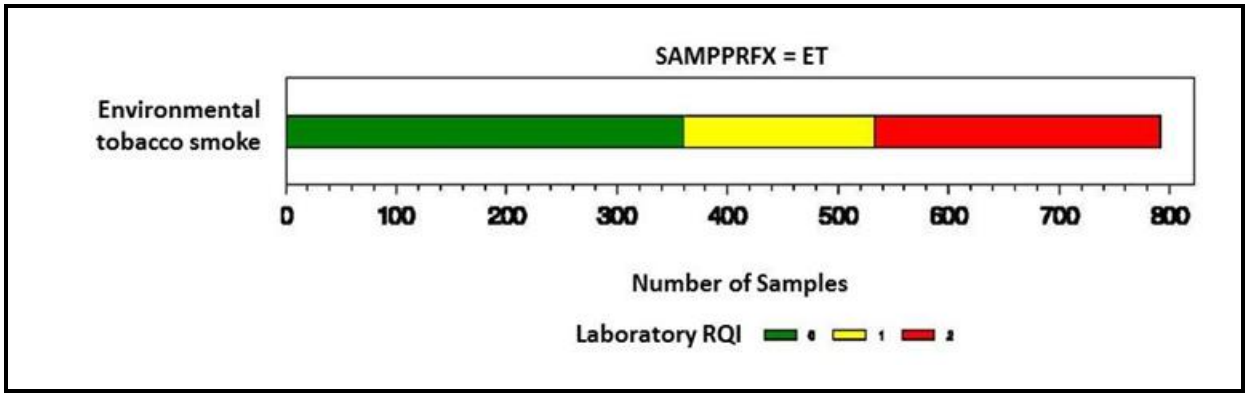


Figure 3-10kk. Distribution of Laboratory Record Quality Indicators for Air µPEM Mass

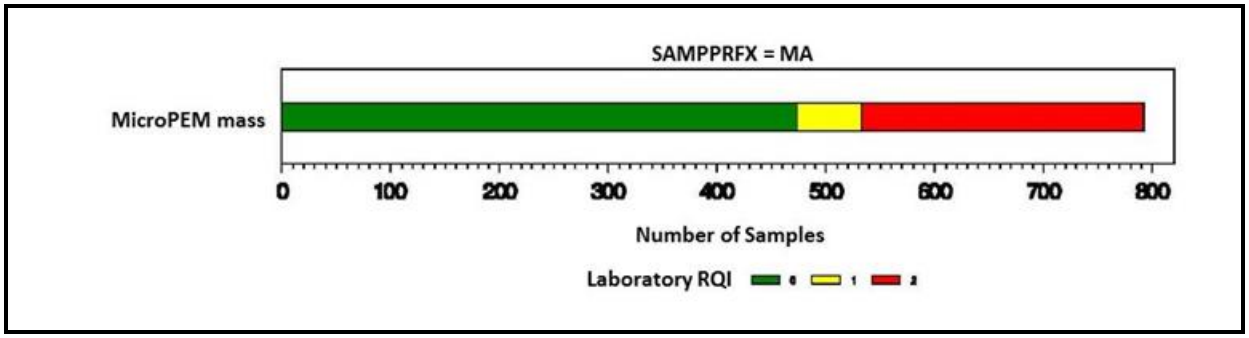


Figure 3-10ll. Distribution of Laboratory Record Quality I

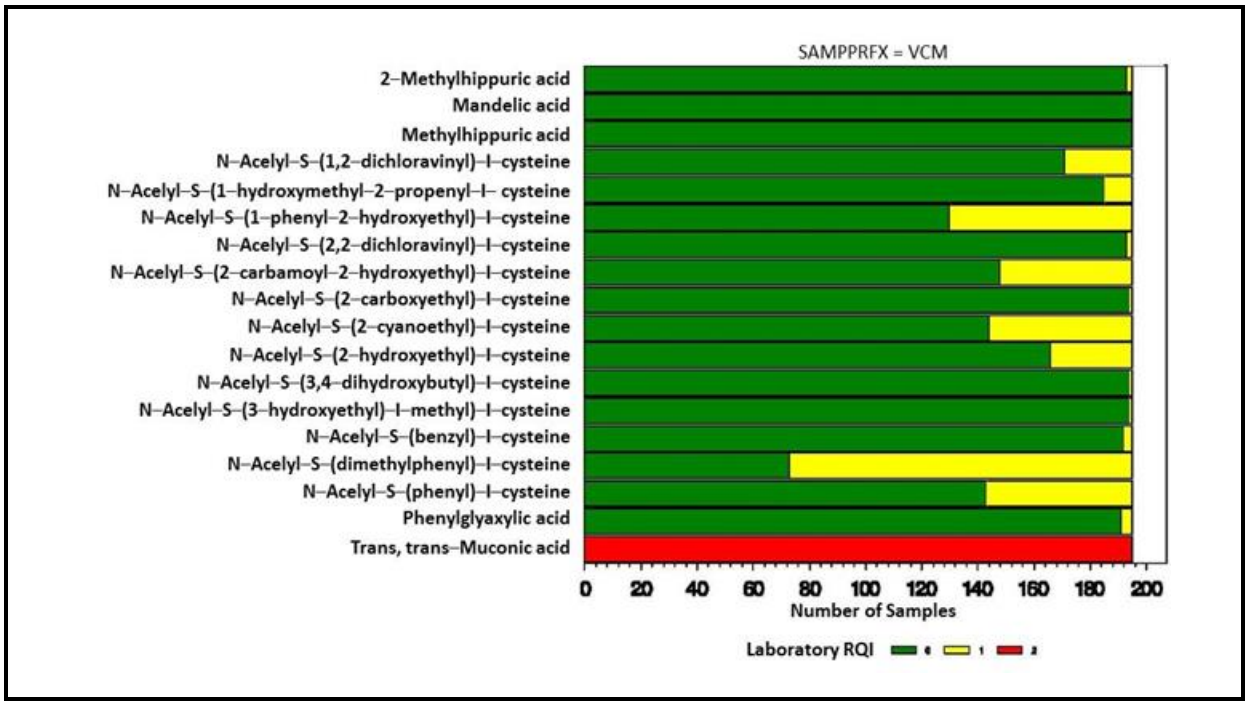


Figure 3-10mm. Distribution of Laboratory Record Quality Indicators for Air Hydrogen Sulfide

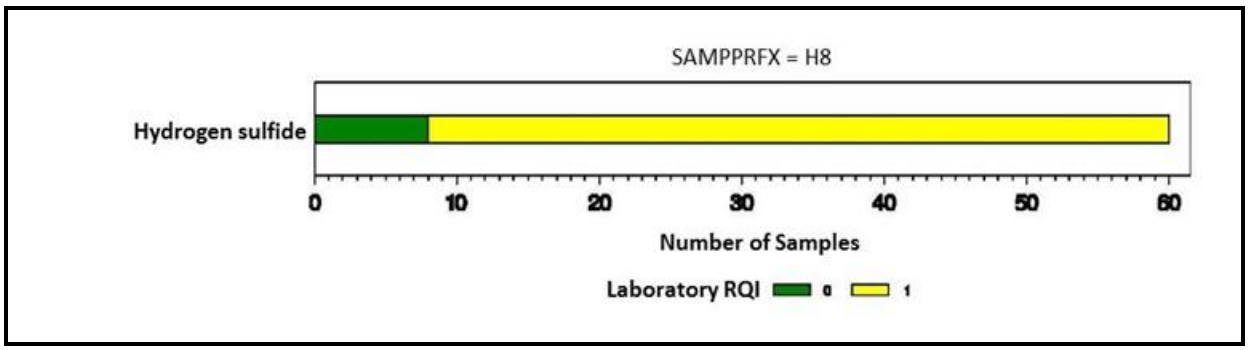


Figure 3-10nn. Distribution of Laboratory Record Quality Indicators for Urinary Cotinine

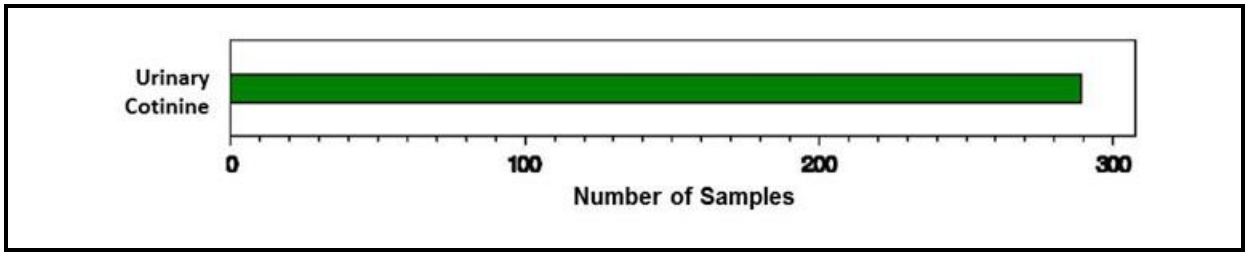


Figure 3-10oo. Distribution of Laboratory Record Quality Indicators for Air Nitrogen Dioxide

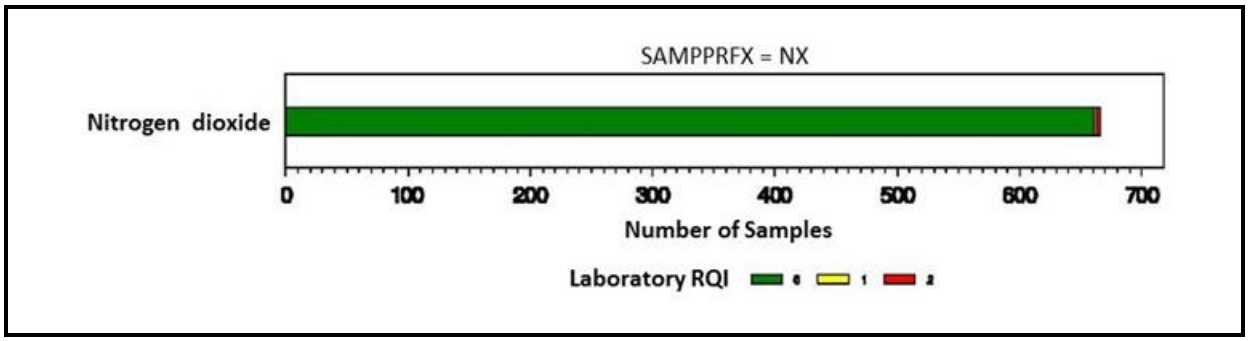
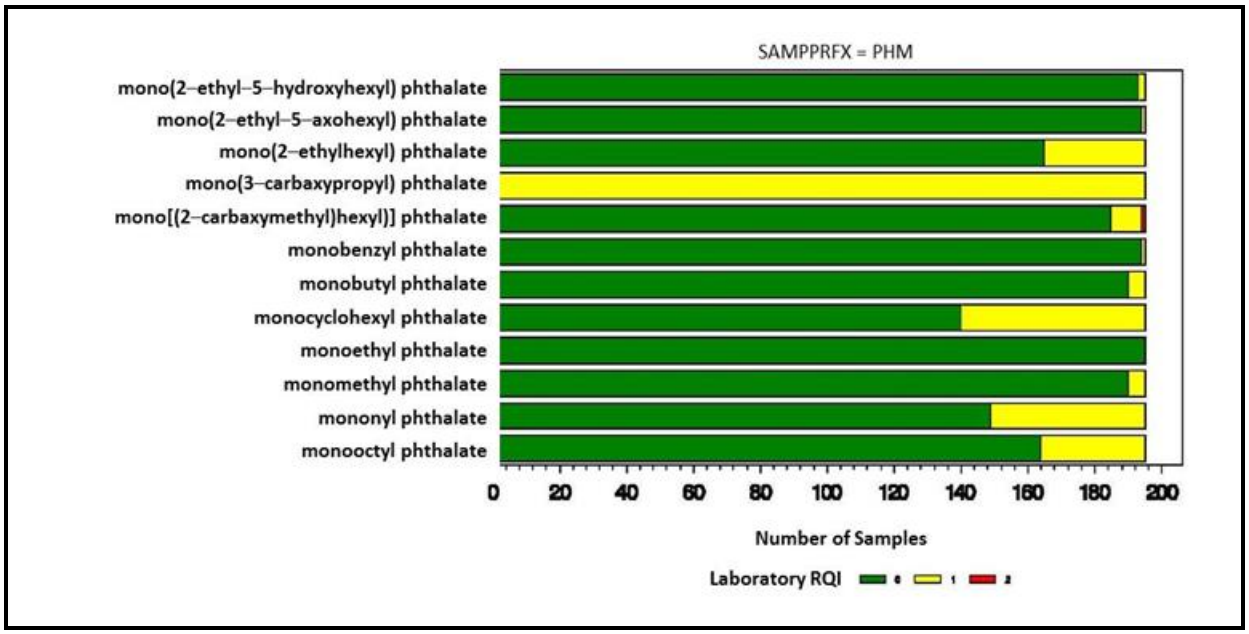


Figure 3-10pp. Distribution of Laboratory Record Quality Indicators by Analyte for Urinary Phthalate Metabolites



3.11 Laboratory Findings

No results shown here were corrected for field blanks because there was not a 1:1 correspondence to field blanks and each extraction batch. All carbonyl results were corrected for the measures from the unexposed section of the badge (that is, each sample had its own blank). All VOC badge data were corrected for the method blank (extraction and processing of an unexposed badge at the time of sample extraction); a method blank was created for each VOC badge extraction set. ***Tables 3-11a*** through ***3-11g*** present summary statistics and percent of samples with measurable results for carbonyls in air, phthalates in dust, environmental tobacco smoke on particles, mass of particles, NO_2 in air, H_2S in air, and VOCs in air,

respectively. ***Tables 3-11h*** through ***3-11l*** present similar summaries for the various biospecimen results. ***Tables 3-11m*** and ***3-11n*** present microbiological findings for the MicroPEM filters and dust. For all tables, the percent of measurable samples in each condition was computed from the ratio of measurable results to all results, exclusive of cases where FINAL_RQI = 2. Descriptive statistics were computed using only values above the laboratory-reported method detection limit.

Table 3-11a. Distribution of Laboratory Measurements for Environmental Samples: Carbonyls in Air

Chemical Analyte	Sampling Location	Study Phase	Meas. Unit	N	Mean Meas.	SD	Min. Meas.	25th Pctl.	Med. Meas.	75th Pctl.	Max. Meas.	% Meas.
Crotonaldehyde	Central Fixed	Baseline	micrograms/m3	8	9.40	1.24	7.51	8.41	9.68	10.32	10.88	10
	Indoor	Baseline	micrograms/m3	5	5.12	2.46	3.06	3.26	4.34	5.93	9.03	10
	Outdoor	Baseline	micrograms/m3	2	1.22	0.87	0.61	0.61	1.22	1.83	1.83	6
	Personal	Baseline	micrograms/m3	19	2.83	2.31	0.34	0.88	1.66	4.90	7.22	17
	Indoor	Follow-up	micrograms/m3	17	3.14	3.88	0.28	0.67	0.89	3.85	11.30	13
	Outdoor	Follow-up	micrograms/m3	7	0.93	0.85	0.16	0.65	0.73	0.77	2.79	7
	Personal	Follow-up	micrograms/m3	11	2.88	3.63	0.29	0.64	1.18	4.62	12.53	8
Acetone	Central Fixed	Baseline	micrograms/m3	2	9.66	1.63	8.51	8.51	9.66	10.81	10.81	2
	Indoor	Baseline	micrograms/m3	8	13.90	27.15	0.78	1.19	4.35	9.45	80.45	16
	Outdoor	Baseline	micrograms/m3	1	0.91	—	0.91	0.91	0.91	0.91	0.91	3
	Personal	Baseline	micrograms/m3	15	3.82	5.67	0.61	1.02	1.72	2.93	22.47	13
	Central Fixed	Follow-up	micrograms/m3	2	21.11	25.18	3.31	3.31	21.11	38.91	38.91	3
	Indoor	Follow-up	micrograms/m3	34	2.96	6.14	0.11	0.36	0.65	2.22	26.81	27
	Outdoor	Follow-up	micrograms/m3	11	1.26	1.55	0.15	0.24	0.54	2.13	5.20	11
	Personal	Follow-up	micrograms/m3	35	2.30	3.36	0.18	0.40	0.87	2.68	14.43	27
Acetaldehyde	Central Fixed	Baseline	micrograms/m3	42	4.33	7.43	1.14	1.84	2.28	2.80	43.84	48
	Indoor	Baseline	micrograms/m3	53	6.96	5.46	0.56	3.20	5.25	8.21	26.13	100
	Outdoor	Baseline	micrograms/m3	11	0.45	0.18	0.22	0.30	0.40	0.66	0.78	33
	Personal	Baseline	micrograms/m3	116	6.73	4.80	1.01	3.67	5.47	8.40	27.29	99
	Central Fixed	Follow-up	micrograms/m3	53	8.36	44.71	0.53	1.66	2.12	2.67	327.57	70
	Indoor	Follow-up	micrograms/m3	125	5.426	4.734	0.372	2.362	3.911	6.785	31.150	99
	Outdoor	Follow-up	micrograms/m3	61	0.399	0.238	0.056	0.236	0.343	0.501	1.162	60
	Personal	Follow-up	micrograms/m3	132	5.660	3.898	0.150	2.846	4.506	7.707	20.571	100

(continued)

Table 3-11a. Distribution of Laboratory Measurements for Environmental Samples: Carbonyls in Air (continued)

Chemical Analyte	Sampling Location	Study Phase	Meas. Unit	N	Mean Meas.	SD	Min. Meas.	25th Pctl.	Med. Meas.	75th Pctl.	Max. Meas.	% Meas.
Formaldehyde	Central Fixed	Baseline	micrograms/m3	87	7.02	4.09	2.13	4.99	6.33	7.93	37.15	95
	Indoor	Baseline	micrograms/m3	67	28.24	10.56	5.36	22.62	27.95	34.96	60.76	99
	Outdoor	Baseline	micrograms/m3	42	2.77	2.98	0.55	1.93	2.47	2.75	20.96	100
	Personal	Baseline	micrograms/m3	137	24.54	10.50	3.52	18.06	23.47	27.90	72.33	100
	Central Fixed	Follow-up	micrograms/m3	66	10.27	48.54	1.67	3.20	4.13	4.86	398.36	87
	Indoor	Follow-up	micrograms/m3	125	30.02	16.26	6.11	19.02	25.91	35.53	100.52	99
	Outdoor	Follow-up	micrograms/m3	101	2.10	0.62	0.23	1.75	2.03	2.50	3.56	99
	Personal	Follow-up	micrograms/m3	131	24.93	14.00	0.79	16.58	22.03	28.94	86.49	99
Propanal	Central Fixed	Baseline	micrograms/m3	78	13.64	6.04	2.07	9.66	13.51	16.23	37.24	88
	Indoor	Baseline	micrograms/m3	48	2.42	1.17	0.70	1.65	2.06	3.30	6.03	94
	Outdoor	Baseline	micrograms/m3	32	5.87	2.25	1.08	4.23	5.50	7.50	10.22	100
	Personal	Baseline	micrograms/m3	111	2.01	1.10	0.30	1.30	1.70	2.47	7.26	96
	Central Fixed	Follow-up	micrograms/m3	54	41.32	221.08	2.28	6.22	9.30	14.47	1634.76	71
	Indoor	Follow-up	micrograms/m3	114	2.20	1.71	0.21	0.97	1.90	2.86	9.54	90
	Outdoor	Follow-up	micrograms/m3	88	5.61	3.11	0.63	2.95	6.32	7.85	12.39	86
	Personal	Follow-up	micrograms/m3	116	1.96	1.30	0.33	1.02	1.72	2.51	7.56	88

Note: Descriptive statistics were computed using only values above the laboratory-reported method detection limit. “% Meas.” is the ratio of measurable results to all results with RQI<2.

Table 3-11b. Distribution of Laboratory Measurements for Environmental Samples: Phthalates in Dust

Chemical Analyte	Sampling Location	Study Phase	Meas. Unit	N	Mean Meas.	SD	Min. Meas.	25th Pctl.	Median Meas.	75th Pctl.	Max. Meas.	% Meas.
Butylbenzyl phthalate	Indoor	Baseline	ng/g	64	59445.90	122210.7	2040.95	8994.64	16493.82	41886.49	574752.2	100
	Indoor	Follow-up	ng/g	40	52753.26	135526.9	1858.91	8198.38	16088.00	36104.91	742572.9	100
Di-n-octyl phthalate	Indoor	Baseline	ng/g	3	3867.72	5446.68	623.74	623.74	823.48	10155.94	10155.94	100
	Indoor	Follow-up	ng/g	2	2863.53	3611.39	309.90	309.90	2863.53	5417.17	5417.17	100
Dibutyl phthalate	Indoor	Baseline	ng/g	98	13457.01	27929.44	1704.81	6037.48	8759.88	13264.91	275258.2	100
	Indoor	Follow-up	ng/g	89	10194.89	15471.09	620.38	4137.61	6673.05	10196.24	135244.0	100
Dicyclohexyl phthalate	Indoor	Baseline	ng/g	65	410.11	345.88	109.48	242.88	333.12	464.74	2073.52	94
	Indoor	Follow-up	ng/g	38	454.07	537.22	108.65	165.62	297.70	440.27	2782.97	79
Diethyl phthalate	Indoor	Baseline	ng/g	80	3100.59	3906.14	153.81	766.41	1728.63	4146.26	25954.70	82
	Indoor	Follow-up	ng/g	73	3337.35	4713.68	133.10	684.33	1751.15	3870.41	25836.90	82
Di(2-ethylhexyl) phthalate	Indoor	Baseline	ng/g	98	175843.3	104999.9	40515.23	104597.4	152769.4	209517.0	574451.9	100
	Indoor	Follow-up	ng/g	89	173656.2	135793.6	20562.37	96948.93	141900.7	204742.4	826264.9	100
Dimethyl phthalate	Indoor	Baseline	ng/g	58	778.93	2369.83	120.31	199.10	251.78	726.15	18149.84	95
	Indoor	Follow-up	ng/g	47	780.17	1547.25	126.67	243.50	364.64	685.33	10352.38	90

Note: Descriptive statistics were computed using only values above the laboratory-reported method detection limit. “% Meas.” is the ratio of measurable results to all results with RQI<2.

Table 3-11c. Distribution of Laboratory Measurements for Environmental Samples: Environmental Tobacco Smoke in PM_{10}

Chemical Analyte	Sampling Location	Study Phase [a]	Meas. Unit	N	Mean Meas.	SD	Min. Meas.	25th Pctl.	Median Meas.	75th Pctl.	Max. Meas.	% Meas.
Environmental tobacco smoke	Central Fixed	Baseline	µg/m3	55	8.72	3.66	1.89	6.45	8.22	10.62	24.37	96
	Indoor	Baseline	µg/m3	39	7.02	6.76	0.64	1.89	4.24	10.69	27.28	91
	Outdoor	Baseline	µg/m3	21	3.28	2.56	0.62	1.57	2.78	3.76	10.16	78
	Personal	Baseline	µg/m3	92	22.09	22.67	1.15	6.71	11.16	34.52	106.45	88
	Central Fixed	Follow-up	µg/m3	52	4.71	2.68	1.00	2.81	3.95	6.53	14.26	91
	Indoor	Follow-up	µg/m3	73	9.99	11.20	0.40	1.83	4.59	15.26	54.47	92
	Outdoor	Follow-up	µg/m3	49	2.34	1.47	0.24	1.24	1.91	2.93	6.88	92
	Personal	Follow-up	µg/m3	92	17.47	20.32	1.03	4.09	9.35	23.00	126.37	96

Note: Descriptive statistics were computed using only values above the laboratory-reported method detection limit. "% Meas." is the ratio of measurable results to all results with RQI < 2. Central Fixed and Outdoor ETS is actually "Brown Carbon."

Table 3-11d. Distribution of Laboratory Measurements for Environmental Samples: Air PM_{10}

Chemical Analyte	Sampling Location	Study Phase [a]	Meas. Unit	N	Mean Meas.	Std. Dev.	Min. Meas.	25th Pctl.	Median Meas.	75th Pctl.	Max. Meas.	% Meas.
MicroPEM mass	Central Fixed	Baseline	µg/m3	56	20.76	8.87	4.97	14.36	17.96	26.49	45.69	100
	Indoor	Baseline	µg/m3	43	25.22	20.25	1.34	11.74	16.41	43.79	87.82	100
	Outdoor	Baseline	µg/m3	26	17.19	7.11	7.82	13.63	15.05	18.97	46.13	100
	Personal	Baseline	µg/m3	104	37.14	27.92	2.82	16.48	28.29	49.76	133.34	100
	Central Fixed	Follow-up	µg/m3	56	20.44	9.32	5.15	14.91	18.75	24.71	58.57	98
	Indoor	Follow-up	µg/m3	77	32.66	28.47	2.83	13.24	19.91	44.28	120.69	100
	Outdoor	Follow-up	µg/m3	53	13.01	3.92	2.44	10.54	13.19	14.83	24.77	100
	Personal	Follow-up	µg/m3	95	39.72	29.25	4.78	18.18	31.46	52.58	196.95	100

Note: Descriptive statistics were computed using only values above the laboratory-reported method detection limit. "% Meas." is the ratio of measurable results to all results with RQI < 2.

Table 3-11e. Distribution of Laboratory Measurements for Environmental Samples: NO_2 in Air

Chemical Analyte	Sampling Location	Study Phase [a]	Meas. Unit	N	Mean Meas.	Std. Dev.	Min. Meas.	25th Pctl.	Med. Meas.	75th Pctl.	Max. Meas.	% Meas.
Nitrogen Dioxide	Indoor	Baseline	ppbv	69	7.65	7.77	1.39	2.71	4.55	9.31	31.88	100
	Outdoor	Baseline	ppbv	41	6.62	3.89	1.75	3.76	5.81	8.33	19.76	98
	Personal	Baseline	ppbv	136	6.06	4.13	1.70	3.26	4.56	7.60	22.01	100
	Indoor	Follow-up	ppbv	132	10.75	19.66	1.37	3.25	4.90	13.89	207.46	100
	Outdoor	Follow-up	ppbv	103	6.12	3.46	1.61	3.23	5.28	8.57	15.15	100
	Personal	Follow-up	ppbv	130	6.89	11.67	1.52	2.93	4.39	7.56	129.89	100

Note: Descriptive statistics were computed using only values above the laboratory-reported method detection limit. "% Meas." is the ratio of measurable results to all results with RQI < 2.

Table 3-11f. Distribution of Laboratory Measurements for Environmental Samples: H_2S

Chemical Analyte	Sampling Location	Study Phase [a]	Meas. Unit	N	Mean Meas.	Std. Dev.	Min. Meas.	25th Pctl.	Med. Meas.	75th Pctl.	Max. Meas.	% Meas.
Hydrogen sulfide	Indoor	Baseline	Ppb	26	1.14	0.24	0.78	0.96	1.09	1.39	1.67	57
	Indoor	Follow-up	Ppb	9	1.15	0.34	0.81	0.85	1.04	1.42	1.70	69

Note: Descriptive statistics were computed using only values above the laboratory-reported method detection limit. "% Meas." is the ratio of measurable results to all results with RQI<2.

Table 3-11g. Distribution of Laboratory Measurements for Environmental Samples: VOC in Air

Chemical Analyte	Sampling Location	Study Phase [a]	Meas. Unit	N	Mean Meas.	Std. Dev.	Min. Meas.	25th Pctl.	Med. Meas.	75th Pctl.	Max. Meas.	% Meas.
Acrylonitrile	Central Fixed	Baseline	µg/m3	3	40.04	65.62	2.12	2.12	2.18	115.81	115.81	3
	Indoor	Baseline	µg/m3	10	0.52	0.44	0.23	0.29	0.36	0.41	1.58	25
	Outdoor	Baseline	µg/m3	1	14.73	—	14.73	14.73	14.73	14.73	14.73	2
	Personal	Baseline	µg/m3	12	1.78	4.85	0.18	0.21	0.29	0.61	17.15	13
	Central Fixed	Follow-up	µg/m3	1	125.21	—	125.21	125.21	125.21	125.21	125.21	1
	Indoor	Follow-up	µg/m3	14	0.53	0.42	0.19	0.33	0.43	0.50	1.86	13
	Personal	Follow-up	µg/m3	9	0.44	0.12	0.28	0.36	0.43	0.48	0.66	9
Benzene	Central Fixed	Baseline	µg/m3	10	4.33	0.78	3.54	3.75	4.07	4.78	5.87	12
	Indoor	Baseline	µg/m3	43	2.70	2.39	0.78	1.08	1.71	3.19	10.88	100
	Outdoor	Baseline	µg/m3	34	1.22	1.10	0.63	0.85	0.97	1.25	7.20	85
	Personal	Baseline	µg/m3	80	2.21	1.95	0.55	1.01	1.55	2.68	11.15	86
	Central Fixed	Follow-up	µg/m3	17	20.42	66.07	1.57	4.19	4.96	5.20	276.76	24
	Indoor	Follow-up	µg/m3	109	2.54	2.42	0.31	1.05	1.77	3.29	13.34	100
	Outdoor	Follow-up	µg/m3	98	1.79	6.27	0.24	0.48	0.77	1.27	46.31	98
	Personal	Follow-up	µg/m3	91	2.10	3.46	0.32	0.65	1.44	2.18	31.11	97
Methyl ethyl ketone	Indoor	Baseline	µg/m3	18	2.63	2.06	0.83	1.49	2.22	2.86	9.40	44
	Personal	Baseline	µg/m3	66	3.07	2.89	0.91	1.58	2.37	3.59	21.27	69
	Central Fixed	Follow-up	µg/m3	10	2.73	0.61	1.79	2.22	3.00	3.18	3.39	14
	Indoor	Follow-up	µg/m3	107	2.93	2.50	0.77	1.63	2.45	3.23	17.76	97
	Outdoor	Follow-up	µg/m3	61	0.67	0.71	0.22	0.35	0.43	0.85	5.59	60
	Personal	Follow-up	µg/m3	90	2.14	1.78	0.40	1.18	1.66	2.30	12.47	93

(continued)

Table 3-11g. Distribution of Laboratory Measurements for Environmental Samples: VOC in Air (continued)

Chemical Analyte	Sampling Location	Study Phase [a]	Meas. Unit	N	Mean Meas.	Std. Dev.	Min. Meas.	25th Pctl.	Med. Meas.	75th Pctl.	Max. Meas.	% Meas.
Naphthalene	Central Fixed	Baseline	µg/m3	23	3.05	4.76	1.55	1.73	1.88	2.23	24.74	26
	Indoor	Baseline	µg/m3	14	0.61	0.49	0.23	0.31	0.46	0.67	2.07	47
	Outdoor	Baseline	µg/m3	6	1.04	1.33	0.22	0.34	0.39	1.21	3.65	15
	Personal	Baseline	µg/m3	46	7.32	19.37	0.19	0.30	0.41	4.31	112.30	64
	Central Fixed	Follow-up	µg/m3	30	45.91	246.99	0.48	0.64	0.71	0.96	1353.66	41
	Indoor	Follow-up	µg/m3	70	10.89	42.70	0.13	0.31	0.40	0.57	250.07	89
	Outdoor	Follow-up	µg/m3	58	0.63	1.87	0.05	0.11	0.16	0.31	9.79	56
	Personal	Follow-up	µg/m3	59	3.15	12.22	0.18	0.29	0.37	0.50	88.64	86
Styrene	Central Fixed	Baseline	µg/m3	23	3.53	1.78	1.42	1.58	4.88	5.12	5.62	26
	Indoor	Baseline	µg/m3	41	1.65	4.46	0.28	0.53	0.84	1.16	29.16	95
	Outdoor	Baseline	µg/m3	8	0.42	0.32	-0.00	0.29	0.30	0.58	1.03	20
	Personal	Baseline	µg/m3	92	1.07	2.21	0.21	0.40	0.64	0.95	17.37	94
	Indoor	Follow-up	µg/m3	97	1.19	1.53	0.17	0.52	0.79	1.25	10.11	89
	Outdoor	Follow-up	µg/m3	5	0.60	0.57	-0.06	0.35	0.46	0.74	1.47	5
	Personal	Follow-up	µg/m3	72	0.78	0.66	0.25	0.42	0.59	0.91	5.08	75
Toluene	Central Fixed	Baseline	µg/m3	79	11.05	24.55	2.30	3.10	4.40	8.45	167.78	84
	Indoor	Baseline	µg/m3	44	16.46	24.72	2.48	3.82	7.24	15.40	110.83	100
	Outdoor	Baseline	µg/m3	39	2.80	3.92	0.69	1.51	2.12	2.76	25.94	98
	Personal	Baseline	µg/m3	99	11.60	16.15	1.90	4.29	6.53	9.92	105.30	100
	Central Fixed	Follow-up	µg/m3	65	98.03	670.96	1.42	2.51	2.96	4.39	5369.43	88
	Indoor	Follow-up	µg/m3	110	21.78	80.71	2.03	4.28	6.73	13.16	788.91	100
	Outdoor	Follow-up	µg/m3	103	6.89	32.07	0.51	1.08	1.59	2.20	240.96	99
	Personal	Follow-up	µg/m3	97	16.39	49.37	1.45	3.60	5.24	10.72	435.98	100

(continued)

Table 3-11g. Distribution of Laboratory Measurements for Environmental Samples: VOC in Air (continued)

Chemical Analyte	Sampling Location	Study Phase [a]	Meas. Unit	N	Mean Meas.	Std. Dev.	Min. Meas.	25th Pctl.	Med. Meas.	75th Pctl.	Max. Meas.	% Meas.
Trichloroethylene	Central Fixed	Baseline	µg/m3	2	2.52	0.47	2.19	2.19	2.52	2.85	2.85	2
	Indoor	Baseline	µg/m3	5	1.32	1.22	0.53	0.54	0.91	1.19	3.46	11
	Outdoor	Baseline	µg/m3	1	0.29	—	0.29	0.29	0.29	0.29	0.29	2
	Personal	Baseline	µg/m3	23	1.87	4.22	0.18	0.29	0.35	0.95	19.98	23
	Central Fixed	Follow-up	µg/m3	45	66.47	394.62	1.65	1.79	1.86	1.98	2643.09	63
	Indoor	Follow-up	µg/m3	89	0.79	2.29	0.21	0.29	0.33	0.42	20.13	82
	Outdoor	Follow-up	µg/m3	79	0.31	0.11	0.19	0.26	0.28	0.33	1.03	77
	Personal	Follow-up	µg/m3	74	0.90	1.91	0.21	0.29	0.33	0.47	12.38	77
Vinyl chloride	Central Fixed	Baseline	µg/m3	1	8.07	—	8.07	8.07	8.07	8.07	8.07	2
	Indoor	Baseline	µg/m3	1	0.44	—	0.44	0.44	0.44	0.44	0.44	8
α-Pinene	Central Fixed	Baseline	µg/m3	61	3.79	3.70	0.98	1.30	1.60	7.32	10.80	65
	Indoor	Baseline	µg/m3	44	10.91	18.54	1.09	3.78	5.81	10.43	120.50	100
	Outdoor	Baseline	µg/m3	40	1.07	1.87	0.25	0.47	0.80	0.99	12.37	98
	Personal	Baseline	µg/m3	99	10.48	15.16	1.11	2.99	5.30	11.06	97.52	100
	Central Fixed	Follow-up	µg/m3	68	73.62	533.19	0.48	2.35	2.66	2.97	4383.67	92
	Indoor	Follow-up	µg/m3	110	12.45	24.14	0.54	2.91	5.03	10.17	196.67	100
	Outdoor	Follow-up	µg/m3	102	0.84	1.06	0.10	0.42	0.61	0.89	9.22	98
	Personal	Follow-up	µg/m3	97	7.47	11.20	0.34	2.13	3.73	6.58	70.47	100

(continued)

Table 3-11g. Distribution of Laboratory Measurements for Environmental Samples: VOC in Air (continued)

Chemical Analyte	Sampling Location	Study Phase [a]	Meas. Unit	N	Mean Meas.	Std. Dev.	Min. Meas.	25th Pctl.	Med. Meas.	75th Pctl.	Max. Meas.	% Meas.
m,p-Xylenes	Central Fixed	Baseline	µg/m3	55	7.78	13.68	1.38	1.76	2.24	3.34	46.59	59
	Indoor	Baseline	µg/m3	44	6.41	8.41	0.56	1.33	2.49	7.91	39.32	100
	Outdoor	Baseline	µg/m3	40	1.72	2.67	0.28	0.69	1.12	2.10	17.30	98
	Personal	Baseline	µg/m3	91	7.35	20.10	0.40	1.39	2.78	5.13	144.84	96
	Central Fixed	Follow-up	µg/m3	42	88.48	509.20	0.76	1.06	1.43	2.57	3291.21	57
	Indoor	Follow-up	µg/m3	110	5.68	10.34	0.51	1.65	2.64	4.84	88.34	100
	Outdoor	Follow-up	µg/m3	103	5.34	25.99	0.12	0.49	0.93	1.31	186.20	99
	Personal	Follow-up	µg/m3	97	4.78	8.41	0.41	1.36	2.18	4.34	61.77	100
n-Octane	Central Fixed	Baseline	µg/m3	40	6.74	5.16	2.90	3.84	5.14	7.24	27.20	43
	Indoor	Baseline	µg/m3	36	1.49	1.29	0.42	0.62	0.89	1.61	4.90	95
	Outdoor	Baseline	µg/m3	22	0.65	0.41	0.34	0.45	0.53	0.71	2.39	65
	Personal	Baseline	µg/m3	80	1.43	1.25	0.37	0.71	0.98	1.63	8.19	90
	Central Fixed	Follow-up	µg/m3	10	5.63	2.37	3.41	3.76	4.87	7.89	10.13	14
	Indoor	Follow-up	µg/m3	94	1.60	2.15	0.33	0.66	0.97	1.56	14.24	86
	Outdoor	Follow-up	µg/m3	53	1.68	4.62	0.40	0.52	0.65	0.74	25.00	53
	Personal	Follow-up	µg/m3	80	1.29	1.42	0.24	0.66	0.88	1.17	9.51	85
p-Dichloro-benzene	Indoor	Baseline	µg/m3	30	4.57	11.97	0.21	0.38	1.11	2.79	64.21	81
	Outdoor	Baseline	µg/m3	17	2.12	3.91	0.17	0.35	0.52	1.08	13.58	47
	Personal	Baseline	µg/m3	79	25.15	103.95	0.17	0.50	1.56	6.88	873.77	86
	Central Fixed	Follow-up	µg/m3	2	4.64	4.93	1.15	1.15	4.64	8.13	8.13	3
	Indoor	Follow-up	µg/m3	95	65.83	238.15	0.11	0.61	2.00	10.83	1472.22	88
	Outdoor	Follow-up	µg/m3	55	7.43	11.45	-0.12	0.54	1.23	9.16	41.90	53
	Personal	Follow-up	µg/m3	84	37.55	127.17	0.11	0.60	2.39	14.99	981.03	89

Note: Descriptive statistics were computed using only values above the laboratory-reported method detection limit. "% Meas." is the ratio of measurable results to all results with RQI < 2.

Table 3-11h. Distribution of Laboratory Measurements for Analyses of Biospecimens: Complete Blood Count (CBC) Results

Chemical Analyte	Sampling Location	Study Phase [a]	Meas. Unit	N	Mean Meas.	Std. Dev.	Min. Meas.	25th Pctl.	Med. Meas.	75th Pctl.	Max. Meas.	% Meas.
Basophil (auto.)	Personal	Baseline	10^{3}/µL	20	0.09	0.04	0.03	0.06	0.08	0.10	0.22	24
Eosinophil (auto.)	Personal	Baseline	10^{3}/µL	91	0.26	0.19	0.05	0.10	0.20	0.40	1.00	97
Hematocrit	Personal	Baseline	%	95	37.90	3.35	31.50	35.40	37.50	40.40	45.20	100
Hemoglobin	Personal	Baseline	gM/dL	96	12.86	1.23	10.10	12.10	12.70	13.80	15.70	100
Lymphocyte (auto.)	Personal	Baseline	10^{3}/µL	96	2.55	0.96	0.23	2.00	2.58	3.00	5.30	100
Mean corpuscular hemoglobin	Personal	Baseline	pg	96	28.96	1.95	22.40	27.95	29.20	30.00	33.70	100
Mean corpuscular hemoglobin conc.	Personal	Baseline	g/dL	96	33.89	0.70	31.90	33.55	33.90	34.30	35.60	100
Mean corpuscular volume	Personal	Baseline	fL	96	85.40	4.96	69.50	82.25	85.65	88.15	96.90	100
Mean platelet volume	Personal	Baseline	fL	96	9.16	1.00	7.10	8.50	9.15	9.80	12.20	100
Monocyte (auto.)	Personal	Baseline	10^{3}/µL	96	0.50	0.24	0.12	0.30	0.50	0.60	1.39	100
Neutrophil	Personal	Baseline	10^{3}/µL	23	2.24	1.20	0.06	1.35	1.96	3.30	4.15	100
Neutrophil (auto.)	Personal	Baseline	10^{3}/L	73	3.50	1.53	0.80	2.50	3.10	4.59	8.00	100
Platelet count	Personal	Baseline	10^{3}/µL	96	271.97	68.96	79.00	226.00	265.00	310.00	493.00	100
Red blood cell count	Personal	Baseline	10^{3}/µL	96	4.45	0.36	3.60	4.15	4.45	4.70	5.20	100
Red blood cell distribution width	Personal	Baseline	%	93	13.43	1.06	11.30	12.70	13.20	13.90	19.10	100
White blood cell count	Personal	Baseline	10^{3}/µL	96	6.57	1.94	2.60	5.40	6.45	7.55	11.70	100

Note: Descriptive statistics were computed using only values above the laboratory-reported method detection limit. "% Meas." is the ratio of measurable results to all results with RQI < 2.

Table 3-11i. Distribution of Laboratory Measurements for Analyses of Biospecimens: Cotinine in Urine Results

Chemical Analyte	Sampling Location	Study Phase a	Meas. Unit	N	Mean Meas.	Std. Dev.	Min. Meas.	25th Pctl.	Median Meas.	75th Pctl.	Max. Meas.	% Meas.
Urinary cotinine	Personal	Baseline	ng/mL	132	22.13	120.09	0.91	2.60	4.18	7.12	1193.18	88
	Personal	Follow-up	ng/mL	137	46.55	209.21	2.05	3.01	4.32	10.78	1777.18	100

Note: Descriptive statistics were computed using only values above the laboratory-reported method detection limit.

Table 3-11j. Distribution of Laboratory Measurements for Analyses of Biospecimens: Clinical Urine Results

Chemical Analyte	Sampling Location	Study Phase [a]	Meas. Unit	N	Mean Meas.	Std. Dev.	Min. Meas.	25th Pctl.	Median Meas.	75th Pctl.	Max. Meas.	% Meas.
Urinary creatinine	Personal	Baseline	mg/dL	151	156.69	87.84	14.58	95.99	147.51	198.65	584.42	100
	Personal	Follow-up	mg/dL	138	174.92	106.87	12.50	102.02	156.95	215.85	568.14	100

Note: Descriptive statistics were computed using only values above the laboratory-reported method detection limit. “% Meas.” is the ratio of measurable results to all results with RQI<2.

Table 3-11k. Distribution of Laboratory Measurements for Analyses of Biospecimens: Immunoglobulin E (IgE) Results

Chemical Analyte	Sampling Location	Study Phase [a]	Meas. Unit	N	Mean Meas.	Std. Dev.	Min. Meas.	25th Pctl.	Med. Meas.	75th Pctl.	Max. Meas.	% Meas.
American cockroach antibody	Personal	Baseline	kU/L	7	0.23	0.10	0.11	0.15	0.24	0.27	0.42	7
Aspergillus fumigatus antibody	Personal	Baseline	kU/L	13	2.80	2.60	0.14	0.58	2.33	3.10	8.69	13
Cat dander antibody	Personal	Baseline	kU/L	18	8.43	19.30	0.10	0.17	0.45	11.40	80.80	18
Dermatophagoides farinae antibody	Personal	Baseline	kU/L	36	16.05	25.83	0.11	0.28	0.97	27.10	88.00	37
Dermatophagoides pteronyssinus antibody	Personal	Baseline	kU/L	30	19.37	29.51	0.10	0.45	1.10	35.50	99.60	31
Total IgE	Personal	Baseline	IU/mL	108	243.28	378.73	2.00	32.50	104.00	263.50	2326.00	100

Note: Descriptive statistics were computed using only values above the laboratory-reported method detection limit. “% Meas.” is the ratio of measurable results to all results with RQI<2.

Table 3-11l. Distribution of Laboratory Measurements for Analyses of Biospecimens: Urinary VOC and Phthalate Metabolites

Chemical Analyte	Sampling Location	Study Phase [a]	Meas. Unit	N	Mean Meas.	Std. Dev.	Min. Meas.	25th Pctl.	Med. Meas.	75th Pctl.	Max. Meas.	% Meas.
2-Methylhippuric acid	Personal	Baseline	ng/mL	102	27.67	27.46	1.87	9.81	16.55	38.87	142.87	100
	Personal	Follow-up	ng/mL	92	26.97	26.42	2.21	11.08	16.68	32.79	145.30	99
Mandelic acid	Personal	Baseline	ng/mL	102	301.26	126.51	4.40	204.21	314.19	390.10	566.32	100
	Personal	Follow-up	ng/mL	92	188.34	128.26	8.01	88.95	169.30	257.24	567.86	99
Methylhippuric acid	Personal	Baseline	ng/mL	102	301.00	357.57	7.10	99.17	170.92	336.58	2043.29	100
	Personal	Follow-up	ng/mL	92	297.84	883.57	13.30	62.79	140.00	265.66	8400.00	99
N-Acetyl-S-(1,2-dichlorovinyl)-l-cysteine	Personal	Baseline	ng/mL	1	1.46	—	1.46	1.46	1.46	1.46	1.46	1
	Personal	Follow-up	ng/mL	1	1.61	—	1.61	1.61	1.61	1.61	1.61	1
N-Acetyl-S-(1-hydroxymethyl-2-propenyl)- l-cysteine	Personal	Baseline	ng/mL	94	11.45	4.06	3.67	9.55	11.26	13.48	29.05	92
	Personal	Follow-up	ng/mL	78	5.63	3.38	2.50	3.28	5.02	6.53	21.78	84
N-Acetyl-S-(1-phenyl-2-hydroxyethyl)-l-cysteine	Personal	Baseline	ng/mL	13	14.24	11.18	4.72	7.44	8.88	16.70	37.42	13
	Personal	Follow-up	ng/mL	6	20.70	17.77	4.63	7.14	16.40	26.75	52.91	6
N-Acetyl-S-(2-carbamoyl-2-hydroxyethyl)- l-cysteine	Personal	Baseline	ng/mL	86	23.76	24.71	5.50	11.12	16.18	27.52	185.90	84
	Personal	Follow-up	ng/mL	70	28.82	27.07	5.32	10.45	18.28	39.70	131.83	75
N-Acetyl-S-(2-carboxyethyl)-l-cysteine	Personal	Baseline	ng/mL	89	140.78	176.33	10.77	31.89	82.74	157.60	791.35	87
	Personal	Follow-up	ng/mL	78	164.79	172.79	12.58	43.82	96.10	236.34	901.06	84
N-Acetyl-S-(2-cyanoethyl)-l-cysteine	Personal	Baseline	ng/mL	96	5.44	8.73	1.13	2.02	3.32	5.37	79.24	94
	Personal	Follow-up	ng/mL	74	4.00	3.99	1.06	1.60	2.83	5.16	24.94	80
N-Acetyl-S-(2-hydroxyethyl)-l-cysteine	Personal	Baseline	ng/mL	57	4.44	1.97	1.81	3.08	4.11	5.20	11.02	56
	Personal	Follow-up	ng/mL	33	3.97	1.75	1.91	2.88	3.51	4.42	9.39	35

(continued)

Table 3-11l. Distribution of Laboratory Measurements for Analyses of Biospecimens: Urinary VOC and Phthalate Metabolites (continued)

Chemical Analyte	Sampling Location	Study Phase [a]	Meas. Unit	N	Mean Meas.	Std. Dev.	Min. Meas.	25th Pctl.	Med. Meas.	75th Pctl.	Max. Meas.	% Meas.
N-Acetyl-S-(3,4-dihydroxybutyl)-l-cysteine	Personal	Baseline	ng/mL	102	588.85	441.66	24.56	313.13	515.96	753.98	2960.06	100
	Personal	Follow-up	ng/mL	92	636.58	456.82	50.43	264.26	524.38	947.61	2001.98	99
N-Acetyl-S-(3-hydroxypropyl-1-methyl)-l- cysteine	Personal	Baseline	ng/mL	102	338.25	314.34	6.05	189.87	292.08	418.72	2945.52	100
	Personal	Follow-up	ng/mL	92	373.31	326.62	29.63	173.64	320.06	444.07	2298.16	99
N-Acetyl-S-(benzyl)-l-cysteine	Personal	Baseline	ng/mL	101	10.76	10.90	1.63	4.92	7.41	11.83	68.43	99
	Personal	Follow-up	ng/mL	90	10.70	11.15	0.67	4.66	7.27	12.48	73.52	97
N-Acetyl-S-(dimethylphenyl)-l-cysteine	Personal	Baseline	ng/mL	6	1.80	0.42	1.47	1.49	1.66	1.95	2.58	6
	Personal	Follow-up	ng/mL	1	1.76		1.76	1.76	1.76	1.76	1.76	1
N-Acetyl-S-(phenyl)-l-cysteine	Personal	Baseline	ng/mL	11	5.91	6.59	1.93	2.28	3.98	6.71	24.82	11
	Personal	Follow-up	ng/mL	13	9.81	9.91	1.87	2.15	8.07	13.76	36.83	14
Phenylglyoxylic acid	Personal	Baseline	ng/mL	99	237.95	182.33	4.04	89.00	229.91	325.62	1001.24	97
	Personal	Follow-up	ng/mL	88	286.84	223.91	4.11	130.20	222.01	422.81	1272.34	95
mono(2-ethyl-5-hydroxyhexyl) phthalate	Personal	Baseline	ng/mL	102	18.50	24.64	0.31	4.48	11.44	26.29	194.33	100
	Personal	Follow-up	ng/mL	91	25.84	26.97	2.48	8.75	16.80	30.20	150.73	98
mono(2-ethyl-5-oxohexyl) phthalate	Personal	Baseline	ng/mL	99	14.78	19.86	0.35	6.18	10.26	18.26	183.07	97
	Personal	Follow-up	ng/mL	92	14.81	17.00	0.51	5.62	9.37	17.02	105.46	99
mono(2-ethylhexyl) phthalate	Personal	Baseline	ng/mL	84	4.20	3.45	0.60	1.72	3.19	5.63	16.79	82
	Personal	Follow-up	ng/mL	78	4.67	4.84	0.62	1.26	3.30	6.09	29.12	84
mono(3-carboxypropyl) phthalate	Personal	Baseline	ng/mL	40	9.91	7.64	2.24	5.63	7.37	11.45	44.40	39
	Personal	Follow-up	ng/mL	48	62.66	312.64	3.71	5.95	8.77	17.44	2170.03	52
mono[(2-carboxymethyl)hexyl] phthalate	Personal	Baseline	ng/mL	88	11.81	20.60	1.70	4.39	6.47	10.37	135.77	87
	Personal	Follow-up	ng/mL	77	333.48	2835.24	0.93	2.59	5.44	12.28	24889.13	83

(continued)

Table 3-11l. Distribution of Laboratory Measurements for Analyses of Biospecimens: Urinary VOC and Phthalate Metabolites (continued)

Chemical Analyte	Sampling Location	Study Phase [a]	Meas. Unit	N	Mean Meas.	Std. Dev.	Min. Meas.	25th Pctl.	Med. Meas.	75th Pctl.	Max. Meas.	% Meas.
monobenzyl phthalate	Personal	Baseline	ng/mL	101	23.47	23.71	0.30	6.60	15.78	29.92	111.69	99
	Personal	Follow-up	ng/mL	90	23.99	26.75	0.53	5.43	15.76	33.55	157.75	97
monobutyl phthalate	Personal	Baseline	ng/mL	102	35.18	33.46	1.21	14.77	26.51	42.70	231.10	100
	Personal	Follow-up	ng/mL	93	34.13	26.55	2.10	13.24	26.47	45.90	139.46	100
monocyclohexyl phthalate	Personal	Baseline	ng/mL	46	0.76	0.42	0.28	0.62	0.67	0.78	2.74	45
	Personal	Follow-up	ng/mL	24	0.55	0.40	0.29	0.33	0.38	0.59	2.12	26
monoethyl phthalate	Personal	Baseline	ng/mL	102	108.05	162.87	2.63	28.12	54.78	111.58	859.83	100
	Personal	Follow-up	ng/mL	93	121.67	261.45	3.59	26.35	42.61	101.28	1970.18	100
monomethyl phthalate	Personal	Baseline	ng/mL	100	13.23	17.14	1.22	4.70	8.79	14.13	120.25	98
	Personal	Follow-up	ng/mL	89	11.35	15.45	1.37	4.24	7.43	13.59	134.18	96
monononyl phthalate	Personal	Baseline	ng/mL	50	1.46	1.45	0.40	0.54	0.85	1.65	6.56	49
	Personal	Follow-up	ng/mL	36	1.20	0.76	0.39	0.62	0.96	1.55	3.51	39
monooctyl phthalate	Personal	Baseline	ng/mL	33	0.76	0.48	0.30	0.43	0.60	0.83	2.14	32
	Personal	Follow-up	ng/mL	29	4.64	7.44	0.58	1.13	2.59	3.94	39.61	31

Note: Descriptive statistics were computed using only values above the laboratory-reported method detection limit. "% Meas." is the ratio of measurable results to all results with RQI < 2.

Table 3-11m. Distribution of Microbiologic Measurements for Analyses of MicroPEM Filters

Chemical Analyte	Sampling Location	Study Phase	Meas. Unit	N	Mean Meas.	Std. Dev.	Min. Meas.	25th Pctl.	Median Meas.	75th Pctl.	Max. Meas.	% Meas.
Aspergillus fumigatus allergen	Indoor	Follow-up	ng/m^3	3	0.31	0.15	0.19	0.19	0.27	0.47	0.47	5
Cat danderallergen	Indoor	Follow-up	ng/m^3	2	0.55	0.27	0.36	0.36	0.55	0.75	0.75	3
	Personal	Follow-up	ng/m^3	2	2.31	1.35	1.36	1.36	2.31	3.27	3.27	3
	Personal	Baseline	ng/m^3	1	2.11	—	2.11	2.11	2.11	2.11	2.11	1
Dust mite Dermatophagoides pteronyssinus allergen	Indoor	Follow-up	ng/m^3	1	1.91	—	1.91	1.91	1.91	1.91	1.91	2
	Personal	Follow-up	ng/m^3	3	12.22	1.25	11.45	11.45	11.55	13.67	13.67	4
	Indoor	Baseline	ng/m^3	1	1.90	—	1.90	1.90	1.90	1.90	1.90	4
	Personal	Baseline	ng/m^3	1	11.79	—	11.79	11.79	11.79	11.79	11.79	1
Endotoxin	Central Fixed	Follow-up	EU/m^3	1	1.54	—	1.54	1.54	1.54	1.54	1.54	100
	Indoor	Follow-up	EU/m^3	56	0.96	1.23	0.03	0.27	0.54	1.11	6.04	100
	Personal	Follow-up	EU/m^3	58	2.64	4.80	0.09	0.93	1.56	2.90	36.34	100
	Indoor	Baseline	EU/m^3	18	1.85	3.11	0.09	0.53	0.96	1.31	13.59	95
	Outdoor	Baseline	EU/m^3	1	0.55	—	0.55	0.55	0.55	0.55	0.55	100
	Personal	Baseline	EU/m^3	72	2.38	2.65	0.11	0.67	1.26	2.95	12.29	100
1,3-β-diglucans	Indoor	Follow-up	pg/m^3	58	132.38	100.62	37.36	73.59	112.09	165.51	612.29	100
	Personal	Follow-up	pg/m^3	67	346.97	245.90	14.82	186.91	266.34	473.18	1442.25	100
	Indoor	Baseline	pg/m^3	24	107.10	56.95	23.33	66.17	97.64	135.60	233.33	100
	Outdoor	Baseline	pg/m^3	1	237.95	—	237.95	237.95	237.95	237.95	237.95	100
	Personal	Baseline	pg/m^3	87	362.11	219.80	56.43	198.53	276.90	475.84	1186.49	100

Note: Descriptive statistics were computed using only values above the laboratory-reported method detection limit. "% Meas." is the ratio of measurable results to all results with RQI < 2.

Table 3-11n. Distribution of Microbiologic Measurements for Analyses of Vacuum Dust

Chemical Analyte	Sampling Location	Study Phase	Meas. Unit	N	Mean Meas.	Std. Dev.	Min. Meas.	25th Pctl.	Med. Meas.	75th Pctl.	Max. Meas.	% Meas.
Aspergillus fumigatus allergen	Indoor	Baseline	ng/g	5	25.08	10.99	13.89	14.40	24.90	35.61	36.62	5
	Indoor	Follow-up	ng/g	9	16.44	6.69	12.56	13.84	14.18	15.19	34.05	10
Cat dander allergen	Indoor	Baseline	ng/g	51	116.76	176.02	23.11	41.26	78.09	87.88	939.96	50
	Indoor	Follow-up	ng/g	44	208.76	238.17	22.97	44.79	83.53	350.09	1024.12	48
Cockroach Blatella germanica allergen	Indoor	Baseline	U/g	35	51.13	64.11	0.32	0.71	1.50	114.18	181.60	35
	Indoor	Follow-up	U/g	35	26.08	65.32	0.31	0.42	0.63	4.15	235.55	38
Dust mite Dermatophagoides farinae allergen	Indoor	Baseline	ng/g	58	311.82	969.13	67.48	79.26	89.99	192.79	7310.05	57
	Indoor	Follow-up	ng/g	50	366.03	511.10	53.30	78.34	123.73	427.54	2354.69	54
Dust mite Dermatophagoides pteronyssinus allergen	Indoor	Baseline	ng/g	17	1400.31	2714.87	80.58	144.67	372.80	1002.41	10914.32	17
	Indoor	Follow-up	ng/g	41	1160.49	2508.92	63.48	156.95	205.88	423.99	9651.80	45
Endotoxin	Indoor	Baseline	EU/mg	73	122.08	183.64	5.05	31.99	60.03	125.83	1083.29	100
	Indoor	Follow-up	EU/mg	59	4151.27	10477.10	7.65	56.53	96.77	252.36	52797.61	98
1,3-β-diglucan	Indoor	Baseline	pg/mg	91	4434.00	3603.57	4.79	2021.48	3265.41	6461.10	18948.75	100
	Indoor	Follow-up	pg/mg	84	3765.65	2924.67	610.39	1996.20	3114.32	4601.18	18613.71	100

Note: Descriptive statistics were computed using only values above the laboratory-reported method detection limit. “% Meas.” is the ratio of measurable results to all results with RQI < 2.

3.12 Special Studies

3.12.1 Air Exchange Rate Calculation

We used the LBLX model to calculate the 24-hour average air exchange rate (AER) for the residences of substudy participants. This model uses air infiltration through the building envelope and natural ventilation through open windows in the AER calculation.

Daily AER values were calculated for 63% and 58% of the residences of Baseline and Follow-up substudy participants, respectively. Daily AER values could not be calculated for some residences because the house square footage, house age, or type of residence was missing. Residence square footage and age are two critical parameters in the LBLX model that were removed from the household questionnaire during streamlining to reduce appointment time and concerns about the quality of the responses. Although municipality tax records, Zillow.com, and Google Maps provided square footage and age information for most residences, one or both pieces were not available for 31% of the residences in Baseline and for 30% of the residence in Follow-up. Also, the LBLX model has been validated only for single-family, detached residences. In the CHATS study, 6% and 12% of the substudy residences in Baseline and Follow-up, respectively, were identified as apartment buildings or duplexes.

Overall, the distributions of calculated daily AERs were similar among summer, fall, winter, and spring (***Table 3-12a***). Modeled daily AERs among CHATS households were lower than residences in North Carolina in 2000 and 2001.[5] We think the mild winter in the Gulf Coast area probably decreased the impact of air infiltration induced by the indoor-outdoor temperature differences on the calculated AER. The prevalence of central air conditioning in the residences reduced air infiltration in the summer as well, thereby suppressing the daily AER.[18]

Table 3-12a. Number of Substudy Participants with Complete Residence Information Required for the Air Exchange Rate Modeling and the Distribution of Modeled Daily Air Exchange Rate (h^{-1}) by Study Phase and Season

Study Phase and Season*	Number of Residences (% of substudy participants)	Total days	Min.	Max.	25th pctl.	50th pctl.	75th pctl.	Mean	Std. Dev.
Baseline	30 (62.5%)	231	0.09	1.01	0.21	0.32	0.48	0.36	0.19
Summer	4 (8.3%)	31	0.16	0.82	0.24	0.27	0.52	0.36	0.19
Fall	26 (54.2%)	200	0.09	1.01	0.21	0.33	0.47	0.36	0.18
Follow-up	60 (57.7%)	487	0.10	1.26	0.27	0.39	0.54	0.42	0.20
Winter	22 (21.2%)	178	0.10	0.97	0.22	0.32	0.50	0.38	0.20
Spring	38 (36.5%)	309	0.11	1.26	0.31	0.41	0.56	0.45	0.20

*Summer was June – August 2012. Fall was September – November 2012. Winter was December 2012 – February 2013. Spring was March – May 2013.

[18] Thornburg, J., Rodes, C.E., Lawless, P.A., Stevens, C.D., & Williams, R.W. (2004). A pilot study of the influence of residential HAC duty cycle on indoor air quality. *Atmospheric Environment,38*:1567-1577.

3.12.2 Exposure Misclassification Substudy

Introduction

One of the primary objectives of the misclassification substudy was to assess how well the integrated samples for indoor and outdoor analytes (PM_{10}, VOCs, carbonyls, NO_2, endotoxin, and the 1,3-β-diglucans) compare with personal measures. Personal monitoring is considered the ideal method when assessing individual exposure to multiple sources of a pollutant in multiple environments that a participant spends time. Personal monitoring is, however, more expensive and more burdensome for participants, but it will likely provide better exposure estimates for comparison to measures of exposure from biological media collected in this study, and as a basis for association studies with health outcomes. Thus, these comparisons aid in identifying the most useful measures to be collected in a full study.

An additional factor in the design of an efficient Full Study is the temporal relationships of environmental exposure to either biological measures or specific health outcomes. In this study, monitors integrated environmental metrics over a nominal 7-day period. Although an integrated measure does represent the overall exposure potential, it does not capture short-term outcome measures, e.g., urinary measures or acute respiratory outcomes, that result from exposures that occur within a couple of days of the biospecimen collection or Health Assessment. A “high” value for an integrated measure could result from a large exposure early in the monitoring period with low exposures subsequently, low exposures early in the period with a high exposure towards the end of the collection period, or consistent exposure throughout the collection period. Each of these scenarios could result in markedly different outcome measures and cloud the true relationships between exposure and outcome. In other words, the time interval between field interviewer visits 1 and 2 might need to be 2 or 3 days, rather than 7, to better understand the cause-effect relationship between exposure and biological outcomes.

The initial statistical analyses shown in the following sections provide some insight to the utility of personal sampling, what exposure information can be captured by the different methods, and the sampling durations that will provide the best data for a particular outcome.

Results

In this section, we present correlations of selected analytes among the various sampling locations. These comparisons help provide insight into the appropriateness of different sampling locations for assessing personal exposure. In addition, comparisons of indoor and outdoor locations provide information about the pollutant source, be it indoor or outdoor. Since this analysis series is a pilot to demonstrate how these data can be used, we selected a

single analyte as a representative example for both the VOC and carbonyl analyte classes. We present each analyte class separately. The final analysis combines all of the data, including the central site data for all analytes except NO_2, which was not collected at the central sites, in a multivariate evaluation.

The measurements for the Baseline occurred between July 17, 2012, and December 10, 2012. These were summer and fall measurements. The measurements for Follow-up occurred between December 10, 2012 and May 24, 2013. These were winter and spring measurements.

Personal, Indoor, Outdoor, and Central Site PM_{10} Mass Concentrations: The conclusions regarding the assessment of PM_{10} mass concentrations followed similar trends during Baseline and Follow-up that also agree with other studies.[19]

Table 3-12b presents both Pearson and Spearman correlations of PM_{10} measurements based on Baseline data. ***Table 3-12c*** presents similar statistics based on Follow-up data. For both periods of time, personal and indoor PM_{10} concentrations are correlated (R = 0.853), but neither are correlated with the ambient PM_{10} concentrations measured at outdoor residential and central site locations (R < 0.2). The stronger Personal:Indoor PM_{10} correlation indicates that a significant portion of the exposure occurs inside the residence. However, the deviation of the personal PM_{10} concentration from the corresponding indoor PM_{10} concentration can be up to 50% (***Figure 3-12a***). The deviation demonstrates the need for personal exposure monitoring, as opposed to indoor stationary monitoring, to accurately capture the child's exposure.

The significant correlation between outdoor residential and central site PM_{10} concentrations suggests that $PM_{2.5}$ makes up a large proportion of the PM_{10} mass in Baseline and Follow-up (R = 0.661, R = 0.488). $PM_{2.5}$ is known to be spatially homogeneous across urban areas whereas coarse particulate matter (particles between 2.5 and 10 micrometers) is more heterogeneous.[20]

[19] Williams, R., Suggs, J., Rea, A., Leovic, K., Vette, A., Croghan, C.et al. (2003). The Research Triangle Park Particulate Matter Panel Study: PM mass concentration relationships. *Atmospheric Environment,37*:5349–5363.

[20] Thornburg, J., Rodes, C. E., Lawless, P.A. &Williams, R.W. (2009). Sources and causes of coarse particulate matter spatial variability in Detroit, MI. *Atmospheric Environment,43*:4251-4258.

Table 3-12b. Pearson (top panel) and Spearman (bottom panel) Correlation Coefficients between the Four PM_{10} Sampling Platforms during Baseline

(For each cell the correlation is shown on the top line, p-value on middle line, and the number of samples is given on the bottom line.)

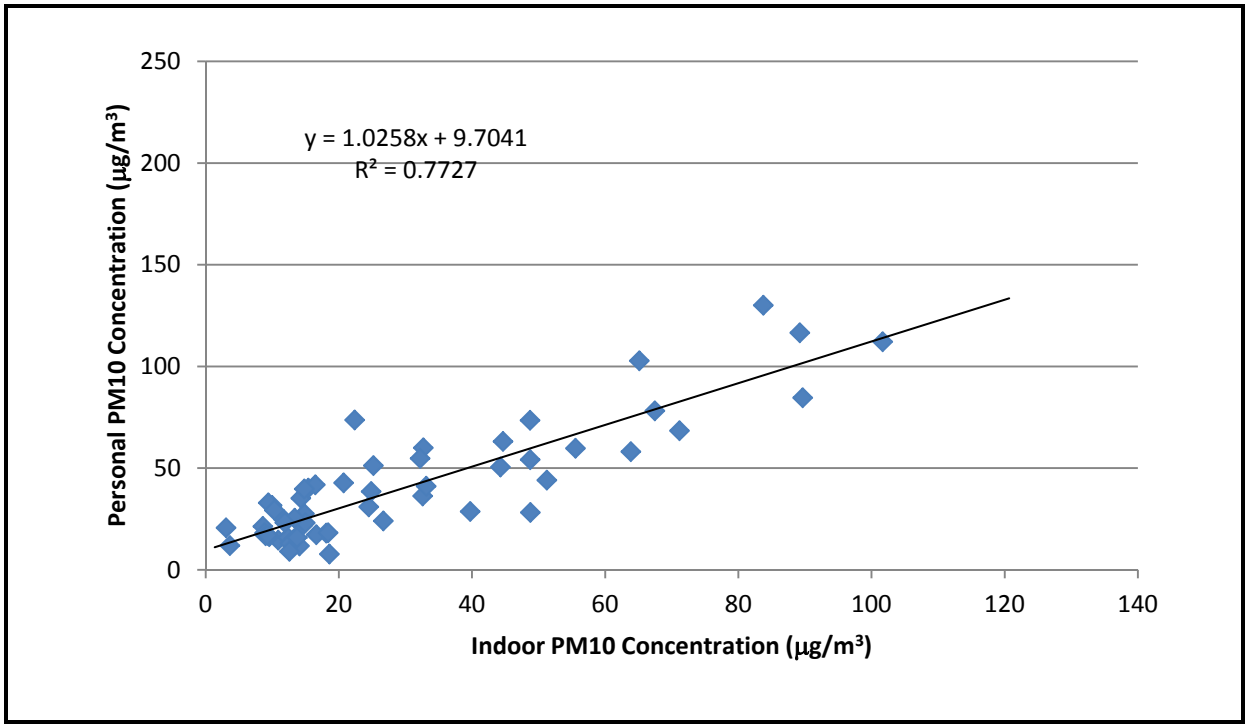
Correlation Coefficients	PM_{10}			
	Personal	Indoor	Outdoor	Central Site
Pearson				
Personal PM_{10} (Total N = 104)	—-	0.853 <0.0001 16	0.199 0.4287 18	0.108 0.4566 50
Indoor PM_{10} (Total N = 43)		—-	-0.207 0.3943 19	0.020 0.9405 16
Outdoor PM_{10} (Total N = 26)			—-	0.661 0.0268 11
Central Site PM_{10} (Total N = 79)				—-
Spearman				
Personal PM_{10} (Total N = 104)	—-	0.718 0.0017 16	0.214 0.3947 18	-0.063 0.6635 50
Indoor PM_{10} (Total N = 43)		—-	-0.142 0.562 19	0.125 0.6444 16
Outdoor PM_{10} (Total N = 26)			—-	0.620 0.042 11
Central Site PM_{10} (Total N = 79)				—-

Table 3-12c. Pearson (top panel) and Spearman (bottom panel) Correlation Coefficients between the Four PM_{10} Sampling Platforms during Follow-up

(For each cell the correlation is shown on the top line, p-value on middle line, and the number of samples is given on the bottom line.)

Correlation Coefficients	PM_{10}			
	Personal	Indoor	Outdoor	Central Site
Pearson				
Personal PM_{10} (Total N = 104)	—-	0.892 <0.0001 43	-0.011 0.9512 36	0.073 0.5948 55
Indoor PM_{10} (Total N = 43)		—-	0.008 0.9637 35	-0.060 0.6878 48
Outdoor PM_{10} (Total N = 26)			—-	0.488 0.0054 31
Central Site PM_{10} (Total N = 79)				—-
Spearman				
Personal PM_{10} (Total N = 104)	—-	0.780 <0.0001 43	-0.080 0.6415 36	0.027 0.8457 55
Indoor PM_{10} (Total N = 43)		—-	-0.011 0.9516 35	-0.059 0.6895 48
Outdoor PM_{10} (Total N = 26)			—-	0.404 0.0242 31
Central Site PM_{10} (Total N = 79)				—-

Figure 3-12a. Comparison of Indoor and Personal PM_{10} Concentrations



Personal, Indoor, and Outdoor Volatile Organic Compound (benzene) Concentrations: Associations among indoor, outdoor, and personal environmental benzene are shown in ***Table 3-12d*** for Baseline and ***Table 3-12e*** for Follow-up.

For the Baseline data, personal benzene correlates with indoor benzene (R = 0.663) but not outdoor benzene (R = -0.062). In addition, indoor benzene does not correlate with outdoor benzene (R = 0.199). For the Follow-up data, personal benzene correlates with indoor air (R = 0.437) and with outdoor air (Spearman, R = 0.451)) but, in contrast to the Baseline data, benzene in indoor air correlates with that in outdoor air (Spearman); benzene in the outdoor environment can contribute to the benzene in indoor air. All together, these data suggest that both indoor and outdoor sources contribute to personal exposure. However, the low coefficient observed in the Follow-up (a larger sample than in baseline) suggests that personal measures for benzene, and presumably other VOCs, such as those released by vehicular emissions, will provide a better estimate than indoor measurements alone.

Personal, Indoor, and Outdoor Carbonyl (formaldehyde) Concentrations: Correlations among formaldehyde measured from indoor, outdoor, and personal platforms are shown in ***Table 3-12f*** for Baseline and ***Table 3-12g*** for Follow-up.

Table 3-12d. Pearson (top panel) and Spearman (bottom panel) Correlation Coefficients of Benzene Concentrations from Three Sampling Platforms during Baseline

(For each cell the correlation is shown on the top line, p-value on middle line, and the number of samples is given on the bottom line.)

Correlation Coefficients	Benzene		
	Personal Air	**Indoor Air**	**Outdoor Air**
Pearson			
Personal Air Benzene (Total N = 92)	—-	0.663 *<0.0001* 39	-0.062 *0.716* 37
Indoor Air Benzene (Total N = 43)		—-	0.199 *0.225* 39
Outdoor Air Benzene (Total N = 40)			—-
Spearman			
Personal Air Benzene (Total N = 92)	—-	0.897 *<0.0001* 39	0.247 *0.140* 37
Indoor Air Benzene (Total N = 43)		—-	0.256 *0.116* 39
Outdoor Air Benzene (Total N = 40)			—-

Table 3-12e. Pearson (top panel) and Spearman (bottom panel) Correlation Coefficients of Benzene Concentrations from Three Sampling Platforms during Follow-up

(For each cell the correlation is shown on the top line, p-value on middle line, and the number of samples is given on the bottom line.)

Correlation Coefficients	Benzene		
	Personal Air	Indoor Air	Outdoor Air
Pearson			
Personal Air Benzene (Total N = 94)	—-	0.437 *<0.0001* 90	-0.032 *0.775* 83
Indoor Air Benzene (Total N = 99)		—-	0.090 *0.405* 88
Outdoor Air Benzene (Total N = 91)			—-
Spearman			
Personal Air Benzene (Total N = 94)	—-	0.824 *<0.0001* 90	0.451 *<0.0001* 83
Indoor Air Benzene (Total N = 99)		—-	0.441 *<0.0001* 88
Outdoor Air Benzene (Total N = 91)			—-

Table 3-12f. Pearson (top panel) and Spearman (bottom panel) Correlation Coefficients of Formaldehyde Concentrations from Three Sampling Platforms during Baseline

(For each cell the correlation is shown on the top line, p-value on middle line, and the number of samples is given on the bottom line.)

Correlation Coefficients	Formaldehyde		
	Personal Air	**Indoor Air**	**Outdoor Air**
Pearson			
Personal Air Formaldehyde (Total N = 134)	—-	0.643 *<0.0001* 43	0.326 *0.040* 40
Indoor Air Formaldehyde (Total N = 67)		—-	0.081 *0.631* 41
Outdoor Air Formaldehyde (Total N = 42)			—-
Spearman			
Personal Air Formaldehyde (Total N = 134)	—-	0.759 *<0.0001* 43	0.321 *0.043* 40
Indoor Air Formaldehyde (Total N = 67)		—-	0.182 *0.255* 41
Outdoor Air Formaldehyde (Total N = 42)			—-

Table 3-12g. Pearson (top panel) and Spearman (bottom panel) Correlation Coefficients of Formaldehyde Concentrations from Three Sampling Platforms during Follow-up

(For each cell the correlation is shown on the top line, p-value on middle line, and the number of samples is given on the bottom line.)

Correlation Coefficients	Formaldehyde		
	Personal Air	Indoor Air	Outdoor Air
Pearson			
Personal Air Formaldehyde (Total N = 128)	—-	0.787 *<0.0001* 94	0.098 *0.366* 87
Indoor Air Formaldehyde (Total N = 116)		—-	0.176 *0.101* 88
Outdoor Air Formaldehyde (Total N = 91)			—-
Spearman			
Personal Air Formaldehyde (Total N = 128)	—-	0.735 *<0.0001* 94	0.192 *0.074* 87
Indoor Air Formaldehyde (Total N = 116)		—-	0.249 *0.019* 88
Outdoor Air Formaldehyde (Total N = 91)			—-

As was seen for benzene, personal exposures to formaldehyde at Baseline AND Follow-up are significantly correlated with indoor levels of formaldehyde (R = 0.643, R = 0.787) and show modest correlations with outdoor air at Baseline (R = 0.326), but not during Follow-up (R = 0.098). The modest correlation coefficients of personal air to indoor air indicate that personal exposure is the superior measure for integrated formaldehyde exposure. The associations of formaldehyde concentrations in the indoor environment with formaldehyde levels in the outdoor environment are not clear. These results suggest that indoor formaldehyde concentrations contribute more to personal exposure than outdoor air and that outdoor concentrations of formaldehyde might contribute to personal exposures.

Personal, Indoor, and Outdoor Nitrogen Dioxide Concentrations: Correlations among indoor, outdoor, and personal environmental measures for NO_2 are shown in ***Table 3-12h*** for Baseline and ***Table 3-12i*** for Follow-up.

Table 3-12h. Pearson (top panel) and Spearman (bottom panel) Correlation Coefficients of Nitrogen Dioxide Concentrations from Three Sampling Platforms during Baseline

(For each cell the correlation is shown on the top line, p-value on middle line, and the number of samples is given on the bottom line.)

Correlation Coefficients	Nitrogen Dioxide		
	Personal Air	Indoor Air	Outdoor Air
Pearson			
Personal Air Nitrogen Dioxide (Total N = 132)	—-	0.916 *<0.0001* 40	0.492 *0.0002* 37
Indoor Air Nitrogen Dioxide (Total N = 69)		—-	0.353 *0.026* 40
Outdoor Air Nitrogen Dioxide (Total N = 41)			—-
Spearman			
Personal Air Nitrogen Dioxide (Total N = 132)	—-	0.899 *<0.0001* 40	0.554 *0.0004* 37
Indoor Air Nitrogen Dioxide (Total N = 69)		—-	0.474 *0.002* 40
Outdoor Air Nitrogen Dioxide (Total N = 41)			—-

For Baseline, personal air NO_2 concentrations are highly correlated with indoor NO_2 concentrations and outdoor NO_2 concentrations. The larger correlation coefficient suggests that the indoor sources of NO_2 are a larger contributor to personal exposures NO_2 r than outdoor sources. Data from the Follow-up show the same patterns, although the significance of Spearman correlations and not Pearson correlations suggests that the Follow-up data contain more extreme values of concentrations. Thus, the measurement of indoor NO_2 may be a good approximation of personal exposure for the studied population.

Table 3-12i. Pearson (top panel) and Spearman (bottom panel) Correlation Coefficients of Nitrogen Dioxide Concentrations from Three Sampling Platforms during Follow-up

(For each cell the correlation is shown on the top line, p-value on middle line, and the number of samples is given on the bottom line.)

Correlation Coefficients	Nitrogen Dioxide		
	Personal Air	Indoor Air	Outdoor Air
Pearson			
Personal Air Nitrogen Dioxide (Total N = 129)	—-	0.957 *<0.0001* 97	0.084 *0.436* 88
Indoor Air Nitrogen Dioxide (Total N = 121)		—-	0.111 0.290 92
Outdoor Air Nitrogen Dioxide (Total N = 92)			—-
Spearman			
Personal Air Nitrogen Dioxide (Total N = 129)	—-	0.898 *<0.0001* 97	0.5388 *<0.0001* 88
Indoor Air Nitrogen Dioxide (Total N = 121)		—-	0.469 *<0.0001* 92
Outdoor Air Nitrogen Dioxide (Total N = 92)			—-

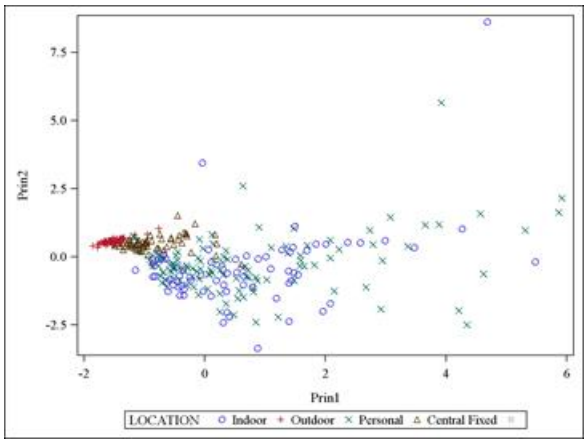
Multivariate Analysis: To help further assess potential differences between the concentrations measured at the personal, indoor, outdoor, and central site locations, we conducted a multivariate analysis, specifically principal component analysis, of selected VOCs and carbonyls (benzene and formaldehyde), PM mass, and PM ETS. We conducted principal components analysis across all platforms (303 observations and 6 variables; SAS PRINCOMP procedure) to examine the possible correlations of measures and observed distinct differences in three principal components between outdoor/central site and personal/indoor locations. ***Table 3-12j*** illustrates that three principal components account for more than 73% of the overall variance in all measurements.

Table 3-12j. Principal Components of Benzene, Formaldehyde, PM_{10}, and ETS Concentrations Measured on Four Sampling Platforms during Baseline

Principal Component	Proportion of Variance Explained	Cumulative Proportion
1	0.383	0.383
2	0.184	0.568
3	0.165	0.733
4	0.130	0.863
5	0.087	0.950
6	0.050	1.000

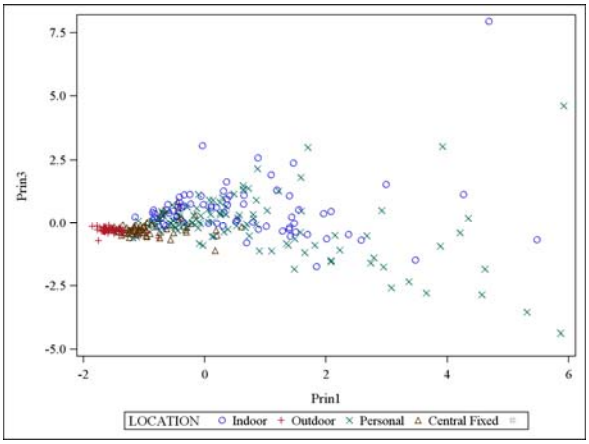
We examined graphical representations of these three principal variance components (components 1, 2, and 3 above) by sampling platform, and the data in ***Figures 3-12b*** and ***3-12c*** clearly show separation of the predominantly outdoor (outdoor and central fixed site) and predominantly indoor (indoor and personal) locations.

Figure 3-12b. Principal Components 1 and 2 for Concentrations of Benzene, Formaldehyde, PM_{10}, and ETS Measured on Four Sampling Platforms during Baseline



Although a more in-depth analysis is warranted, this limited multivariate assessment of measurements of airborne pollutants on the four platforms suggests that correctly selected centralized locations may have value as surrogates for more participant-proximate (i.e., traditional outdoor) locations, particularly when coupled with appropriate spatial modeling techniques.

Figure 3-12c. Principal Components 1 and 3 for Concentrations of Benzene, Formaldehyde, PM_{10}, and ETS Measured on Four Sampling Platforms during Baseline



Microbiologicals on PM Filters and in Vacuum Dust: This section presents a discussion of three types of comparisons. We first compare indoor PM_{10} from the MicroPEM filter on the indoor platform to personal PM_{10} from the MicroPEM filter on the personal platform to evaluate the respirable bioaeresols, similar to the analyses for benzene and formaldehyde above. Second, we compare indoor dust and indoor PM_{10}. Intuitively, one would think that any allergens, endotoxins, or mold markers (e.g., 1,3-β-diglucan) in indoor dust would contribute to personal exposure. Although this is true, the inhalation exposure from dust depends on the resuspension of these nonvolatile biological markers adsorbed onto dust particles. The chemical masses measured in MicroPEM dust depends on the fraction of the floor dust that gets resuspended and is measureable in inhalable PM_{10}. It is unlikely, therefore, that the larger particles will be resuspended and measurable on the indoor or personal PM_{10} sampling platforms. In addition, vacuum dust collected for CHATS was passed through a 60-mesh sieve prior to extraction and analysis. This sieving removed “dust bunnies”, hair, insect parts, etc., that did not contribute to inhalation exposure. It is important for the comparisons presented here to keep in mind the dramatically different sample constitution of floor dust as compared to PM_{10} filter. Our third comparison evaluates endoxtoxin and glucan concentrations between personal PM_{10} filters and vacuum dust to include the potential for exposures to endotoxin and glucan from other environments that the personal samples capture. If there are correlations of microbiologicals between dust and indoor PM, then a comparison of dust to personal PM can provide some measure of the relative contributions of indoor exposure at home to exposures from all of the microenvironments the person encountered during the sample collection period.

Endotoxin and glucan were measureable in a very high proportion of both dust and PM_{10} filters. Allergens were rarely detected on filters, so comparisons of allergens among locations and media were not possible. Meaningful comparisons of allergens on filters to other media require either larger volumes of air to be collected or more sensitive measures for allergens.

Endotoxin: Correlations between concentrations measured on indoor platforms (MicroPEM filters and dust) and personal platform (MicroPEM filters) for endotoxin are shown in ***Table 3-12k*** for Baseline and ***Table 3-12l*** for Follow-up.

Table 3-12k. Pearson (top panel) and Spearman (bottom panel) Correlation Coefficients of Endotoxin Concentrations from Three Sampling Platforms during Baseline

(For each cell the correlation is shown on the top line, p-value on middle line, and the number of samples is given on the bottom line.)

Correlation Coefficients	Endotoxin		
	Personal Filter	**Indoor Filter**	**Dust**
Pearson			
Personal Filter Endotoxin (Total N = 72)	—-	0.558 *0.118* 9	0.096 *0.512* 49
Indoor Filter Endotoxin (Total N = 18)		—-	-0.127 *0.653* 15
Dust Endotoxin (Total N = 73)			—-
Spearman			
Personal Filter Endotoxin (Total N = 72)	—-	0.717 *0.030* 9	0.115 *0.430* 49
Indoor Filter Endotoxin (Total N = 18)		—-	0.229 *0.413* 15
Dust Endotoxin (Total N = 73)			—-

Table 3-12l. Pearson (top panel) and Spearman (bottom panel) Correlation Coefficients of Endotoxin Concentrations from Three Sampling Platforms during Follow-up

(For each cell the correlation is shown on the top line, p-value on middle line, and the number of samples is given on the bottom line.)

Correlation Coefficients	Endotoxin		
	Personal Filter	Indoor Filter	Dust
Pearson			
Personal Filter Endotoxin (Total N = 72)	—-	0.421 *0.015* 33	0.040 *0.816* 36
Indoor Filter Endotoxin (Total N = 18)		—-	-0.042 *0.814* 33
Dust Endotoxin (Total N = 73)			—-
Spearman			
Personal Filter Endotoxin (Total N = 72)	—-	0.598 *0.0002* 33	0.263 *0.122* 36
Indoor Filter Endotoxin (Total N = 18)		—-	0.082 *0.651* 33
Dust Endotoxin (Total N = 73)			—-

Endotoxin in personal air associates with indoor PM (Spearman) for Baseline and by both correlation methods in the Follow-up data. There is no association of either personal PM filter or indoor PM filters with endotoxin in dust. Based on our findings, indoor dust measurements cannot be used to estimate exposure to endotoxin. Dust can be used to screen for pollutants that are associated with particulates, but analysis of potential exposures will require monitoring at the personal level.

1,3-β-diglucans: Associations among indoor (PM_{10} and dust) and personal PM_{10} measures for glucans are shown in ***Table 3-12m*** for Baseline and ***Table 3-12n*** for Follow-up.

Table 3-12m. Pearson (top panel) and Spearman (bottom panel) Correlation Coefficients of Glucan Concentrations from Three Sampling Platforms during Baseline

(For each cell the correlation is shown on the top line, p-value on middle line, and the number of samples is given on the bottom line.)

Correlation Coefficients	Glucan		
	Personal Filter	Indoor Filter	Dust
Pearson			
Personal Filter Glucan (Total N = 87)	—-	0.444 *0.085* 16	0.114 *0.333* 74
Indoor Filter Glucan (Total N = 24)		—-	-0.186 *0.383* 24
Dust Glucan (Total N = 91)			—-
Spearman			
Personal Filter Glucan (Total N = 87)	—-	0.550 *0.027* 16	0.142 *0.227* 74
Indoor Filter Glucan (Total N = 24)		—-	-0.046 *0.831* 24
Dust Glucan (Total N = 91)			—-

In general, the glucan data are very similar to those for endotoxin. Associations between personal PM filters and indoor PM filters are shown, with higher degrees of significance shown in the Follow-up study with the greater number of samples; the coefficients are similar. No associations are seen for either personal or indoor air with indoor dust. However, the Follow-up data show near-significant (P = 0.067) and significant (P = 0.006) correlations for personal and indoor PM filters, respectively, with indoor dust, rebased on Pearson correlations. However, correlations are not significant when Spearman correlations are performed, indicating that extreme values are found in the data. As for endotoxin, indoor dust cannot be used to estimate inhalation exposure to glucan.

Table 3-12n. Pearson (top panel) and Spearman (bottom panel) Correlations of Glucan Concentrations from Three Sampling Platforms during Follow-up

(For each cell the correlation is shown on the top line, p-value on middle line, and the number of samples is given on the bottom line.)

Correlation Coefficients	Glucan		
	Personal Filter	Indoor Filter	Dust
Pearson			
Personal Filter Glucan (Total N = 87)	—-	0.489 *0.0014* 40	0.249 *0.067* 55
Indoor Filter Glucan (Total N = 24)		—-	0.389 *0.006* 48
Dust Glucan (Total N = 91)			—-
Spearman			
Personal Filter Glucan (Total N = 87)	—-	0.476 *0.002* 40	0.147 *0.283* 55
Indoor Filter Glucan (Total N = 24)		—-	0.095 *0.521* 48
Dust Glucan (Total N = 91)			—-

Indoor and Personal, Environmental, and Biological Measures: The comparison of environmental monitoring data for pollutants to their corresponding metabolites in urine helps to shed light on the extent of absorption of specific pollutants from the environment. Ultimately, the associations of exposure measure, derived either from environmental OR biological samples, with health outcomes are the true measure of utility for further studies. The linkage between an environmental and biological measurement depends on the temporal nature of the exposure and the elimination/metabolic factors of the exposed population. The introduction to this section summarized these considerations. Similarly, any lag times between exposure and health outcome are important; the integrated monitoring data from CHATS are not adequate to address this question. However, the data are sufficient to make some initial assessments that can guide future work. The following analyses are presented with that goal in mind and focus on exposures to ETS, VOCs, and phthalates with the urinary metabolites of

nicotine (cotinine), benzene (N-Acetyl-S-(phenyl)-L-cysteine or PMA), di-ethylhexyl phthalate (mono-ethylhexyl phthalate, MEHP) and di-benzyl phthalate (mono-benzyl phthalate, MBP).

Personal and Indoor ETS Compared to Urinary Cotinine: CHATS is the first study to report whether personal or indoor environmental ETS measurements are related to urinary cotinine data (see ***Table 3-12o*** for Baseline data and ***Table 3-12p*** for Follow-up data). In general, Spearman correlations show more significance relationships than Pearson correlations. The ranked Personal and Indoor ETS concentrations are significantly correlated to the urinary cotinine levels (both unadjusted and creatinine adjusted) in both Baseline and Follow-up. However, the paired ETS-Cotinine concentrations are not.

Table 3-12o. Pearson (top panel) and Spearman (bottom panel) Correlations between ETS Concentrations from Three Sampling Platforms, Urinary Cotinine, and Urinary Cotinine Adjusted for Creatinine from Biospecimens and Samples Collected During Baseline

(For each cell the correlation is shown on the top line, p-value on middle line, and the number of samples is given on the bottom line.)

Correlation Coefficients	ETS		Urinary Cotinine	Urinary Cotinine Adjusted for Creatinine
	Personal	Indoor		
Pearson				
Personal ETS (Total N = 94)	—-	0.913 *<0.0001* 14	0.045 *0.7083* 71	-0.018 *0.8808* 71
Indoor ETS (Total N = 39)		—-	0.429 *0.0227* 28	0.267 *0.1700* 28
Urinary Cotinine (Total N = 132)			—-	0.983 *<0.0001* 132
Urinary Cotinine Adjusted for Creatinine (Total N = 132)				—-
Spearman				
Personal ETS (Total N = 94)	—-	0.749 *0.002* 14	0.508 *<0.0001* 71	0.509 *<0.0001* 71
Indoor ETS (Total N = 39)		—-	0.463 *0.0132* 28	0.557 *0.0021* 28
Urinary Cotinine (Total N = 132)			—-	0.738 *<0.0001* 132
Urinary Cotinine Adjusted for Creatinine (Total N = 132)				—-

Table 3-12p. Pearson (top panel) and Spearman (bottom panel) Correlations between ETS concentrations from Three Sampling Platforms, Urinary Cotinine, and Urinary Cotinine Adjusted for Creatinine from Biospecimens and Samples Collected During Follow-up

(For each cell the correlation is shown on the top line, p-value on middle line, and the number of samples is given on the bottom line.)

Correlation Coefficients	ETS		Urinary Cotinine	Urinary Cotinine Adjusted for Creatinine
	Personal	Indoor		
Pearson				
Personal ETS (Total N = 94)	—	0.802 *<0.0001* 41	-0.042 *0.7136* 80	-0.028 *0.8066* 80
Indoor ETS (Total N = 39)		—	-0.026 *0.8405* 64	-0.017 *0.8959* 64
Urinary Cotinine (Total N = 132)			—	0.960 *<0.0001* 137
Urinary Cotinine Adjusted for Creatinine (Total N = 132)				—
Spearman				
Personal ETS (Total N = 94)	—	0.812 *<0.0001* 41	0.566 *<0.0001* 80	0.424 *<0.0001* 80
Indoor ETS (Total N = 39)		—	0.578 *<0.0001* 64	0.500 *<0.0001* 64
Urinary Cotinine (Total N = 132)			—	0.727 *<0.0001* 137
Urinary Cotinine Adjusted for Creatinine (Total N = 132)				—

Differences in statistical significance with type of correlation (Pearson versus Spearman) may be attributable to temporal differences in the sample collection periods for the PM_{10} and urine samples. The PM_{10} samples were integrated over 5 to 9 days; the urine sample was usually collected on the last day. Since urinary cotinine has a half-life of approximately 20 hours (depending on individual metabolisms), cotinine measurements will likely not be representative of the full period of environmental monitoring and weak Pearson correlations between ETS exposure and urinary cotinine measurement may be expected from statistical analysis of the continuous measurements. Conversely, the somewhat less powerful, nonparametric Spearman

ranked correlation may show a stronger association because the test assumes all of the data are aligned monotonically. The statistically significant ETS-Cotinine Spearman correlations suggest that statistically significant Pearson correlations might be observed if ETS samples are collected over the 24 hours prior to urine sample collection. The adjustment of urinary metabolite concentrations for creatinine had minimal impact on the correlations.

Personal VOC and Urinary VOC Metabolite Concentrations: The correlations between benzene in personal air and urinary PMA, not corrected for creatinine, are shown in ***Table 3-12q,*** which provides data for both Baseline and Follow-up.

Table 3-12q. Pearson and Spearman Correlations between Benzene Concentrations from the Personal Platform and Urinary Metabolite PMA from Biospecimens Collected During Baseline and Follow-up

(For each cell the correlation is shown on the top line, p-value on middle line, and the number of samples is given on the bottom line.)

	Baseline Data	Follow-up Data
Pearson Correlation Coefficients	0.0414 0.834 28	-0.109 0.605 25
Spearman Correlation Coefficients	0.128 0.516 28	0.288 0.162 25

The data show no association between benzene in air and the urinary metabolite PMA most likely because the integrated benzene concentration in air was compared to a urinary metabolite with a half-life of only a day or two. Varying concentrations of benzene in air, when integrated over 7 days, will inadequately reflect the urinary concentration of PMA. A caveat to this is the method detection limit of PMA in urine, which appears to be an issue here, i.e., many of the urinary PMA measures are at or near the method level of detection (LOD). Investigations of the relationships of benzene in air and PBA in urine with health outcome assessments should be evaluated to better identify the most appropriate measure of exposure.

Dust Phthlates and Urinary Metabolite Concentrations: Correlations between DEHP and DBP in dust and their corresponding metabolite (MEHP and MBP, respectively) in urine are shown in ***Table 3-12r*** for both Baseline and Follow-up.

The data show no relationship between the parent phthalates in dust and the corresponding metabolites in urine. This result is not surprising, given the lack of association of indoor dust with personal measures for endotxins or glucans (***Tables 3-12k*** through ***3-12n***).

However, as for benzene, associations with health outcome need to be assessed to identify the most appropriate measures of exposure for any follow-on study.

Table 3-12r. Pearson and Spearman Correlations between Phthalate Concentrations from Dust and Urinary Metabolites from Biospecimens and Samples Collected During Baseline and Follow-up

(For each cell the correlation is shown on the top line, p-value on middle line, and the number of samples is given on the bottom line.)

	Baseline Data	Follow-up Data
DEHP: MEHP		
Pearson Correlation Coefficients	0.106 0.392 89	0.106 0.392 67
Spearman Correlation Coefficients	0.114 0.357 89	0.114 0.357 67
DBP: MBP		
Pearson Correlation Coefficients	-0.068 0.530 88	-0.052 0.663 73
Spearman Correlation Coefficients	0.113 0.295 88	0.030 0.799 73

4. RECOMMENDATIONS

4.1 Study Design

4.1.1 Stratification on Trailer Site

Although the Feasibility Study did not provide sufficient data to assess significance, a number of indicators, in addition to the anecdotal information, show that the THU living experience of those who lived in the group sites differed from those living on private property. From the FEMA database, the total lease days differed substantially: group site THUs were leased for an average of 393.6 days (N = 11,453) while those on private property were leased for about 2 months longer, for 450.4 days (N = 92,485). Also, the children in the group sites lived in the THUs every day, whereas 8% of the children in private sites did not live in the THUs every day. We recommend that the same stratification be maintained in the Full Study.

4.1.2 Longitudinal Design

The primary rationale for the longitudinal design has been the realization that the incidence of health effects varies by age of the child. Consequently, the assessment of a cohort of children as they age would provide a fuller picture of the impact of a historical exposure in the context of current exposures that change over time. For many different reasons, the initiation of this study has been delayed repeatedly. Almost 8 years have elapsed since Hurricanes Katrina and Rita, so the children who lived in the FEMA-provided THUs have aged. In the Feasibility Study, about a third were teenagers (aged 13 to 17 years), and only 3% were preschool (younger than 5 years of age). With a delay in the initiation of a Full Study of only 1 year, the majority of children are likely to be teenagers at the beginning of the study. The intense follow-up of a longitudinal design will be increasingly challenging as the teenagers age and leave the home. We recommend that the Full Study consist of a Baseline Assessment of a sufficient number of children to answer questions of association of health effects with THU living experiences.

4.1.3 Collection of Self-reported Historical Information

The instrument used in CHATS asked the parent to recall residential history, characteristics and regular activities in past homes, as well as the health history of the child. To assess whether the parents felt confident in their ability to answer these questions, immediately following these sections of the questionnaire, we asked the parents about their confidence. ***Table 4-1a*** outlines the interviewee responses to certain modules of the Baseline Assessment instrument and indicates that the interviewees were very confident in their answers. Although this instrument does not assess the validity of their answers, we believe the

parents feel they are able to provide meaningful answers and recommend that the Full Study incorporate the detailed questions that were used in the Feasibility Study instruments.

Table 4-1a. Confidence of Interviewee Regarding Responses to Certain Modules of the Baseline Instrument

Question	Total Responses	Percent, %			
		Very Confident	Somewhat Confident	Somewhat Uncertain	Very Uncertain
How confident are you of your answers about housing?	181	64	31	4	1
How confident are you of your answers about your child's health?	181	64	33	3	0
How confident are you of your answers about characteristics of and activities in the home?	181	61	37	2	0

4.2 Sample Design

4.2.1 Frame Quality

The sample frame for the Feasibility Study turned out to be of very high quality, free of duplicates, accurate, and quite complete. We were able to locate most of the exposed sample households through our tracing operation, with a successful trace rate of 86%. We recommend that the same file be used for the Full Study.

4.2.2 Use of GIS

The original plan for the Feasibility Study involved extensive use of GIS technology to locate the exposed households and the CBGs in which they were located. We recommend that similar GIS approaches be used for the Full Study to help link the sample design with the data collection process.

4.2.3 Unexposed Sample

The original sample design plan for the Feasibility Study included a separate and independent sample of unexposed households within each CBG in which there was at least one exposed household. Ultimately, this approach was revised to search for unexposed households within close proximity of the exposed starter household. This strategy turned out to be cost-effective and efficient, and we recommend that the same strategy be used for the Full Study.

4.2.4 Eligibility Rate

The final data from the Feasibility Study strongly suggest that the eligibility rate was overestimated. Some of this difference was due to definition—exactly how households were designated eligible or ineligible (see ***Section 4.3*** for a detailed discussion)—but some indications also showed that the sample survey data did not correspond to census figures in terms of the proportion of households with at least one eligible child. For the Full Study, we recommend using a lower level of eligibility rate based on an in-depth analysis of the results of the Feasibility Study.

4.3 Subject Recruitment Recommendations

4.3.1 Staffing: Field Interviewer Experience

Attrition was a primary concern early in data collection. Of the original 15 field interviewers hired in April 2012, only 6 remained on staff for the entire data collection period, for an attrition rate of 60%. Moreover, most of this attrition occurred within the first 3 months of data collection. Of the 9 who left the project, 7 did so prior to the end of the third month of data collection. This issue occurred in New Orleans particularly, where 63% of the initial hires left within the first 3 months, and only 1 hire remained on staff through the conclusion of the Baseline Assessment phase and the beginning of Follow-up data collection. These early losses prompted a second round of recruiting and training in New Orleans in August 2012.

The initial candidates hired in April had relatively limited field interviewing experience. Our goal was to hire staff with prior field interviewing experience, but the pool of candidates in the Gulf Coast area was very low. While attrition among this second group remained high, the rate of 43% was lower than the overall attrition rate of the initial April session (60%) and substantially lower than the attrition rate among April hires in New Orleans (88%). Additionally, the number of hours worked each week, the overall costs per case, and the rate at which work was completed improved dramatically once these more experienced field interviewers began contributing to the data collection effort in September.

After field interviewers gained some experience in the field, their attrition rate decreased dramatically. In the final 6 months of data collection, only one field interviewer left the project. In the 6 months prior to that point, at least one field interviewer left the project each month.

Future studies that conduct work in the Gulf Coast area should plan for a longer time period to recruit and hire field interviewers. We also recommend that hiring outside of the New Orleans metro area, with field interviewers traveling into the area by car, be considered.

4.3.2 Training

Although field interviewers left training with a strong overall understanding of the CHATS instruments, they experienced some confusion between the various types of deployment and retrieval processes. Simple mistakes, unfortunately, have led to invalid data collection on the MicroPEM™, aldehyde, VOC, and NO_2 sampling devices. As a result, we recommend more training for future CHATS work in a variety of ways.

All 22 field interviewers hired for CHATS completed an extensive 5-day training, reviewing all protocols involved in conducting the screening, both sessions of the interview, and the various types of deployments. Given the complexity of the study, covering all the requisite material in 5 days proved challenging and limited the amount of time that could be devoted to supervised practice of the deployment and retrieval procedures in the classroom. We recommend adding one day to training to allow significantly more time for repetition of these procedures and hands-on practice, which should help prevent errors in the field.

The small sample size selected for Baseline and Follow-up deployments also created a problem with field interviewer retention of the relevant procedures. Often, field interviewers went several weeks, and in a few occasions, months, between deployments; thus, refreshing field interviewers on procedures during these stretches became necessary. In late January 2013, an environmental expert hosted a group call on the deployment procedures and common errors. This call was followed by a question-and-answer session. In March 2013, boxes with dummy samplers were shipped to all field interviewers. The interviewers could use the contents of these boxes to progress through a practice interview, allowing them to work independently on mock cases and thus have repeated exposure to the deployment procedures before they conducted actual field work. The field interviewers' responses to both of these efforts were positive, and following both reviews, the frequency of failures due to field interviewer-generated deployment was reduced. Having training kits available from the beginning of data collection and scheduling routine group calls throughout data collection as refreshers on these procedures and any observed common errors should assist in limiting these problems further.

4.3.3 Screening Procedures

The screening process conducted on the iPAQ worked as designed, but unanticipated issues with the exposed and unexposed samples warrant changes to the protocol prior to fielding a Full Study.

The primary hindrance to data collection was the rate at which households were selected for participation in the Baseline Assessment. The eligibility rate was initially projected

to be 28%, but in fact, was only 19% among exposed households and 13% among unexposed households. This diminished eligibility rate meant that 10.6 screenings had to be completed for each Baseline participant. Had the eligibility rate for these two populations been the expected 28%, the same Baseline sample could have been drawn from a screening population of 1,082 households, 41% fewer than the 1,851 actually screened. The result of the low eligibility rate was significantly higher cost and time devoted to the screening effort. Moreover, by design, the unexposed sample was not released in a geographic area until exposed cases were discovered and completed. The diminished number of exposed Baseline sample caused by this eligibility shortfall delayed the beginning of work on the unexposed sample. The final release of unexposed cases came only 10 days prior to the cessation of screening activities.

Some of this eligibility shortfall could be corrected through a change in the way the screening program identifies eligible participants. By design, a household designated as "exposed" at the sampling stage was not able to select a child for participation who met the eligibility criteria for the "unexposed" cohort. The same was true for households designated as "unexposed," where children who had been "exposed" resided. By changing the screening program to identify children meeting either sampling criteria, both the exposed and unexposed samples could be identified more quickly and efficiently. This approach would reduce the overall number of screenings that need to be conducted, and as identifying the exposed Baseline sample should be more expedited, allow the release of the unexposed screening sample earlier in the data collection process.

4.3.4 Participant Burden

Those individuals who refused to participate and frequently rescheduled appointments expressed concern about the length of time required to complete the CHATS Feasibility Study. The actual average time for completion was longer than the estimated burden calculated from initial testing. The estimated time range was 2 hours, 15 minutes to 3 hours, 15 minutes. The actual average time required for the Baseline Session 1 interview was 140 minutes. The average amount of time for the Session 2 interview was 88 minutes for the field interviewer portion and 68 minutes for the nurse portion, though more often than not, these portions could be conducted simultaneously. The average times to complete the Follow-up Session 1, Session 2, and nurse portion were 89, 68, and 54 minutes, respectively. Again, the Session 2 and nurse interviews were usually conducted simultaneously. A person who participated in both the Baseline and Follow-up devoted 385 minutes, on average, to the CHATS Study, or roughly 6.5 hours, discounting any time spent on the Time and Activity Diary, gathering medical records, wearing the PEM platform, or devoting more time for another appointment should a nurse not be available at the time of Session 2. Participants found that clearing their schedules for these

long time periods was quite difficult, especially because both child and parent needed to be present for these interviews.

The largest single section of either Baseline or Follow-up was the informed consent process. This process included the parent or guardian consent, as well as the assent for the child. For the Feasibility Study, the assent from the child was completed only after the consent from the parent or guardian was secured. The scripts were tailored based on the age of the child; for example, only the households with a child aged 7 years or older was told about the personal MicroPEM, but because of the very complex and numerous components of the study, the consent and assent scripts required significant detail to ensure the study met the requirements for informed consent. For the Baseline, these scripts took an average of 26 minutes. For the Follow-up, these scripts took an average of 19 minutes.

Additionally, consents for specific placement of environmental samplers were collected throughout the deployment sections of the Baseline and Follow-up Session 1 assessments. We developed these consents to ensure that the participants agreed to each subcomponent of the environmental sampling, such as the actual placement of the indoor platform in a specific location within the home. From participants' feedback we learned that in most cases, participants felt they had already agreed. Many participants thought these additional scripts to confirm continued agreement were overly repetitive.

Because of the smaller-than-expected sample size of Baseline and Follow-up participants, a disproportionate number of households were selected for the substudy. This substudy required the field interviewer to perform three different deployments (personal, indoor, and outdoor), which substantially increased the amount of time it took to both deploy and retrieve the equipment. This factor contributed to the longer-than-expected average time overall. The substudy was only designed as part of the Feasibility Study and would not be included in the Full Study.

Overall, should the interview session be shortened or streamlined, levels of participation and adherence to appointment times may rise accordingly.

4.4 Environmental Assessment

One result from the Feasibility Study provided insight into the PEM platform design. A focus group of RTI employees' children indicated that children preferred a variety of options for wearing the personal platform. The final design was a bag where the environmental samplers could be clipped to a waistpack, a lanyard, the child's backpack, or stand freely on a surface (e.g., desk or table). A child's age and gender also influenced how the PEM platform was carried, and subsequently wearing compliance. Feedback from CHATS participants collected

during the Baseline and Follow-up determined the lanyard option was the least popular. We found that the waistpack was not suitable for slender children. By default, the preferred options were clipping the PEM platform to the backpack or carrying it by hand. More data analysis is required to understand the PEM platform preferences' relationship to compliance with the study protocol for the personal platform that determined wearing compliance. Recognizing that every child cohort may be different, future children's exposure studies should establish focus groups to understand personal exposure platform design and functionality for children that will maximize compliance with study protocols. Another area of improvement of the exposure study is to engage children more throughout the study so that they understand the purpose, value the sampling activities and compliance to protocols, and have ownership of research.

We also learned better communication with schools is necessary to obtain cooperation from teachers. Although we gave every participant a letter to explain the study, teachers did not understand the purpose of the study and the importance of personal platform-wearing compliance. Field interviewers remarked for a few cases that the participants were not allowed to bring the personal platforms to school or certain classes. These types of incidents are common across all child and adult cohorts. Similar anecdotal incidents have been reported for schools, banks, theaters, federal and municipal buildings, and amusement parks. Future studies should try to apply new informative tools for dissemination to schools and the participants.

Another insight is that a just-in-time mail approach to an environmental exposure study can provide quality data for addressing research hypotheses. In a just-in time study, a central laboratory (RTI) prepares the environmental exposure samples and ships them when field interviewers request them. The cost-effective approach is for the field interviewer to conduct a screener, then schedule an appointment for Session 1; RTI then mails environmental samples to arrive at the interviewer's home on the day before the appointment. This approach still provided 7 to 10 days for field interviewers to complete Session 1 before the MicroPEM had returned to RTI for refurbishing. Studies that do not use a MicroPEM could have an extended period, depending on type of passive gas samplers, before unused samplers had to be returned.

A successful environmental exposure study design requires careful consideration of the impact on four key aspects: data quality, participant burden, field interviewer burden, and sample collection costs. The length and complexity of the questionnaires and environmental sample deployment/retrieval are the determining factors. RTI recommends a careful review of the Baseline data collected to determine what items are critical and should be retained for a Full Study implementation and what items could be dropped to reduce the burden. Additional time in training would improve efficiency and quality from the initial assessments completed. Ideally the home visit~~s~~ time would be reduced to 60 minutes per session.

RTI developed robust procedures for handling environmental and biospecimen samples. The sample handling protocol tracked the preparation, deployment, retrieval, receipt, and laboratory analysis of each sample or electronic file. This approach required effective communication among data collection, laboratory analysis, and database management teams. CHATS generated a large database of high-quality information that will allow analysis of numerous hypotheses into the relationship among survey data, biomarkers of exposure, environmental exposure, and health measures for a diverse children's cohort.

4.5 Health Assessment

The complexity of the data collections protocol, combined with the challenge of collecting data in the home, required a registered nurse to conduct the Health Assessment. We conducted exit interviews with the nursing staff, and they perceived the training, supervision, and availability of clinical support as helpful and appropriate. The challenges that we initially experienced in maintaining the nursing staff may be minimized with enhanced screening during nurse recruitment to ensure clearer understanding of the position requirements.

Although the difficulties the nursing staff experienced with the eNO measurement improved as they learned how to deal with the programming requirements, simplification of those requirements is recommended. An alternative to a programming revision is a protocol revision to allow manual entry of the measurement results into the data collection instrument.

Anecdotal reports from the nursing staff reveal that many participants exhibited heightened anxiety during the visits in anticipation of the venipuncture, which was the visit's last activity. Consequently, we recommend that in future studies, venipuncture be obtained as the initial data collection activity.

4.6 Laboratory Analysis

The laboratory methods established generally performed well. For this Feasibility Study, timely delivery of biological specimens to the LSU laboratory was variable. Delays in delivery impacted data quality, especially for CBC measures. As the study progressed, we resolved these issues, but consistent and timely receipt, log-in, and analysis are critical if quality data are to be generated. For environmental samples, extraction and analysis within the required holding time was, with rare exceptions, accomplished.

The original supposition that VOC badge extracts could be stored prior to analysis was shown to be true for some, but not all, analytes. Vinyl chloride and acrylonitrile were most affected by extract storage. In general, if a VOC badge is collected, it should be extracted and

analyzed for the best data. Also, the collection efficiency of the badge with deployment times that vary from 5 to 9 days should be evaluated to insure linearity.

The carbonyl badges performed very well for formaldehyde, even with harsh predeployment conditions. Unsaturated carbonyls, including acrolein, did not perform well, but this result was expected. There is little reason to anticipate nonlinear sampling behavior for formaldehyde for sampling periods longer than 5 to 7 days. However, prior to the conduct of a Full Study with longer deployment periods, such as 7- to 9-day deployments, we suggest a methods study to verify linear sampling behavior.

The urine-based VOC metabolite determinations generally performed quite well. The method is straightforward and appears to provide reliable data for the metabolite of acrolein, an analyte that has been shown to be problematic with the DNPH sampler (this study and others). Alternate approaches, such as those based on DNSH derivatization, have not performed well and reliable standard preparation has been problematic. The urinary metabolite showed good agreement between laboratories; urine appears to be a valid matrix for assessing recent exposure to this unstable parent carbonyl.

In cases where multiple matrices provide overlapping data on exposure to the same compounds, e.g., VOC badges vs. VOC metabolites in urine, additional data analysis should be performed to determine the most appropriate measure for the health outcome under study. For example, a respiratory outcome that results from exposure to VOCs might be more clearly represented by urinary VOC metabolites (recent exposures) than by integrated 7-day air samples. Similarly, exposures to allergens were assessed by floor dust, personal PM_{10} samples, and specific IgE measures. Although easily measured in floor dust, the personal PM samples almost always provided allergen measures near or below the limit of detection. Additional data analysis should provide insights into whether or not floor dust allergens correlate with specific IgE responses. Modified personal sampling approaches should be considered if inhalation exposure to allergens is to be assessed. Measureable concentrations of endotoxin and glucan in both personal and floor dust samples will likely provided insights into the potential use of floor dust as a surrogate for inhaled allergen. Particle size distributions that impact resuspension of a subset of available allergens are likely to be important in obtaining accurate estimates of exposure.

The submission of data in the feasibility study was accomplished, but adherence to format requirements is critical to provide for facile data processing. Extra attention at the lab level should be paid via additional QA, so that more efficient processing can be accomplished.

4.7 Outreach

A Community Advisory Panel (CAP) was an essential component that added depth to the team's understanding of the impact of Hurricanes Katrina and Rita, and the housing conditions and the feelings associated with unaddressed health and related concerns many of the residents experienced in the aftermath. By inviting respected leaders of organizations that had and continued to provide much-needed services to the community to serve on the CAP and welcoming their honest feedback on all aspects of the study and similarly, the team's willingness to share limitations about what the study could and could not do, the CAP members became front-line advocates for the study and their support was essential to raising and sustaining public awareness about CHATS and encouraging support for the study. We recommend that any future studies engage leading community members to serve on a CAP.

RTI developed linkages with over 900 community leaders and organizations serving the targeted areas in both states, and engage them to support the study and lend their dissemination channels to raise awareness about the study. As a result, the participation of community organizations in helping spread the word about CHATS via their listservs, community bulletin boards, newsletters, and waiting rooms provided a much broader reach than could have been achieved alone. We recommend engaging well-regarded community organizations and leaders as information dissemination channels and supporters to expand the reach of awareness efforts in future studies.

4.7.1 Public Forums

RTI planned and produced five public forums, and through these events, received invitations to conduct two additional presentations to introduce the public to CHATS. While turnout to the five forums was less than optimal, attendees were engaged and overall positive and supportive of the study. The forums were held during Easter Week, which also serves as spring break in the region, to avoid the many festivals that were certain to compete with the launch of CHATS. While spring break was not a desirable time, as many area families use the opportunity to travel out of the area, it was the only option available to introduce the study to the public prior to the launch of the advertising campaign and data collection. Despite limited attendance, a number of key organizations were represented at the forums and the events garnered important media coverage. Our recommendation for future studies in this region is to schedule public forums to avoid major festivals, holidays, and other key events—a particular challenge during the spring and summer. In addition, due to the length of time since the Hurricanes struck, we also recommend partnering with major family and/or health-related events scheduled by other leading organizations and collaborating with them to present the

study. In this way, the team can go to the places where the public will already be in attendance versus working to draw the public to their own events.

RTI developed and disseminated media and outreach materials, and the team secured an equal number of earned media placements as paid for the radio public service announcements. Other materials included CHATS brochure, website FAQs/content, General Information Card, Schools Information Card, community billboard, newspaper, radio, and Facebook advertisements. A small media budget that limited choice of media outlets and the length of exposure to campaign messages (1 month) reduced the effectiveness of the awareness campaign. As a result, both the overlap time with start of data collection and work of field interviewers were limited: only about2% of the potential participants had heard about CHATS before RTI field interviewers contacted them. Our recommendation is to increase dollars allocated to the media/campaign budget, which will significantly help in extending the reach and depth of a paid campaign—the most effective way to broadly raise public awareness.

APPENDIX A

LIST OF ANALYTES, MEDIA, AND SOURCE OF SAMPLES

The CHATS study included a number of different protocols. Consequently, results on analytes are not available for all of the children and sites. In the table provided here, the media and sources for the samples are indicated for all of the analyte results included in this report. In the case of "Substudy Children 7 and older", multiple ambient samples were analyzed for each child: from personal, indoor, and outdoor platforms

Class (abbreviation)	Analyte Name (synonym/acronym)	Media	Source of Sample or Biospecimen				
			Children 3-4 years	Children 5-6 years	All Children 7 and older	Only on Substudy Children 7 and older	Central Site
Allergens	Aspergillus fumigatus allergen (asp f1)	Dust				X	
	Aspergillus fumigatus allergen (asp f1)	MicroPEM filter				X	
	Cockroach Blatella germanica allergen (bla g1)	Dust				X	
	Cockroach Blatella germanica allergen (bla g1)	MicroPEM filter				X	
	Dermatophagoides farinae allergen (der f1)	Dust				X	
	Dermatophagoides farinae allergen (der f1)	MicroPEM filter				X	
	Dermatophagoides pteronyssinus allergen (der p1)	Dust				X	
	Dermatophagoides pteronyssinus allergen (der p1)	MicroPEM filter				X	
	Cat dander allergen (fel d1)	Dust				X	
	Cat dander allergen (fel d1)	MicroPEM filter				X	
Brown Carbon	Environmental Tobacco Smoke (ETS)	MicroPEM filter	X	X	X		X
Carbonyls (or Aldehydes) (CA)	Ethanal (Acetaldehyde)	Air	X	X	X		X
	2-Propanone (Acetone)	Air	X	X	X		X
	(2E)-But-2-enal (Crotonaldehyde)	Air	X	X	X		X
	Methanal (Formaldehyde)	Air	X	X	X		X
	Propionaldehyde (Propanal)	Air	X	X	X		X
Complete Blood Count (CBC)	Basophil (BASO)	Blood		X	X		
	Eosinophil (EOS)	Blood		X	X		
	Hematocrit (HCT)	Blood		X	X		
	Hemoglobin (HGB)	Blood		X	X		
	Lymphocyte (LYMPH)	Blood		X	X		
	Mean corpuscular hemoglobin (MCH)	Blood		X	X		
	Mean corpuscular hemoglobin concentration (MCHC)	Blood		X	X		
	Mean corpuscular volume (MCV)	Blood		X	X		
	Mean platelet volume (MPV)	Blood		X	X		

Class (abbreviation)	Analyte Name (synonym/acronym)	Media	Source of Sample or Biospecimen				
			Children 3-4 years	Children 5-6 years	All Children 7 and older	Only on Substudy Children 7 and older	Central Site
	Monocyte (MONO)	Blood		X	X		
	Neutrophil (NEUT)	Blood		X	X		
	Platelet count (PLTC)	Blood		X	X		
	Red blood cell count (RBC)	Blood		X	X		
	Red blood cell distribution width (RDW)	Blood		X	X		
	White blood cell count (WBC)	Blood		X	X		
Endotoxin	Endotoxin	Dust				X	
	Endotoxin	MicroPEM filter				X	
Glucans	1,3-Beta-di-glucans	Dust				X	
	1,3-Beta-di-glucans	MicroPEM filter				X	
Immunoglobulin E (IgE)	Total IgE (IGE)	Blood		X	X		
	Aspergillus fumigatus antibody	Blood		X	X		
	Cockroach antibody (COCKR)	Blood		X	X		
	Cat dander antibody (CATDAN)	Blood		X	X		
	Dermatophagoides farinae antibody (DERFAR)	Blood		X	X		
	Dermatophagoides pteronyssinus antibody (DERPTE)	Blood		X	X		
Exhaled nitric oxide (NO)	eNO	Breath		X	X		
Nitrogen Dioxide (NX)	NO_2	Air	X	X	X		X
Particulate Matter	PM_{10} (MASS)	MicroPEM filter	X	X	X		X
Phthlates	Butyl benzyl phthalate (BBP)	Dust				X	
	Di-2-ethylhexyl phthalate (DEHP)	Dust				X	
	Dibutyl phthalate (DBP)	Dust				X	
	Dicyclohexyl phthalate (DCP)	Dust				X	
	Diethyl phthalate (DEP)	Dust				X	
	Dimethyl phthalate (DMP)	Dust				X	
	Di-n-octyl phthalate (DNOP)	Dust				X	
	Monocyclohexyl phthalate (MCHP)	Urine				X	
	Mono(3-carboxypropyl) phthalate (MCPP)	Urine				X	

Class (abbreviation)	Analyte Name (synonym/acronym)	Media	Source of Sample or Biospecimen				
			Children 3-4 years	Children 5-6 years	All Children 7 and older	Only on Substudy Children 7 and older	Central Site
	Mono(2-ethylhexyl) phthalate (MEHP)	Urine				X	
	Mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP)	Urine				X	
	Mono(2-ethyl-5-oxohexyl) phthalate (MEOHP)	Urine				X	
	Monoethyl phthalate (MEP)	Urine					
	Mono[(2-carboxymethyl)hexyl] phthalate (MCMHP)	Urine				X	
	Monomethyl phthalate (MMP)	Urine				X	
	Monononyl phthalate (MNP)	Urine				X	
	Monooctyl phthalate (MOP)	Urine				X	
	Monoisononyl phthalate (MIP)	Urine				X	
	Monobutyl phthalate (MBP)	Urine				X	
	Monobenzyl phthalate (MBzP)	Urine				X	
VOC and VOC metabolites (VC)	1,3-Butadiene	Air				X	
	Acrylonitrile	Air				X	
	a-pinene	Air				X	
	Benzene	Air				X	
	m,p-Xylenes	Air				X	
	Methyl ethyl ketone (MEK)	Air				X	
	Methyl t-butyl ether (MTBE)	Air				X	
	Naphthalene	Air				X	
	n-Octane	Air				X	
	p-Dichlorobenzene	Air				X	
	Styrene	Air				X	
	Toluene	Air				X	
	Trichloroethylene (TCE)	Air				X	
	Vinyl chloride	Air				X	
	N-Acetyl-S-benzyl-1-cysteine (BMA)	Urine				X	
	N-Acetyl-S-(2-carboxyethyl)-1-cysteine (CEMA)	Urine				X	
	N-Acetyl-S-(2-cyanoethyl)-1-cysteine (CYMA)	Urine				X	

Class (abbreviation)	Analyte Name (synonym/acronym)	Media	Source of Sample or Biospecimen				
			Children 3-4 years	Children 5-6 years	All Children 7 and older	Only on Substudy Children 7 and older	Central Site
	N-Acetyl-S-(1,2-dichlorovinyl)-1-cysteine (1,2DCVMA)	Urine				X	
	N-Acetyl-S-(2,2-dichlorovinyl)-1-cysteine (2,2DCVMA)	Urine				X	
	N-Acetyl-S-(3,4-dihydroxybutyl)-1-cysteine (DHBMA)	Urine				X	
	N-Acetyl-S-(2,4-dimethylphenyl)-1-cysteine (DPMA)	Urine				X	
	N-Acetyl-S-(2-hydroxy-3-pripionamide)-1-cysteine (GAMA)	Urine				X	
	N-Acetyl-S-(2-hydroxyethyl)-1-cysteine (HEMA)	Urine				X	
	N-Acetyl-S-(3-hydroxyropyl-1-methyl)-1-cysteine (HPMMA)	Urine				X	
	Mandelic Acid (MA)	Urine				X	
	2-Methylhippuric acid (2MHA)	Urine				X	
	3-Methylhippuric acid; 4-Methylhippuric acid (34MHA)	Urine				X	
	N-Acetyl-S-(1-hydroxymethyl-2-propenyl)-l-cysteine and N-Acetyl-s-(2-hydroxy-3-buteny 1)-1-cysteine (MHBMA)	Urine				X	
	trans, trans-Muconic acid (MU)	Urine				X	
	Phenylglyoxylic acid (PGA)	Urine				X	
	N-Acetyl-S-(1-phenyl-2-hydroxyethyl)-1-cysteine (PHEMA)	Urine				X	
	N-Acetyl-S-(phenyl)-1-cysteine (PMA)	Urine				X	

In addition to those listed above, hydrogen sulfide was assessed in the homes of a subsample of 60 participants.

Appendix B:

Protocols for Laboratory Analyses Conducted in the CHATS Feasibility Study

Protocol Table of Contents

Recommended Operating Procedure CANE-CHATS-009 for Gravimetric Analysis of Mass Collected on Filter Media in Children's Health after the Storms (CHATS) B-4

Recommended Operating Procedure CANE-CHATS-010 for Sampling and Analysis of Aerosols for Black Carbon and Environmental Tobacco Smoke Using Optical Absorbance in Children's Health after the Storms (CHATS) B-17

Center for Microbial Communities System and Health Research - Research Operating Procedure 03: Extraction and Analysis of Dust and PM Filter Samples for Environmental (Asp f 1, Bla g 1, Der f 1, Der p 1, and Fel d1) Antigens for CHATS B-28

Recommended Operating Procedure CANE-CHATS-012 for Temperature and Relative Humidity Collection Using the HOBO U10 Data Logger for Children's Health after the Storms (CHATS) B-37

Center for Microbial Communities System and Health Research - Research Operating Procedure 02: Extraction and Analysis of Dust and PM Filter Samples for (1,3)-β-D-Glucan for Children's Health after the Storms (CHATS) B-45

Center for Microbial Communities System and Health Research - Research Operating Procedure 01: Extraction and Analysis of Dust and PM Filter Samples for Endotoxin for Children's Health after the Storms (CHATS) B-52

Center for Microbial Communities System and Health Research - Research Operating Procedure 04: Sieving of Dust Samples for Children's Health after the Storms (CHATS) B-59

Research Operating Procedure EAR-CHATS-001 – Procedure for Determining Carbonyls from Passive Samplers for Children's Health after the Storms (CHATS) B-65

Research Operating Procedure EAR-CHATS-002 – Procedure for Determining Volatile Organic Compounds from Passive Samplers for Children's Health after the Storms (CHATS) B-77

Research Operating Procedure 09: Procedure for Determining Hydrogen Sulfide (H2S) from Passive Samplers for Children's Health after the Storms (CHATS) B-91

Research Operating Procedures for Handling and Analysis of Passive NO2 Samplers for Children's Health after the Storms (CHATS) B-98

Research Operating Procedures CORE-HEME-1 Automated Complete Blood Count (CBC) on the Coulter LH750 for Children's Health after the Storms (CHATS) B-112

Research Operating Procedure for Determination of Specific IgEs using the IMMULITE® 2000 3gAllergy™ Specific IgE Universal Kit for Children's Health after the Storms (CHATS) B-167

Research Operating Procedure Total IGE for Determination of Total IgE in CHATS using the Beckman Coulter Access Immunoassay System for Children's Health after the Storms (CHATS) B-177

Research Operating Procedure CORE-CHEM-DXC-24 for Quantitative Determination of Creatinine in Human Plasma, Serum Using the SYNCHRON® System(s) for Children's Health after the Storms (CHATS) B-191

Research Operating Procedure PHTHMET-U01 – Phthalate Metabolites in Urine by High Resolution-Accurate Mass Spectrometry for Children's Health after the Storms (CHATS) B-204

Protocol Table of Contents, continued

Research Operating Procedure MERACIDVOC-U01 Synopsis – Mercapturic Acid Metabolites in Urine by High Resolution-Accurate Mass Spectrometry for Children's Health after the Storms (CHATS) B-214

Nicotine Cotinine in Urine by Turboflow LC-Electrospray Tandem Mass Spectrometry B-223

Research Operating Procedure EAR-CHATS-22 – Determination of Phthalate Metabolites in Urine for Children's Health after the Storms (CHATS) B-226

Research Operating Procedure EAR-CHATS-23 – Determination of VOC Metabolites in Urine for Children's Health after the Storms (CHATS) B-238

Recommended Operating Procedure CANE-CHATS-009

for

Gravimetric Analysis of Mass Collected

on Filter Media

in Children's Health after the Storms (CHATS)

Prepared by: _____ Date: 05/10/2011
Seung-Hyun Cho

Reviewed by: _____ Date: 05/10/2011
Ryan Chartier

Reviewed by: _____ Date: 12/13/2011
Cynthia Salmons

Reviewed by: _____ Date: 12/14/2011
James Raymer

Approved by: _____ Date: 12/8/2011
Diane K. Wagener

RTI International
3040 Cornwallis Road
Research Triangle Park, NC 27709
USA

List of Revisions

CHATS Number	Revision Number	Changes	Date
CANE-CHATS-009	0	Original (derived from prior projects; CANE ROP No. 095)	5/10/2011
CANE-CHATS-009	1	Data quality review section added	12/9/2011

Table of Contents

1.0 Scope & Application 4
2.0 Summary of Method 4
3.0 Cautions 4
4.0 Interferences 4
5.0 Personnel Qualifications 5
6.0 Apparatus & Materials 5
7.0 Measurement Procedures 6
7.1 Activities (in chronological order) 6
7.1.1 Filter Preparation for Precharacterization 6
7.1.2 Gravimetric Analysis (Primary) 6
7.1.3 Gravimetric Analyses (Primary) 6
7.2 Quality Assurance/Quality Control 11
7.2.1 Chain-of-Custody 11
7.2.2 Calibration Check 11
7.2.3 Accuracy Check 11
7.2.4 Evaluation Criteria 12
7.2.5 Corrective Action 12
7.2.6 Record keeping 12
7.2.7 Calculations 12
7.2.8 Data Quality Review 13
7.3 Short Form Directions 14
7.3.1 Preliminary 14
7.3.2 Balance Initiation 14
7.3.3 Weighing 15
7.3.4 Printing 16

1.0 Scope & Application

Particulate matter in ambient air is captured onto filters and measured by pre- and post-weighing of the filters. Mass data are useful in determining total particulate matter loading for a given airmass. The same analytical balance should be used to ensure consistency when weighing the filters before and after sample collection. This ROP is written with the Mettler AT20, AT261, or similar balance in mind. Alternative procedures for the Mettler UMT2 and UMX2 balance are included within this ROP as well.

2.0 Summary of Method

Ambient aerosols are collected onto Teflon filters and the mass of the aerosols is determined by weighing the filters before and after sample collection.

3.0 Cautions

Radioactivity of polonium static discharging strip

4.0 Interferences

Positive interferences may arise due to contamination from airborne indoor dust and particles shed from clothing. The absolute amount of particulate matter captured onto Teflon filters is normally quite small (10 µg-100 µg) and, thus, relatively easy to contaminate. It is imperative that filters are always handled in a clean, dust-free environment to prevent contamination from airborne dust.

Negative interferences may be caused by evaporative losses of volatile or semi-volatile components of the aerosols. These interferences commonly occur during sampling procedures, but further evaporative losses may be minimized by storing and shipping the filters at low temperatures (-20 °C for long-term storage).

Positive and negative interferences may occur due to evaporation or adsorption of water vapor. It is imperative that both pre- and post-weighing be conducted at the same temperature and relative humidity.

Static charge on the filter almost always increases the apparent weight of the filter. If the filter can rest completely on the pan (37 mm filters or smaller on UMX2; 47 mm filters or smaller on AT20), then no static discharging is required. If the filter overhangs the edge of the pan, discharging of the filter is required (10 seconds passing over the static strips).

5.0 Personnel Qualifications

All training required is provided by RTI through a demonstration and hands-on training sessions. Training consists of the following steps:

1. Review of the ROP for the weighing.
2. Demonstration of the microbalance programs.
3. Demonstration of proper set up of microbalance, environmental chamber, and ancillary equipment for acceptable gravimetric measurements.
4. All individuals need to be evaluated by the PI or supervising scientist to ensure correctness.

6.0 Apparatus & Materials

1. Teflon Filters (Pall Corporation; Teflo PTFE Membrane Filters; 3 µm pore size; 25 mm diameter; Pall # R2PI025)
2. Metal forceps (e.g. Broad-Tipped, Fischer Scientific Catalog No. 10-300)
3. Petri Dish (50 mm x 11 mm) (e.g. Fisherbrand 50 mm x 11 mm, Fisher Scientific Catalog No. 09-753-52A)
4. Thermometer (direct reading; -10 to 260 °C, 1 °C graduation, 76 mm immersion, 405 mm long, Mercury; Meets accuracy requirements of ANSI/SAMA Z236.1-1983) (e.g. Fisher Scientific Catalog No. 14-985-5E)
5. Labels (e.g. for Petri dishes)
6. Pen (Black, Permanent Ink)
7. Barometer (e.g. Digital barometer, Fischer Scientific Catalog No. 02-400)
8. Microbalance with accuracy ± 1 µg (e.g. Mettler UMX2; Fisher Scientific Catalog No. 01- 913-275)
9. ASTM Class 1 (or NBS class P), 100 mg weight (e.g. Fisher Scientific Catalog No. 02-225- 35D)

Powder free nitrile gloves should be worn during loading & unloading filters. Gloves should be worn for normal gravimetric analysis, except in special cases. The manual dexterity required for careful weighing of very lightly loaded filters (i.e., MicroPEM filters) argues against the use of gloves.

7.0 Measurement Procedures

7.1 Activities (in chronological order)

7.1.1 Filter Preparation for Precharacterization

1. Using stainless steel tweezers, remove the filters from packages, and place each filter into a plastic Petri dish. Cover the dish loosely.

2. Place these Petri dishes into a controlled environmental chamber for a minimum of 24 hours, undisturbed to allow the filters to equilibrate. The temperature must remain between 19 °C – 20 °C +/- 2 °C and the humidity must be 30 - 40% and must remain constant +/- 5% for a minimum of 24 hours.

7.1.2 Gravimetric Analysis (Primary)

1. Allow a minimum of 1 hour (or instrument specific) electronic warm-up of the balance prior to use.

2. Using the Mettler Balance Program (Figure 1), enter the operator ID, temperature, relative humidity, barometric pressure, filter lot number, project and balance type at the appropriate spaces.

3. Position the balance bubble and remove all obvious dust and particles.

4. Using the Mettler Balance Program, calibrate the balance using the internal Mettler AT20 calibration or similar, instrument specific procedure.

5. Using the Mettler Balance Program, perform a manual audit of the calibrated balance by placing a 100 mg mass (ASTM Class 1 or NBS Class P weight) onto the balance pan. Use the “Standard Weight” procedure. The balance is successfully audited if the resulting value is within 2 µg of the expected value. If a passing value is not obtained, this procedure is repeated. If a second attempt fails, obvious sources of error should be investigated (i.e., operator, mechanical, etc.). If the error is not discovered, the balance should be considered inoperable and not utilized. An adequate replacement should be found and the calibration procedures repeated.

6. Using the Mettler Balance Program, place a reference filter on the balance with tweezers. Use the "Reference Weight" procedure. The reference test is successful if the resulting value is within 5 µg of the expected value. If a passing value is not obtained, this procedure is repeated. It is possible that the reference filter weight may change in time, but the reasons for the change should be sought.

7. Using the Mettler Balance Program, perform a precision test with the reference filter. The precision test measures the operator filter handling technique. At the end of the 5 measurements, the precision level for the AT20 balance should be less than 3 µg (0.000003 g); for the UMT2 or UMX2 balance, the precision should be less than 1 µg (0.0000010 g). If these are not achieved, repeat the precision test.

8. Using the Mettler Balance Program, prepare to weigh a filter. Place a filter label on a Petri dish with a filter in it. Scan the filter label into the filter number box using a light pen (or enter the number carefully by hand if the pen won't scan and note that it was hand entered). After weighing the filter, be sure the weight is displayed in the data window, place the filter back in the Petri dish, and close the lid firmly. Place the Petri dish back into the Petri dish box from which it came.

9. Repeat step 8 for each new filter.

10. After twenty-five sample filters have been weighed, the program will request a re-weigh of a previous filter as a QA function. Perform the re-weigh with the requested filter. If the weighing session ends before 25 filters have been weighed, the re-weigh of a single filter must be initiated with the "Reweigh" button in the program. The filter reweigh should be within ±6 µg of the original weight or all the filters must be reweighed. The program does not alert the technician if the acceptance criterion is not met. The technician must be alert and check the session summary table displayed by the program.

7.1.3 Data Uploading

1. After data quality review, post validated data files on CHATS server.
2. Upload the validated data files to ESN.

7.2 Quality Assurance/Quality Control

7.2.1 Chain-of-Custody

1. The instrument operator must record the data onto the proper log, which currently is a computer file. After the filters are pre-weighed, they will be stored in their respective Petri dishes at ambient temperature in the laboratory until they are ready for transport into the field.

2. Collected filters will be returned to the lab as soon as the sampling teams return. A copy of the chain of custody form must accompany the field filters and the receiving party will must initial the form to indicate receipt of the materials.

3. Subsequent gravimetric analysis will be indicated on the sample form by responsible parties. Copies of all original chain-of-custody forms will be maintained in the project files.

7.2.2 Calibration Check

The Mettler AT series balance will periodically indicate a need for automatic re-calibration. When this is indicated, the re-calibration can be initiated with the "Exercise Balance" option of the program, by letting the balance sit without use for about 5 minutes, or by using the balance controls to select the calibration operation. For UMX2, perform automatic re-calibration before starting a weighing session.

7.2.3 Accuracy Check

1. To ensure accuracy and measure precision, re-weigh every 25th sample as prompted by the balance program. The mass values should agree within ±6 µg. If disagreement occurs, then gravimetric re-analysis of the previous 25 filters should be performed. The program does not alert the technician if the acceptance criterion is not met. The technician must be alert and check the session summary table displayed by the program

2. If a session requires fewer than 25 filters to be weighed, randomly select one filter for re-weigh. You will need to select the "Reweigh" button.

3. A minimum of 5 filters of a group should be prepared as lab blanks. Field blanks will be drawn at random from the normal filter population. One lab blank should be weighed for every weighing session. The particular lab blank to be weighed will be determined by the program on a rotating basis.

4. Field blanks will be prepared as normal samples, transported to the field, left without being used for sampling and returned with other sample filters for weighing.

7.2.4 Evaluation Criteria

If the instrument is capable of being successfully calibrated with the internal weights, the values are acceptable for calibration. Inability to be calibrated with internal weights will require servicing of the balance.

7.2.5 Corrective Action

Perform a Standard Weight measurement of the calibrated balance by placing a 100 mg mass (ASTM Class 1 or NBS Class P weight) onto the balance pan. The value will be recorded by the program. The balance is successfully audited if the resulting value is within 10 µg of the expected value. If a passing value is not obtained, this procedure is repeated. If a second attempt fails, obvious sources of error should be investigated (i.e., operator, mechanical, etc.). If the error is not discovered, the balance should be considered inoperable and not utilized. An adequate replacement should be found and the calibration procedures repeated.

7.2.6 Record keeping

Record all data concerning the gravimetric analysis immediately after the data are collected in the Gravimetric Analysis Data book. (This may consist of printed sheets from the weighing session.) Back up the electronic copies of the data files to a network server computer for further analysis in spreadsheet format.

The gravimetric analysis electronic database will be retained on an RTI server for 10 years following the completion of the project.

7.2.7 Calculations

The barometric pressure correction accounts for the change of air density between preweighing and postweighing of samples. The correction is applied to the preweight of the filter only. The balance program applies the correction to the net weight recorded in the sample weight file, but the uncorrected pre- and postweights are also in the file, in case changes in the correction need to be applied. The computer correction assumes a filter density of 830 kg/m, the density of the polymethylpentene filter support ring.

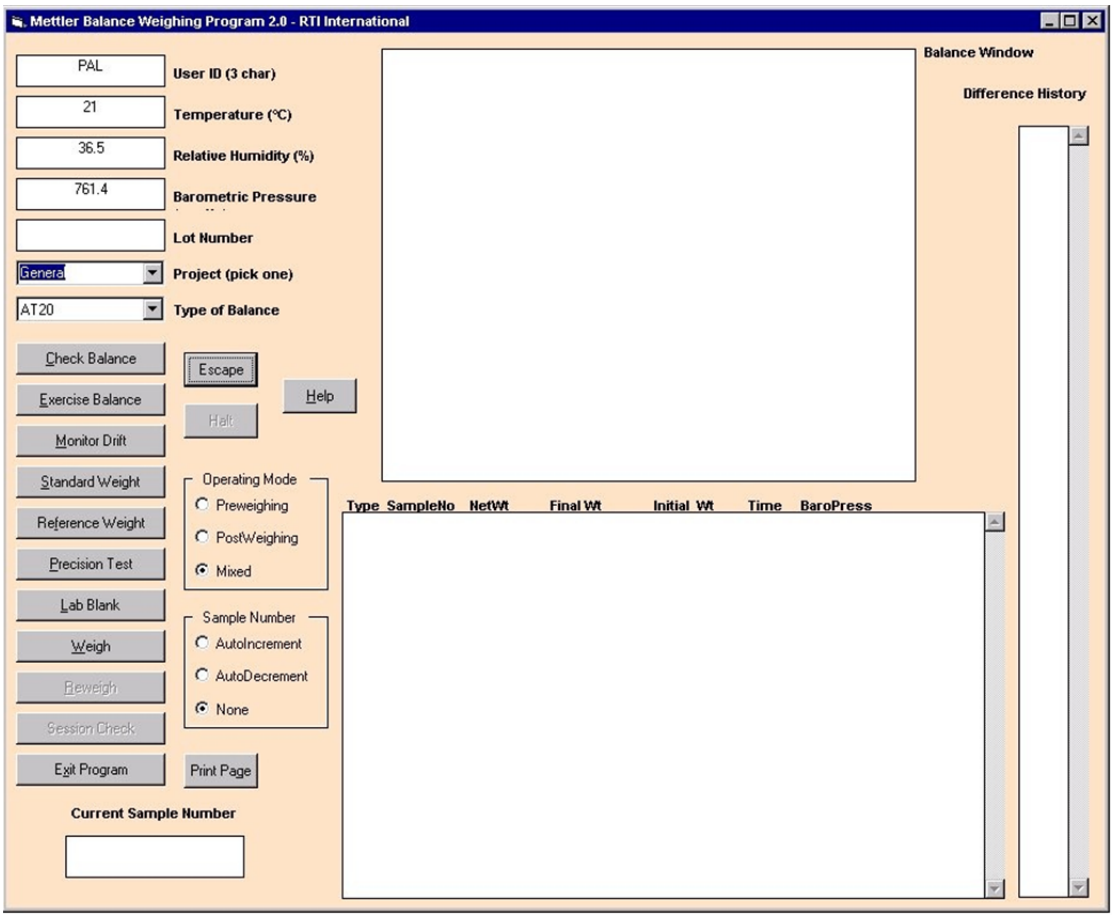


Figure 1 Screen of the Mettler Balance Program

7.2.8 Data Quality Review (CANE staff)

1. Each Weighing Session
    a. Plot the time series of reference filter weights. Notify project management if reference filter weight is not within ± 6 µg of the cumulative average.
    b. Plot the time series of standard reference weights. Notify project management if standard weight is not within ± 3 µg of the specified value.
2. Pre-weight Measurements
    a. Plot a histogram for the frequency of pre-weight to identify any negative values or exceptionally high or low measurement values.

3. Net Weight Assessment
    a. Plot a histogram for the frequency of net weight to identify any negative values or exceptionally high or low measurement values.
    b. Calculate standard deviation of net weight and identify any extreme values that are beyond 3-standard deviation.
    c. For negative values, check the net weight of field blank filter to investigate if any loss of filter material has occurred during sample handling.
    d. For extremely high values, examine the color of the sample for ETS, which shows brownish color as opposed to typical gray colored samples. This sample needs further investigation.
    e. For other extreme values, examine MicroPEM metadata to investigate any malfunction of the device.

7.3 Short Form Directions

7.3.1 Preliminary

1. If necessary, turn on the computer and log on to the network for later file transfer.

2. Start the Mettler Balance program by choosing its name on the Start Menu. For filters marked with "SP" numbers, use the Mettler Special Balance program.

3. Required: User ID (3 or more characters), Temperature, Relative Humidity, Barometric Pressure. Temperature and RH may be read from the weighing room control panel. Barometric pressure should be read from the weighing room mercury barometer. Barometric pressure should be updated at least every two hours, or when noticeable (± 1 mmHg) changes occur.

4. Enter the lot number of the filters to be weighed. This number should be updated if the lot changes in the middle of the weigh session.

5. Be sure the project for the filter weights is properly selected.

6. Be sure the type of balance is properly selected.

7.3.2 Balance Initiation

1. Perform "Check Balance". If "OK" is returned, other buttons will be enabled.

2. Perform "Exercise Balance". After about 20 seconds, data from the balance should appear. The Halt button may be pressed at this time to stop the process after one calibration. If the balance is to be left on, but not weighing for any length of time, select "Exercise Balance" to keep it ready for weighing.

3. Perform "Standard Weight" one time with the standard 100 mg weight. The value should be within ± 2 µg of the previous values. Repeat if it is not.

4. Perform "Reference Weight" for the reference filter left by the balance. The value should be within ± 5 µg of the previous values. Repeat if it is not.

5. Perform "Precision Test" with the reference filter. The degree of precision is determined by the balance type. If the desired precision is not reached, please repeat the Precision Test until it is.

6. Perform "Lab Blank". The program will request that a particular lab blank sample be readied for weighing. Do not proceed until it is ready. One lab blank per weighing session is adequate. More may be done if desired. (Lab blanks are taken from the lot of filters being weighed. They are used to track weight changes during the measurement season. Each project will have a new set of five lab blanks.)

7.3.3 Weighing

1. Begin weighing filters for the project. Do not mix preweights with postweights during a session. All filters should be either preweights or postweights, but not both. For convenience, select the radio button describing the session as "Preweight" or "Postweight." This will reduce the number of confirming responses needed.

2. For preweights, it is desirable to label the filter Petri dish at time of scanning in the sample number. If desired, the "Autoincrement" or "Autodecrement" button may be selected. This will automatically update the sample number by 1 in the desired direction at the end of each measurement.

3. When 25 filters have been weighed, the program will call for a reweigh of one of the filters. If the reweigh is within proper limits, the weighing session may continue. If not, all the filters in that group of 25 must be reweighed, with some file deletions required before doing so. Contact Jonathan Thornburg for this operation.

4. If fewer than 25 filters are weighed (in the session or at the end of the session), select the "Reweigh" button to initiate the reweigh sequence manually.

7.3.4 Printing

If you need to see the printed results before the session is over, press the "Print Page" button. Otherwise the page(s) are printed when the "Exit Program" button is pushed. If the program is left running, print the pages at the end of the session manually.

Recommended Operating Procedure CANE-CHATS-010

for

Sampling and Analysis of Aerosols for Black Carbon and Environmental Tobacco Smoke Using Optical Absorbance in Children's Health after the Storms (CHATS)

Prepared by: _____ Date: 6/7/2011
Seung-Hyun Cho

Reviewed by: _____ Date: 12/12/2011
Ryan Chartier

Reviewed by: _____ Date: 12/13/2011
Cynthia Salmons

Reviewed by: _____ Date: 12/13/2011
James Raymer

Approved by: _____ Date: 12/8/2011
Diane K. Wagener

RTI International
Aerosol Physics and Exposure Program
Center for Aerosol and Nanomaterials Engineering
3040 Cornwallis Road
Research Triangle Park, NC 27709
USA

List of Revisions

CHATS Number	Revision Number	Changes	Revised By	Date
CANE-CHATS 010	0	Original (derived from prior projects; CANE ROP No. 105)	SHC	5/10/2011
CANE-CHATS 010	1	Data quality review section added	SHC	12/9/2011

Table of Contents

1.0 Scope and Application 4
2.0 Summary of Method 4
3.0 Pre-sampling evaluation 5
4.0 Sampling procedure 5
5.0 Transmissivity Measurement 6
5.1 Start-up Instructions (One time per measurement session) 6
5.2 Filter Measurements 7
5.2.1 ..... Lab Blanks 7
5.2.2 ..... Filter Syntax 7
6.0 Analysis Procedure 8
7.0 Data Quality Review (CANE supervisor) 10
8.0 Data Uploading (CANE supervisor) 10
9.0 References 11

1.0 Scope and Application

Carbon can exist in atmospheric aerosols primarily in three separate classifications: as organic carbon compounds (OC), as elemental carbon ("soot") particles (EC) or, more rarely, as inorganic carbonate carbon (CC). The ability to analyze the aerosol for carbon and properly speciate it into one of the above forms can be important for assigning sources of the aerosol, such as Environmental Tobacco Smoke (ETS) or diesel particulate matter (PM).

2.0 Summary of Method

Aerosols are captured onto Pall Teflo filters, and the elemental carbon content is determined using the optical absorbance method. This method was designed to measure black carbon (BC) and environmental tobacco smoke (ETS) in aerosols by optically measuring the absorption of the particulate matter on the filter at several wavelengths of light (Table 1).

Table 1. Available wavelengths

Color Name	Wavelength	Program Designator
Infrared	940 nm	OD0
Red	660 nm	OD1
Orange	620 nm	OD2
Yellow	587 nm	OD3
Green	565 nm	OD4
Cyan	460 nm	OD5
Blue	430 nm	OD6

The current specifications for this analysis are as follows in Table 2:

Table 2. Method limitations

BC Detection Limit	~0.3 ϒg/filter
ETS Detection Limit	~1.7 ϒg/filter
Precision	~0.45%
Accuracy	~5%

The value for accuracy has been determined from comparing the absorbance method with the thermal-optical EC/OC method on collocated filters; it is approximately what is expected from the uncertainties in the volumetric flow. The instrument is calibrated by collecting BC or ETS directly onto pre-characterized (weight and optical absorbance) Teflo filters. The measured absorbances are used with a fitting function to generate calibration curves that are used in the analysis. (See reference for details.)

Samples received are stored as needed in a cold storage refrigerator until analyzed. The optical analysis is performed after 24-hr equilibration in the temperature/humidity controlled chamber and gravimetric analysis on each filter to avoid disturbing or losing carbon particles before the filters have been weighed.

To assure proper accuracy in quantification of BC and ETS the following procedures are followed:

1. Repeat measurements are done on approximately 4% of the samples to determine precision (consistency).

2. Instrument blanks are performed as a part of each measurement. This defines the instrument zero and is done in order to make sure detection limits are of the proper low magnitude.

3. Blank filters are analyzed in order to determine values which may be subtracted from the samples. These may be either static-field blanks (those taken to the field, but kept in the containers) or dynamic-field blanks (those placed in the sampling instruments, but not having ambient air drawn through them).

3.0 Pre-sampling evaluation

Each filter will have its transmissivity measured before it is preweighed. The transmissivity is determined according to Section 5.0. Its presampling transmissivity will be recorded by the sample number, just as the preweight is recorded by the same sample number.

If 25 filters from the same lot are determined to have the same initial transmissivity, within the variability of the measurement (CV of 15%), the remaining filters in the lot need not be measured in the same detail, but only at a subset of the measurement wavelengths. This is done with the Red and Blue wavelengths.

4.0 Sampling procedure

The sampling procedure is governed by the CHATS ROP 009 for personal exposure monitoring (PEM) gravimetric sampling. After sampling, the filters should be conditioned and postweighed before the transmissivity measurements. Filters will be stored according to project requirements following transmissivity measurements.

5.0 Transmissivity Measurement

The transmissivity measurement is conducted in an integrating sphere photometer with a filter holder. Each of seven light emitting diodes (LED) emits light for different wavelength (see Table 1) and illuminate the sphere interior. The filter under measurement is laid in the filter holder. The cap containing a white-painted collector with a photocell in the center is placed atop the filter. The photocell current is read with an IL1700 Research Radiometer (International Light, Peabody, MA).

5.1 Start-up Instructions (One time per measurement session)

1. The LEDs are powered by a 4-cell battery. It should be plugged in for at least 5 minutes before measurements begin to allow it to stabilize.

2. The computer program to be used is “Photometer". Start the program; enter the user initials for identification. If the filter lot number is known, enter it in the next box. Select the proper project for storing the data. Other choices on this list require reconfiguring the instruments and should not be selected.

3. Click the *Zero* button. This monitors the voltage with the lights off. Wait 10 seconds and click *OK* in the dialog box. The low voltage will be stored in the IL1700 and subtracted from subsequent readings.

4. Check all the colors of light that will be used for the measurements. For the first 25 pre-measurements in a single lot, all checks will be forced on. A running CV is displayed for each wavelength at the bottom of the results box. If the CV is less than 15% after 25 filters, the wavelengths used can be reduced to Red and Blue.

5. Measure the *standard filter*. The *standard filter* is used to calibrate the photometer on every project and as such makes comparisons between projects all the more reliable. This will confirm proper operation of the instrument. At the start of the measurement, all the checked wavelengths will be measured and then the prompt for loading the filter will appear. The standard filter typically has an Infrared transmission of 51%, and visible light transmissions of 27 – 31%. These numbers will appear in the colored boxes at the bottom of the program window. If the numbers are greatly different from these values, there is something wrong with the unit, and the unit will need to be re-stabilized and re-zeroed. If the unit fails again after these steps, consult Jonathan Thornburg.

6. Measure the *reference filter*. The *reference filter*, like the *standard filter*, is a filter that is kept with the photometer and referenced as a universal standard for any measurements done with the photometer. Choose a reference filter from the same lot where filters that are going to be analyzed were taken. The transmissions for this filter range from roughly 0.95 to 0.93 going from Infrared to Blue. For the filters of 25 and 37 mm diameter, these values are slightly lower. This measurement checks on the stability of the reference filter and cleanliness of the lab environment.

7. Perform the *Precision Test*. This test measures the same filter (the *reference filter*) 5 times and computes the average and standard deviation. This process is a confirmation of the operator skill in handling the filters. The standard deviation of five measurements should be less than 0.0010 for adequate precision. Repeat the test, if necessary. Seek help from the lab supervisor if the test cannot be performed within limits after two attempts. The operator might need to be re-trained.

5.2 Filter Measurements

5.2.1 Lab Blanks

Lab blanks may or may not be called for, depending on the project. If they are called for, select the *Lab Blank* button. The program will prompt for loading a specific filter. The filter will then be measured like all the other filters.

5.2.2 Filter Syntax

Routine filter measurements are initiated with the *Measure* button. The operator will be prompted for the filter number, which is two letters (UP) followed by four digits with leading zeros as needed (UP0001, for example). After 25 filters, remeasurement of a randomly selected filter will be conducted and a letter will be appended to the sample number (UP0001a). If the operator needs to do so, a letter may be appended to indicate a second measurement on the same filter (UP1102b.) The data manager needs to be informed of this action.

For convenience and speed, two features affect the measurement of filters. A set of buttons can be used to describe the general type of measurement being done: *Pre*- and *post*-exposure. Use of these buttons turns off certain messages and reduces the operator's need to answer the same questions over and over. For example, when measuring new filters, select the *Premeasure* button to keep a query confirming "premeasuring" from appearing. If the sample has already been premeasured, a query announcing this will be raised, but otherwise, there will be no interruption.

The other speedup feature is the *Auto-Increment/Decrement* selection. It automatically increases (or decreases) the filter number by one with each new measurement. The operator needs merely to confirm the new filter number each time. It is important to visually check the number with each filter, to avoid propagating a series of wrong filter numbers. While the number dialog box is active, the filter number can be changed as needed.

While premeasuring new filters, it has been found that most filters from the same lot are quite similar. As a result, after 25 or more from the same lot have been measured in 7 colors, the number of colors can be reduced to 2 widely separated colors (usually Red and Blue) to speed up the measurements without affecting the accuracy much at all.

After completing the measurement, the program will display the values and ask if they are acceptable. Select "okay" if they fall into the acceptable range as determined by the supervisor. Select "no" if they fail to fall in to this range and repeat the filter reading. If they fail a second time, alert the supervisor for further action.

Warning! Pressing the *Measure* button before filter reading appears on the screen display will result in a failure to record the values; the filter reading will need to be repeated.

Remeasuring is required periodically. In a long session, a remeasure will be required for every 25 filters (a full box). The remeasure is requested before the box is to be put away. If the session is over and before a remeasure is required by the system, it is good practice to activate the *Remeasur*e button and perform the remeasure manually.

Exiting the program will print a hard copy of the session. (The *Print Page* button will perform the same action.)

6.0 Analysis Procedure

The optical density of the filter (assumed to be due to absorption) is computed by:

$$OD = -\ln \frac{T_{post}}{T_{pre}} \tag{1}$$

where the presample transmission is either the measured value or the lot average value.

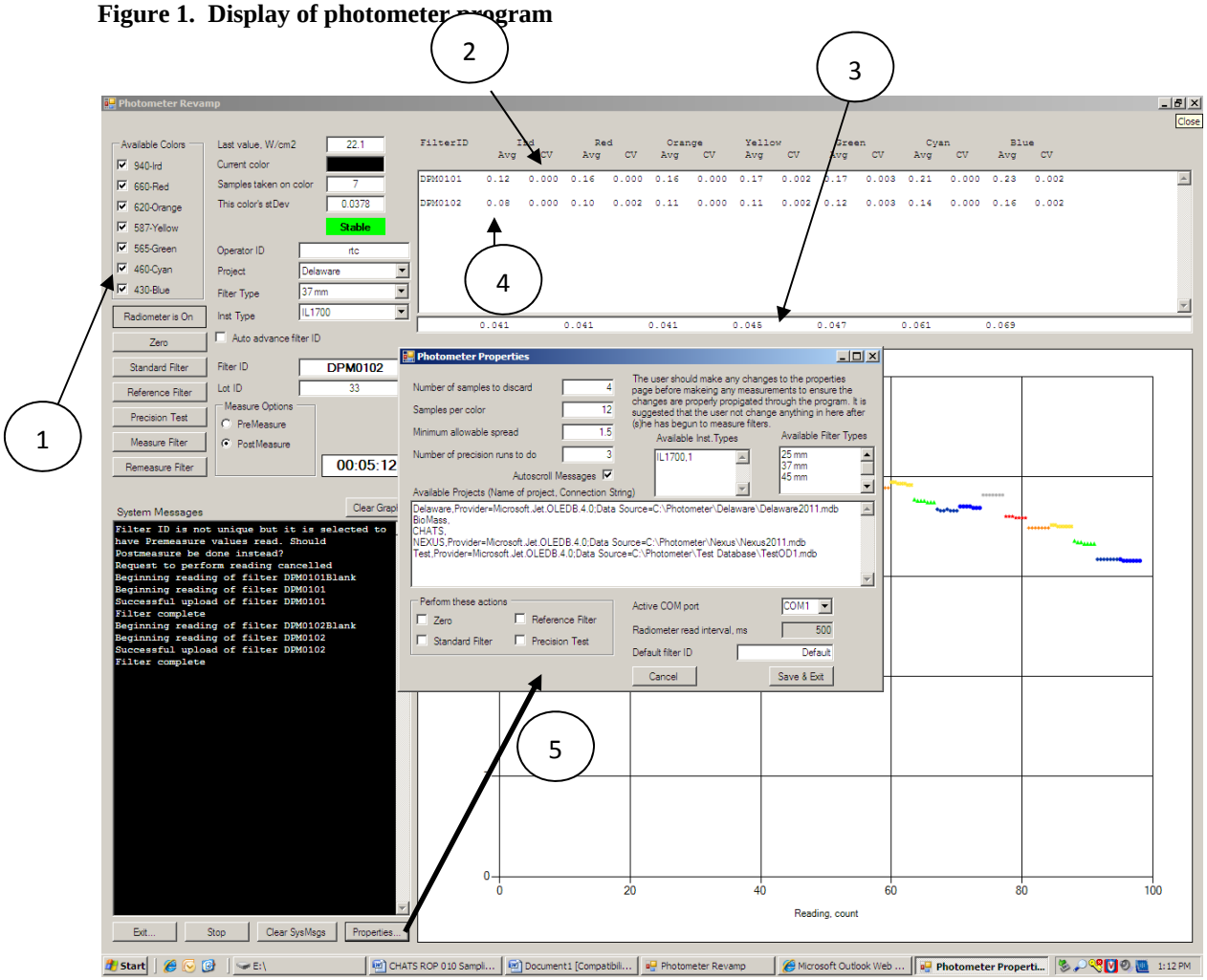
The estimated BC mass on the filter from a single wavelength measurement is given by:

$$BC = \frac{OD \cdot FilterArea}{B_a} \tag{2}$$

where the filter area is the particle deposit area and B_a is a calibration factor giving the absorption cross-section in m^2/g. Values in the literature range from 10 m^2/g to 25 m^2/g.

The multicolor analysis is performed in specialized spreadsheets, and while it is similar to these simple analysis methods, it makes use of the extra information gathered with the multiple measurements.

Figure 1. Display of photometer program



1. Wavelength select
2. CV for data points from current measurement of wavelength (CV of samples per color)
3. CV for wavelengths – use during premeasure of filters from same lot – should be less than 15% for 25 filters
4. Calculated OD
5. Photometer properties

7.0 Data Quality Review (CANE supervisor)

1. Each Analysis Session
    a. Plot the time series of transmissivity of standard filter for each wavelength. Notify project management if the measurement is greatly different from 51% for Infrared and 30 – 32% for visible light.
    b. Plot the time series of reference filter. The transmissions for this filter range from roughly 0.95 to 0.93 going from Infrared to Blue. Notify project management if the transmission is not within ± 10% of cumulative average.
2. Pre-Measurements of Transmissivity
    a. Plot a histogram for the frequency of pre-measurement transmissivity to identify any exceptionally high or low measurement values. A filter with Red and Blue wavelengths not within 15% of CV will be analyzed for all wavelengths.
3. Mass Assessment
    a. Plot a histogram for the frequency of estimated mass of BC and ETS values to identify any exceptionally high values.
    b. Calculate standard deviation of BC and ETS mass and identify any extreme values that are beyond 3-standard deviation.
    c. Examine MicroPEM metadata for the sample that shows extreme values to investigate any malfunction.
    d. Compare the BC and ETS values with the net weight of the sample. If these values are greater than the net weight, the sample needs further investigation.

8.0 Data Uploading (CANE supervisor)

1. Merge validated information of analysis date in datasheet “ODens” and estimated BC and ETS mass in datasheet “OFit” into one data file.
2. Post the validated data files on CHATS server.
3. Upload the validated data files to ESN in proper format.

9.0 References

Lawless, P.A., Rodes, C.E., and Ensor, D.S. "Multiwavelength Absorbance of Filter Deposits for Determination of Environmental Tobacco Smoke and Black Carbon", *Atmospheric Environment* 38:3373-3383, 2004.

Center for Microbial Communities System and Health Research
Research Operating Procedure 03

Extraction and Analysis of Dust and PM Filter Samples for Environmental (Asp f 1, Bla g 1, Der f 1, Der p 1, and Fel d1) Antigens for Children's Health after the Storms (CHATS)

Prepared by: Date: 2/13/2013

Reviewed by: Cynthia Ann Salmons Date: 7-29-13

Reviewed by: James W. Rayman Date: 7-30-13

Approved by: Date: 7-31-13

List of Revisions

Revision Number	Changes	Date
0	Original from RTI	12/14/2011
1	Title and 1.0- Added Feline (Fel d1); 5.2 - changed tube volume to 15 mL; 5.7 - updated data review and submission text	2/13/2013

Table of Contents

1.0 Scope & Application 4
2.0 Summary of Method 4
3.0 Apparatus & Materials 4
4.0 Personnel Qualifications 5
5.0 Procedures 5

1.0 Scope & Application

This research operating procedure (ROP) establishes procedures for quantifying antigens (Aspergillus (Asp f 1), Cockroach (Bla g 1), Dust Mite (Der f 1 and Der p 1), and Feline (Fel d1) that occur naturally in the environment. The ROP is applicable to bulk dust samples, and dust collected on filters.

2.0 Summary of Method

Dust and filter samples are weighed for dust mass. The procedures in this ROP follow the package insert guidelines in the ELISA test kits commercially available from Indoor Biotechnologies, Inc. (Charlottesville, VA). Antigens are quantitated based on antigen-antibody binding, using a known monoclonal antibody reference for comparison.

3.0 Apparatus & Materials (identify manufacturer, model city, state of what's actually used)

Balance
Microplate reader (405 nm)
Multichannel pipettor (8-12 channel)
pH meter
Vortex
Mechanical Shaker
Sample extraction tubes
Plate washer
Pipettors capable of multiple volume settings (i.e. 20 µL, 100 µL, etc.)
Refrigerator
Microcentrifuge
Buffers and solutions on attached recipe page (all salts included)
Distilled water
Microtiter plates – 96-well
Pipet tips to fit pipettors above
Pipets, serological, various volumes as needed
Dilution tubes in volumes needed
Microcentrifuge tubes
Assay kit components (monoclonal antibody, standard antibody, biotinylated monoclonal antibody)
pH buffer solutions
Humid box or styrofoam cooler

Reagent reservoirs, 50-mL
Test tube racks
Weighing paper
Spatulas
Stir bars
Glass beakers
Aluminum foil
Centrifuge tubes
Permanent markers
Project notebook
Gloves
Timer

4.0 Personnel Qualifications

Personnel must have completed coursework in microbiology, and/or satisfactory training by supervisor, including sterile technique. All staff performing this method will have demonstrated proficiency. For this method, proficiency is demonstrated by performing assays with standard curves and controls that meet specified acceptance criteria, as explained in section 5.5 below.

5.0 Procedures (depending on the method)

5.1 Sample handling
Samples are received, logged in, and stored appropriately

5.2 Preparation/Extraction

Solution Recipes (all solutions should be labeled and dated)

<u>50 mM Carbonate/Bicarbonate Buffer, pH 9.6</u>
0.4 g Na_2CO_3
0.73 g $NaHCO_3$
250 mL distilled water

Stir ingredients well in clean container with clean stir bar and adjust pH to 9.6 as needed. Citric acid or sodium phosphate are fine for adjusting pH. DO NOT use HCl as it will inhibit the assay. Store in refrigerator for up to one month.

Phosphate Buffered Saline, pH 7.4, containing 0.05% Tween 20 (PBS-T)
8.00 g NaCl
0.2 g KH_2PO_4
1.15 g Na_2HPO_4
0.2 g KCl

Add dry ingredients in 1-L volumetric flask. Bring up to almost 1 L with distilled water, then add 0.5 mL Tween 20. Bring up to 1 L with distilled water, add clean stir bar, and stir well. Final pH should be around 7.4. Citric acid or sodium phosphate are fine for adjusting pH. DO NOT use HCl, as it will inhibit the assay. Can be stored in small carboy in lab for wash solution (or other container suitable for washing plates), and extra solution can be stored in refrigerator up to one month.

1% BSA PBS-T
1 g BSA
100 mL PBS-T

Dissolve BSA into PBS-T in clean container, such as a flask or bottle. Store in refrigerator for up to one month.

1 mM ABTS in 70 mM Citrate-Phosphate Buffer
Solution A: 4.8 g anhydrous citric acid
250 mL distilled water

Solution B: 7.1 g anhydrous Na_2HPO_4 (or 13.4 g $Na_2HPO_4 \cdot 7H_2O$)
250 ml distilled water

Mix both solutions well. Mix together 29.4 mL of Solution A with 20.6 mL Solution B, measured with a serological pipet, in 100-mL volumetric flask. Bring p to 100 mL volume with distilled water. Add 54.8 mg ABTS. Adjust pH to 4.2 as needed with either Solution A or B.

- For bulk dust samples, weigh and record a portion of dust to be extracted, and place it in a sample tube (e.g., 15 mL polypropylene screw cap tube).
- For PM filter samples, place the post-weighed filter in a sample tube.
- Add the appropriate amount of extraction fluid (water or buffer) to the sample tube, and shake the tube vigorously for at least 5 minutes with mechanical shaker.
- Remove the sample extract with a pipet and transfer to a clean tube for storage.

5.3 Analysis

- Prepare assay buffers according to recipes from Indoor Biotechnologies. These recipes are included in section 5.2 above.

<u>Day 1 – Coating of Microtiter Plate with mAb</u>

- Dilute the monoclonal antibody (mAb) specified in the test kit 1:1000 in 50 mM carbonate/bicarbonate buffer, pH 9.6 (i.e, 10 uL/10 mL).
- Pipet 100 uL of the diluted antibody into each microtiter plate wells to be used.
- Store plate in humid environment overnight at 4 °C. This is simply to help minimize evaporation of the solution in the plate.

<u>Day 2 – Assay Procedure</u>

- Wash plate wells 3 times with PBS-T.
- Block the microtiter plate by adding 100 uL of BSA PBS-T to each well.
- Incubate plate 30 minutes at room temperature in humid box.

<u>Performing the Assay</u>

- Antigen standard dilutions are based on kit instructions, but range can be modified.
- Wash wells 3 times with PBS-T.

- Example of standard dilution, 250 – 0.5 ng/ml antigen, starting concentration 2500 ng/ml: Pipet 20 µL standard into 180 µl 1% BSA PBS-T into wells A1 and B1 of the microtiter plate. Mix well with pipet (up and down pipetting), and transfer 100 µL across t Page 1 of 9 into 100 µL 1% BSA PBS-T diluent to make 10 serial dilutions. Discard final 100 µL.
- Pipet 100 µL of samples into appropriate wells, including dilutions if needed.
- Pipet 100 µL of 1% BSA PBS-T into at least 2 wells for negative controls.
- Incubate plates for 1 hour at room temperature in humid box.
- Dilute secondary monoclonal antibody (biotinylated for Der f 1 and Der p 1) 1:1000 in 1% BSA PBS-T.
- Wash plate well 3 times with PBS-T.
- Pipet 100 µL of the antibody solution into each well.
- Incubate plate for 1 hour at room temperature in humid box.
- Conjugate must be reconstituted and divided into storage aliquots before use. For Asp f 1 and Bla g 1 assays, use Goat anti-rabbit IgG (Jackson Laboratories Cat. #111-036-046), reconstituted in 1 mL distilled water and 1ml glycerol. For Der f 1 and Der p 1, use steptavidin-peroxidase (Sigma Cat. #S5512), 0.25 mg reconstituted in 1 mL distilled water.
- Dilute appropriate conjugate 1:1000 in 1% BSA PBS-T.
- Wash plate 3 times with PBS-T.
- Pipet 100 µL of conjugate solution into each well.
- Incubate plate 30 minutes at room temperature in humid box.
- Turn on microplate reader set at 405 nm and make sure it is ready to take readings before proceeding. Readiness of the reader depends on the instrument being used.
- Wash wells 3 times with PBS-T.
- Make a 10 uL/10 mL solution of 30% H_2O_2 in 1 mM ABTS in 70 mM citrate-phosphate buffer, pH 4.2
- Pipet 100 µL of the solution into each well.
- Begin taking readings of plates at 405 nm.
- Stop readings when wells with highest concentration of the standard reach 2.0-2.4 optical density (OD).

5.4 Calibration

Equipment is calibrated according to procedures maintained in the laboratory SOPs.

5.5 Quality Control

Solution components should be stored as directed by the manufacturer and used before stated expiration date. Solutions (except PBS-T in carboy) and kits should be stored refrigerated. Reagents are usually stored for less than 1 month, and kit expiration dates vary with the kit. Clean glassware is used, and sterile plasticware is used whenever possible. All troubleshooting should be done by qualified personnel. If samples, positive controls, or negative controls are out of range, there may be contamination or interference. Negative controls should have an OD of less than 0.15. See laboratory supervisor for appropriate action. If wells are not developing color change properly and quickly, check that solutions were prepared according to recipes, and are fresh and at the proper pH.

5.6 Calculations

- Duplicate OD values for the standard curve can be entered in an Excel spreadsheet and averaged.
- Mean values are plotted as log concentration (ng/mL) vs. measured OD.
- Sample values are averaged if they have been measured in duplicate. The measured values can be plotted against the straightest part of the standard curve to obtain the sample concentration.
- This concentration must be multiplied by any dilution used.
- Values can be reported as ng/mL or ng/g, etc., depending on desired measurement and sample type.

5.7 Data Review and Reporting

Supervisor or delegate will give final review of data. Following internal supervisor review, data are then sent via email to the project QA/QC prior to final submission to the specified reporting system according to EAR-CHATS-021.

5.8 Sample/Extract Archiving

If archiving is included in the scope of the project and feasible, extracts are stored in the refrigerator for short term storage, and in the freezer for long term storage.

Recommended Operating Procedure CANE-CHATS-012

for

Temperature and Relative Humidity Collection

Using the HOBO U10 Data Logger for

Children's Health after the Storms (CHATS)

Prepared by: _____ Date: 12/09/2011
Seung-Hyun Cho

Reviewed by: _____ Date: 12/09/2011
Cortina Johnson

Reviewed by: _____ Date: 12/13/2011
Cynthia A. Salmons

Reviewed by: _____ Date: 12/14/2011
James H. Raymer

Approved by: _____ Date: 12/14/2011
Diane K. Wagener

RTI International
3040 Cornwallis Road
Research Triangle Park, NC 27709
USA

List of Revisions

CHATS Number	Revision Number	Changes	Date
CANE-CHATS 012	0	Original (derived from prior projects; CANE ROP No. 124)	12/9/2011

Table of Contents

1.0 Scope and Application 4
2.0 Summary of Method 4
3.0 Materials and Supplies 4
4.0 Sampler Launching (at RTI) 4
4.1 Setting Date/Time 5
4.2 HOBO Launching 5
5.0 Sample Deployment (Session 1) 5
5.1 Inputting Sample Information 5
5.2 Attaching the HOBO to the Sampling Platform 6
5.3 Place the sampling platform 6
6.0 Sampler Retrieval (Session 2) 6
6.1 Inputting Completion Information 6
6.2 Sample Collection and Packing 6
7.0 Data Downloading and Uploading (at RTI) 6
7.1 Reading Out the Data 6
7.2 Plotting the Data 7
7.3 Uploading the Data 7
8.0 Data Quality Review 8
9.0 References 8

1.0 Scope and Application

The HOBO is designed to measure relative humidity and temperature, providing direct and continuous readout as well as electronic recording of the information. In the CHATS, Field Interviewer will deploy the unit in participant's home, and indoor temperature and relative humidity measurements are collected on a 5-minute basis for 5-9 days of record of the participant's indoor conditions. These data are used in computing the air exchange rate using PFT/CATS. The unit will be shipped back to RTI, and the relative humidity and temperature data from these units will be downloaded by RTI staff.
The HOBO monitors are factory calibrated and subject to on-site verification prior to field placement. Batteries for these monitors should be replaced every six months. The technician should be grounded prior to handling the HOBO to prevent static charge.

2.0 Summary of Method

The monitor must be located away from heating zones, zones of air movement and fixed lighting sources. Attaching the monitor to the indoor sampling cage should meet these requirements.

3.0 Materials and Supplies

The following list of equipment and supplies are necessary for HOBO deployment:

A. U10-003 HOBO
B. Laptop computer with HOBOware Lite software
C. Pawclock software
D. USB interface cable
E. Barcode scanner

4.0 Sampler Launching (at RTI)

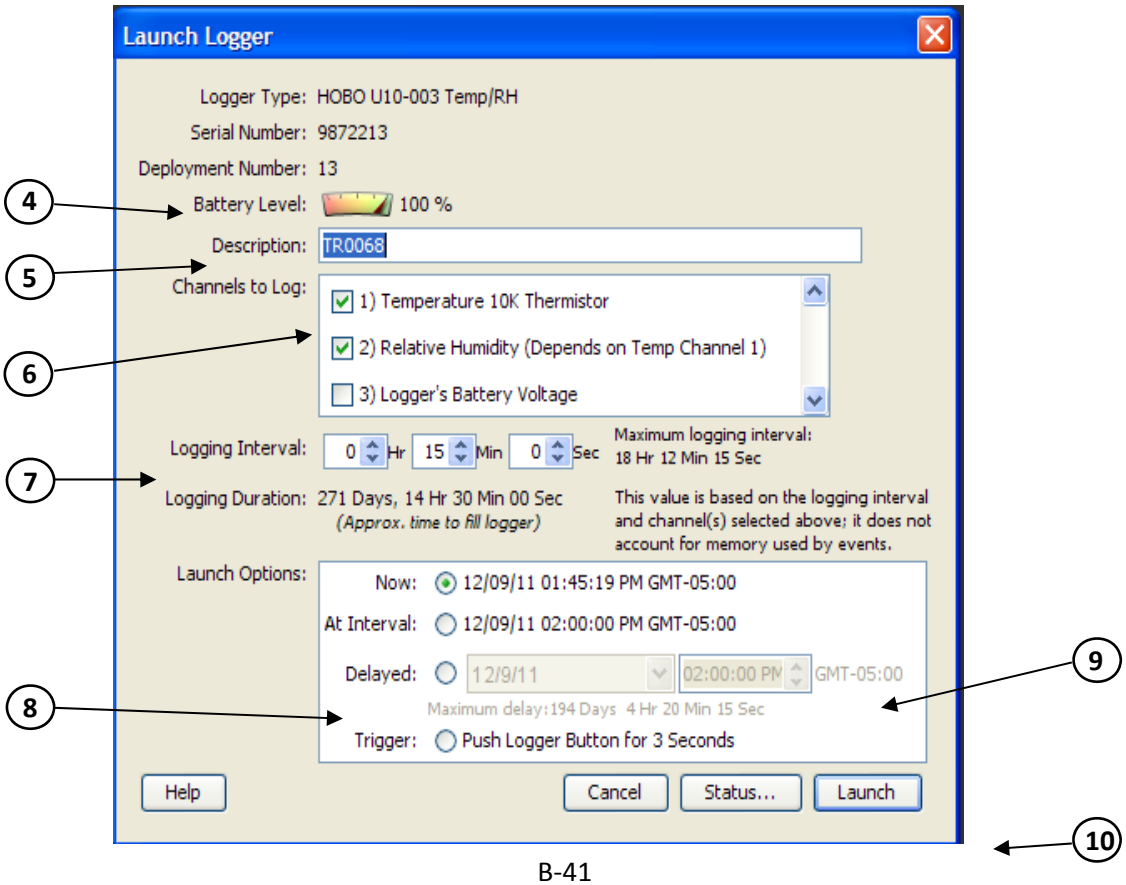
Note: make sure to discharge static electricity from your body before handling the HOBO, especially in winter. A strong static shock can damage the unit. Touch a grounded metal object if possible.

4.1 Setting Date/Time

The HOBO unit internal clock automatically synchs to the laptop clock when the HOBO is launched. It is important that the laptop clock be synchronized with the NIST master clock on a daily basis using the Pawclock software.

4.2 HOBO Launching

1. Plug the large end of the USB interface cable into a USB port on the computer.
2. Plug the small end of the USB interface cable into the side of the logger.
3. Start the HOBOware lite software by clicking the "Launch" icon.
4. Check the battery level. If below 50% change battery before continuing to step 5.
5. Description: Enter filename according to CHATS format (TRxxxx).
6. Channels to Log: Select Temperature and RH
7. Logging interval: "5 minute"
8. Launch options: Select Delayed.
9. Enter the start time to be the nearest even 5-minute time.
10. Select LAUNCH
11. When the delayed start time has passed, confirm that the red LED is blinking slowly (every 1 to 4 seconds). Fast blinking or no blinking means it is necessary to re-launch HOBO.



5.0 Sample Deployment (Session 1)

5.1 Inputting Sample Information

1. Open the electronic datasheet in the laptop for the appropriate sampling platform.
2. Scan the pre-assigned sample ID barcode on the front of the HOBO. It will automatically record the sample ID, sampling date, and start time in the datasheet.

5.2 Attaching the HOBO to the Sampling Platform

1. Remove HOBO from foam tray in participant box.
2. Attach HOBO by making contact of the magnetic strip in the back of HOBO to the metal sheet in the platform.
3. Make sure the HOBO is not covered, obscured, or facing upward, nor is in danger of being obscured during the sample collection period.

5.3 Place the sampling platform

1. Make sure the platform is not adjacent to any observable sources; including ashtrays, shelves or cabinets with cleaners or chemicals, etc.
2. Make sure that air from heating or air conditioning systems will not blow directly on the sampling platform.

6.0 Sampler Retrieval (Session 2)

6.1 Inputting Completion Information

At the end of the sampling period, attach the scanner to the computer and scan the barcode on the HOBO. It will automatically record the finish date and time into the datasheet of the appropriate sampling platform.

6.2 Sample Collection and Packing

1. Remove HOBO from platform by pulling the HOBO.
2. Place HOBO in its designated area in the participant box.

7.0 Data Downloading and Uploading (at RTI)

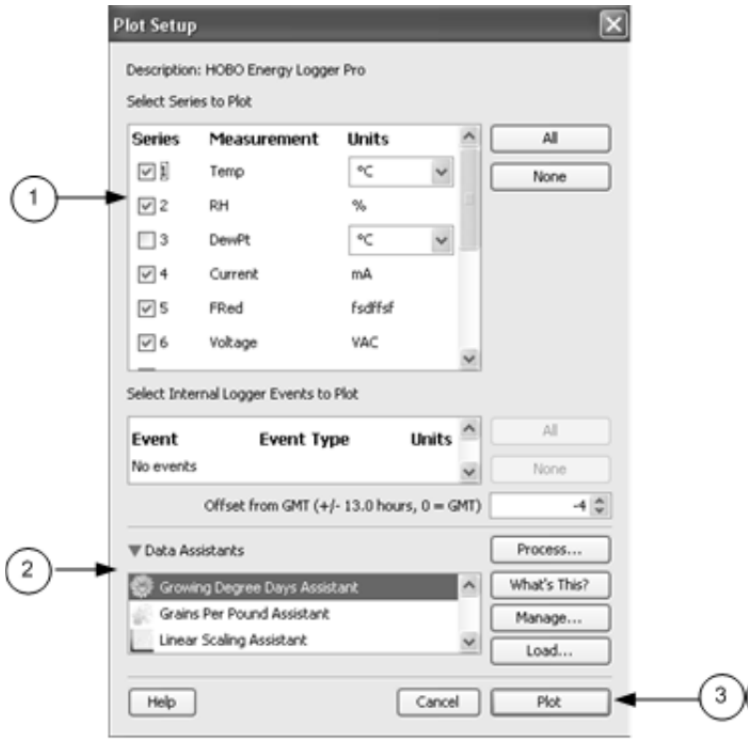
7.1 Reading Out the Data

Prior to handling the HOBO, the technician should touch a grounded conductor to prevent static charge from being transferred to the HOBO. Previous field studies have shown that static charge can cause the HOBO to fail and all data will be lost.

1. Click the Readout icon on the toolbar.
2. At the prompt for Stop – follow rules described above.
3. At the prompt for name and location, set the location to be the desktop.
4. Confirm filename matches appropriate data being downloaded and is the correct format (TRxxxx).
5. Make sure both Temp and RH boxes are selected.
6. Select OK

7.2 Plotting the Data

After the data is read out and the file is saved, the Plot Setup window appears. You must plot the data in order to save it another format. This is also the opportunity to verify that the logger has been operating properly.



1. Select the series to plot (both T and RH).
2. Select the units (°C and % RH).
3. Click **Plot**, and the plot will appear.

7.3 Uploading the Data

1. After data quality review, post validated data files on CHATS server.

2. Upload the validated data files to ESN in the proper format.

8.0 Data Quality Review

1. Count data points in each data file. The number of collected data points should be greater than 95% of the expected value for the file to be valid.
2. Plot a histogram for the frequency of measurement values with increments of 5 degree for temperature and 5 percent for RH to identify the distribution. Exceptionally high or low measurement values that lie outside 3 standard deviations of the mean will be treated as outliers.
3. Plot the time series of values to examine any abnormality of data logging.
4. Conduct calibration check for batches of HOBO every 6 months by exposing them to 2 or 3 different temp/RH conditions. Any HOBOs measuring temp/RH beyond the accuracy level (± 0.4°C for temperature; ±5% for RH) will be further examined.

9.0 References

HOBO® U10 Temp/RH Data Logger (Part #U10-003). MAN-U10-003, Doc #: 11196-A. © 2005, 2006 Onset Computer Corporation.

HOBOware 3.0 Users Guide. ©2010 Onset Computer Corporation.

HOBOware 3.0 Getting Started Guide. Part #: MAN-BHW-GS; Doc #: 12284-D. ©20090-2010 Onset Computer Corporation.

Center for Microbial Communities System and Health Research
Research Operating Procedure 02

Extraction and Analysis of Dust and PM Filter Samples for (1,3)-β-D-Glucan for Children's Health after the Storms (CHATS)

Prepared by: Date: 2/27/2013

Reviewed by: Cynthia Ann Salmons Date: 7-29-13

Reviewed by: Date: 7-29-13

Approved by: Date: 7-31-13

List of Revisions

Revision Number	Changes	Date
0	Original from RTI	12/9/2011
1	Updated section 5.7 to include QA step in data review and submission process.	2/27/2013

Table of Contents

1.0 Scope & Application…… 4

2.0 Summary of Method…… 4

3.0 Apparatus & Materials…… 4

4.0 Personnel Qualifications…… 5

5.0 Procedures…… 5

1.0 Scope & Application

This research operating procedure (ROP) establishes procedures for measuring (1-3)-β-D-glucan, one of the constituents of fungal cell walls. While (1-3)-β-D-glucan is not found in all fungal cell walls nor is it fully unique to fungi, it has been used to provide a reasonable comparison within and between locations. The ROP is applicable to analysis of (1-3)-β-D-glucan in bulk dust samples and dust collected on filters.

2.0 Summary of Method

Dust and filter samples are weighed for dust mass. (1-3)-β-D-glucan is quantitated using Glucatell® (Associates of Cape Cod, Inc., Falmouth, MA), a commercially available assay. Quantitations are based on the reaction of glucan in the specimen with lysate, producing a color change over time at 540 nm, as compared to similar reactions of a standard reference of known glucan content.

3.0 Apparatus & Materials

Microplate reader at 540 nm
Pipettors
Vortex mixer
Pipettors (including repeating pipettor)
Glucatell kit containing: glucan standard, Glucatell® reagent, Pyrosol® buffer
Serological pipets
LAL Reagent Water (LRW), available from the kit manufacturer and other vendors
Parafilm
Microplate, 96-well
Reaction tubes, borosilicate glass
Test tube racks
Pipet tips
Reagent reservoirs
Timer
Permanent marker
Analysis software
Pyrogen-free polypropylene tube

4.0 Personnel Qualifications

Personnel must have completed coursework in microbiology, and/or satisfactory training by supervisor, including sterile technique. All staff performing this method will have demonstrated proficiency. For this method, proficiency is demonstrated by performing assays with standard curves and controls that meet specified acceptance criteria, in section 5.5 below.

5.0 Procedures (depending on the method

5.1 Sample handling

Samples are received, logged in, and stored as appropriate

5.2 Preparation/Extraction

- For bulk dust samples, weigh a portion of dust to be extracted, and place it in a pyrogen-free polypropylene tube.
- For PM filter samples, the post-weighed filter is placed in a pyrogen-free polypropylene tube.
- Add the appropriate amount of extraction fluid to the sample tube and shake the tube vigorously for at least 1 minute with a mechanical shaker.
- Remove the sample extract with a pipet and place into a borosilicate glass tube, covered, for storage.

5.5 Analysis

<u>Reconstitution of Glucan Standard</u>

- Reconstitute glucan standard by pipetting the appropriate volume (will vary for each lot, see vial label) of LRW to make a 100 pg/mL solution. Vortex well. Label container.
- Store unused glucan standard at 4-8 °C for up to 4 weeks (Do not freeze).
- Record the source and lot # (and preparer's initials) in project notebook.

<u>Reconstitution of Glucatell® Reagent</u>

- Reconstitute Glucatell with 2.8 mL of LRW followed by 2.8 mL of Pyrosol® Buffer. DO NOT VORTEX or mix vigorously. Gently swirl to dissolve the pellet.

- Store cold (2-8 °C) and use within 2 hours.

Performing the Assay

- Preparing a standard series by making serial dilutions of the glucan standard in either LRW or dilution buffer. Cover tubes with Parafilm and vortex each dilution vigorously before removing an aliquot and pipetting into the next dilution tube.
- Prepare 4 dilutions total at desired concentrations, unless another standard series is being used. Label the tubes appropriately. An example standard curve might be 50 pg/mL, 25 pg/mL, 12.5 pg/mL, and 6.2 pg/mL.
- A dilution from the middle of the standard curve is appropriate to use as a positive control (e.g., 25 pg/mL).
- LRW or the dilution buffer is used as the negative control.
- Remove the sample extract and pipet into a labeled borosilicate glass tube for storage.
- Make dilutions of samples if needed.
- Turn on the plate reader, start the software on the computer, and select the kinetic protocol for the experiment. Include a 5 second minimum shake step before readings are taken, and incubate the microtiter plate at 37 °C The software should be set up to measure time of onset at 0.03 OD units. Onset time is defined as the intervals (seconds) required for the reaction mixture to achieve a pre-set optical density
- Input sample names into the software plate layout matrix.
- Load 25 uL of the standards, samples, and controls into the microplate according to the matrix.
- Vortex each tube before opening, and use a new pipette tip to transfer solution to the appropriate microplate wells.
- Use a repeating pipettor to add 100 uL of Glucatell® to each well used. Do this as quickly as possible to ensure uniformity of the test.
- Immediately place the uncovered plate into the microplate reader and start the automatic data point collection to obtain optical density measurements at 540 nm.

5.6 Calibration

Equipment is calibrated according to procedures maintained in the laboratory SOPs.

5.5 Quality Control

Glassware is depyrogenated before use according to MMBD SOP 014. LRW should be certified to be <0.005 EU/mL, which is below the detection limit of the test. All troubleshooting should be done by qualified personnel. The correlation coefficient of the standard curve should be 0.980 or greater. If samples, positive controls, or negative controls are out of range, there may be contamination or interference. See laboratory supervisor for appropriate action.

5.6 Calculations

Readings are saved and exported to an Excel spreadsheet for analysis. Exported data includes a generated standard curve and individual sample data. Data is further analyzed in the spreadsheet and the endotoxin concentration of the samples calculated.

5.7 Data Review and Reporting

Supervisor or delegate will give final data review. Following internal supervisor review, data are then sent via email to the project QA/QC prior to final submission to the specified reporting system according to EAR-CHATS-021.

5.8 Sample/Extract Archiving

If archiving specimens is included in the scope of the project and is feasible, extracts should be archived in covered borosilicate glass tubes in storage racks in the -20 °C freezer.

Center for Microbial Communities System and Health Research
Research Operating Procedure 01

Extraction and Analysis of Dust and PM Filter Samples for Endotoxin for Children's Health after the Storms (CHATS)

Prepared by: Date: 2/27/2013

Reviewed by: Cynthia Ann Dalmons Date: 07-29-13

Reviewed by: Date: 07-30-13

Approved by: Date: 07-31-13

List of Revisions

Revision Number	Changes	Date
0	Original from RTI	12/14/2011
1	Updated section 5.7 to include QA step in data review and submission process.	2/27/13

Table of Contents

1.0 Scope & Application…… 4

2.0 Summary of Method…… 4

3.0 Apparatus & Materials…… 4

4.0 Personnel Qualifications…… 5

5.0 Procedures…… 5

1.0 Scope & Application

This research operating procedure (ROP) establishes procedures for quantifying endotoxins, components of Gram-negative bacteria cell walls that occur naturally in soil, water and air. Human health implications for endotoxin exposures have been well documented. Environmental exposure is associated with respiratory symptoms and pulmonary inflammation. The ROP is applicable to bulk dust samples and dust collected on filters.

2.0 Summary of Method

Dust and filter samples are weighed for dust mass. Endotoxin is quantitated based on the sample reaction with Pyrochrome® (Associates of Cape Cod, Falmouth, MA), a commercially available Limulus Amebocyte Lysate (LAL) assay. The reaction causes a color change at 405 nm over time, and is compared to similar reactions of a known standard endotoxin reference.

3.0 Apparatus & Materials

Microplate reader at 405 nm
Vortex mixer
Pipettors
Pyrochrome, lyophilized
Pyrochrome Reconstitution Buffer
Control Standard Endotoxin (CSE)
Serological pipets
LAL Reagent Water (LRW)
Parafilm
Microplate, 96-well
Reaction tubes, borosilicate glass
Test tube racks
Pipet tips
Reagent reservoirs
Timer
Permanent marker
Analysis softwareError! Bookmark not defined.

4.0 Personnel Qualifications

Personnel must have completed coursework in microbiology, and/or satisfactory training by supervisor, including sterile technique. All staff performing this method will have demonstrated proficiency. For this method, proficiency is demonstrated by performing assays with standard curves and controls that meet specified acceptance ranges specified in section 5.5 below.

5.0 Procedures (depending on the method)

5.1 Sample handling

Samples are received, logged in, and stored according to SOP XX

5.2 Preparation/Extraction

- For bulk dust samples, weigh a portion of dust to be extracted, and place it in a pyrogen-free polypropylene tube
- For PM filter samples, place the post-weighed filter in a pyrogen-free polypropylene tube
- Add the appropriate amount of extraction fluid to the sample tube and shake the tube vigorously for a least 1 minute with a mechanical shaker.
- Remove the sample extract with a pyrogen-free pipet and place into a borosilicate glass tube for storage.

5.3 Analysis

<u>Reconstitution of CSE –Label containers appropriately.</u>

- Reconstitute CSE with the volume of LRW specified in the Certificate of Analysis (C of A, which gives the potency of the CSE) and as directed on the package insert. The C of A and the potency stated on it are specific to a combination of Pyrochrome and CSE lot. **Be sure to use the correct C of A and potency.**
- Vortex vigorously for one minute, at 5-10 minute intervals over a 30-60 minute period at room temperature.
- Store reconstituted CSE at 2-8 °C for not more than four weeks.
- Vortex the CSE for at least 30 seconds immediately before making the first dilution, and then make appropriate dilutions to achieve desired concentrations.
- Record the source and lot # and analyst's initials in project notebook.

Reconstitution of Pyrochrome

- Gently tap the vial of Pyrochrome to cause loose LAL to fall to the bottom before opening. Gently break the vacuum by carefully lifting the gray stopper. Cover opened vial with Parafilm.
- Reconstitute Pyrochrome with 3.2 mL Pyrochrome Reconstitution Buffer. DO NOT VORTEX or mix vigorously. Gently swirl contents to dissolve the pellet.
- Store cold (2-8 °C or on ice) when not in use.
- Pyrochrome must be used within 8 hours of reconstitution.

Performing the Assay

- Make 1:10 serial dilutions of CSE in either LRW or appropriate dilution buffer. Use as few dilutions as possible with appropriate pipet volumes to maximize accuracy. Cover tube with Parafilm and vortex each dilution vigorously before removing an aliquot and pipetting into the next dilution tube.
- Prepare 5 dilutions total, unless another standard series is being used. Label the tubes appropriately.
- A dilution from the middle of the standard curve is appropriate to use as a positive control (e.g. 0.5 EU/mL).
- LRW or the dilution buffer is used as the negative control.
- Remove the sample extract and pipet into a labeled borosilicate glass tube for storage.
- Make dilutions of samples if needed.
- Turn on the plate reader, start the software on the computer, and select the kinetic protocol file for the experiment. The protocol file should include a 10-second shake step before readings are taken, and the plate should be incubated at 37 °C. The test is run until all of the samples have incubated for significantly longer than the time required for the lowest standard endotoxin concentration to reach the onset OD at 405 nm.
- Input sample names into the protocol file.
- Open the plate layout matrix in the protocol file and load 50 µL of the standards, samples, and controls into the microplate accordingly.
- Vortex before opening each tube and use a new pipette tip for each transfer.
- Use a repeating pipettor to add 50 µL of Pyrochrome to each well used. Do this as quickly as possible to ensure uniformity of the test.
- Immediately place the uncovered plate into the microplate reader and start the automatic data point collection to obtain optical density measurements at 405 nm.

- Determine the time taken for specimens to reach a particular OD threshold (usually 0.03 OD units relative to an initial reading taken to be zero OD units) after any data corrections have been made. The time taken to reach the OD value is called the onset time.

5.4 Calibration

Each piece of equipment used in this assay is calibrated according to procedures maintained in the overall laboratory SOPs.

5.5 Quality Control

- Glassware is depyrogenated before use according to MMBD SOP 014. LRW should be certified to be <0.005 EU/mL, which is below the detection limit of the test.
- For the test to be valid: the endotoxin concentration of the negative controls should be significantly lower than that of the lowest standard concentration. The mean measured endotoxin concentration of the positive controls must be within 25% of the nominal concentration. The correlation coefficient of the standard curve should have an absolute value of greater than 0.980.
- All troubleshooting should be done by qualified personnel.

5.6 Calculations

Readings are saved and exported to an Excel spreadsheet for analysis. Exported data includes a generated standard curve and individual sample data. Data is further analyzed in the spreadsheet and the endotoxin concentration of the samples calculated.

5.7 Data Review and Reporting

Supervisor or delegate will give final data review. Following internal supervisor review, data are then sent via email to the project QA/QC prior to final submission to the specified reporting system according to EAR-CHATS-021.

5.8 Sample/Extract Archiving

If archiving is included in the scope of the project and feasible, extracts are archived in borosilicate glass tubes storage racks in the freezer at -20 °C.

Center for Microbial Communities System and Health Research

Research Operating Procedure 04

Sieving of Dust Samples for

Children's Health after the Storms (CHATS)

Prepared by: Date: 7-26-13

Reviewed by: Cynthia Ann Dalmous Date: 7-29-13

Reviewed by: Date: 7-30-13

Approved by: Date: 7-31-13

List of Revisions

Revision Number	Changes	Date
0	Original from RTI	5/20/2011

Table of Contents

1.0 Scope & Application…… 4

2.0 Summary of Method…… 4

3.0 Apparatus & Materials…… 4

4.0 Personnel Qualifications…… 5

5.0 Procedures…… 5

1.0 Scope & Application

This research operating procedure (ROP) establishes procedures for sieving the dust samples collected in the cone-shaped HEPA filter collected for CHATS using protocol (insert Task 5 protocol for dust) and shipped to RTI according to protocol (insert sample shipment protocol reference).

2.0 Summary of Method

The collected HEPA sock dust sample is opened and placed in the sterile sieve. It is sieved for 30 minutes, and the collected dust is weighed and saved for analysis.

3.0 Apparatus & Materials

HEPA sock dust sample (from CHATS Field protocol CANE-CHATS-061)
250 um sieve (#60) (8 inches in diameter), brass lid and collection pan
Shaker
Timer
Balance (at least 3 places)
Autoclave
Autoclave time tape
Sterile Nitrile or latex gloves
Sterile scraper
Sterile scissors
Sterile foreceps
Sterile spatula
Weighing paper
Collection tubes (Depyrogenated glass)
Permanent marker
Notebook

4.0 Personnel Qualifications

Personnel must have completed coursework in microbiology, and/or satisfactory training by supervisor, including sterile technique.

5.0 Procedures

5.1 Sample handling

Samples are received from the primary custodian by a chain of custody transfer. They are logged in to the microbiology laboratory sample tracking system, and stored at -20°C until processed.

5.2 Preparation/Extraction

- The sieve, lid and collection pan must be cleaned in hot water, alconox and rinsed with distilled water prior to use.
- Place collected HEPA sock dust sample into 250 um sieve using sterile gloves. This should be done in a clean area with low air flow to avoid dispersing the dust. The collection pan should already be in place below the sieve for this step.
- Carefully open the HEPA sock and gently distribute the contents onto the sieve. Try to transfer the captured dust from the HEPA sock onto the sieve, especially removing the dust from the tip of the sock. The opening of the HEPA sock can be done by tearing while wearing sterile gloves, or by cutting using sterile scissors. The dust removal can be facilitated by using sterile forceps or a spatula. If there are items that are clearly not dust, for example, a piece of cereal, tap them gently to shake dust from them, and then remove and discard.
- Place the opened and mostly empty HEPA sock face down on the sieve and try to lay it flat.

- Put the lid on the sieve, and apply masking tape to all the joints between sieves to prevent loss of dust.
- Place sieve pans on shaker, secure them, and shake for 30 minutes. Several sets of sieve/pans containing HEPA socks may be stacked on the shaker and shaken simultaneously.
- After shaking is completed, remove tape, open up sieves, and scrape dust out of collection pan and weigh to at least the nearest milligram. Record this weight in the appropriate notebook and on the collection tube (if desired). Sieved dusts are then aliquotted, with a daughter sample placed in a labeled jar for transfer for further analysis. A second extraction sample is weighed out for analysis in lab.
- Discard the pieces of the sock filter and the larger pieces of sample that did not pass through the 250 um sieve.
- The sieves and capture pan must be cleaned between different samples. After cleaning, rinse out sieves and capture pan thoroughly in DI filtered water.

5.7 Calibration

Balance is calibrated daily before use according to procedures maintained in the laboratory SOPs.

5.4 Quality Control

Sieves are cleaned before use. Scissors, forceps, scrapers, and spatulas are cleaned and subject to sterilization via autoclave before the next use.

5.5 Calculations

Weights of dust samples are recorded in appropriate notebook.

Research Operating Procedure EAR-CHATS-001

Procedure for Determining Carbonyls from Passive Samplers for

Children's Health after the Storms (CHATS)

Prepared by: Michelle McCombs Date: 6/4/13

Reviewed by: Cynthia Ann Salmons Date: 6/13/2013
Cynthia A. Salmons

Reviewed by: James H. Raymer Date: 6/13/2013
James H. Raymer

Approved by: Diane K. Wagener Date: 7/31/2013
Diane K. Wagener

RTI International
Exposure Analysis Research
3040 Cornwallis Road
Research Triangle Park, NC 27709

List of Revisions

Revision Number	Changes	Date
0	Original from RTI	12/8/11
1	Deleted compounds not included with analysis. Updated calibration curve information and part numbers for supplies. Corrected typographical errors. Add text for PDA wavelength and equivalent HPLC system can be used.	6/4/13

Table of Contents

1.0 Scope & Application 4
2.0 Summary of Method 4
3.0 Definitions 5
4.0 Cautions 5
5.0 Interferences 6
6.0 Apparatus & Materials 6
7.0 Personnel Qualifications 7
8.0 Procedures 7
9.0 Method performance including MDLs 11
10.0 References 12

1.0 Scope & Application

The analytical procedures described in this protocol are intended for the determination of selected carbonyls from passive badge samples that will be collected as part of the Children's Health after the Storms (CHATS) Study. This protocol addresses:

- Extraction of passive badge samples
- Analysis of carbonyl sample extracts by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detector
- Laboratory quality control (QC) procedures
- Data processing and documentation

Target analytes include:

- Acetaldehyde
- Acetone
- Crotonaldehyde
- Formaldehyde
- Propionaldehyde

2.0 Summary of Method

The procedure is taken from EPA's Compendium Method TO-11A, *Determination of Formaldehyde in Ambient Air Using Adsorbent Cartridge Followed by High Performance Liquid Chromatography (HPLC) and SKC Update to EPA Compendium Method IP-6A, Determination of Formaldehyde and Other Aldehydes in Indoor Air Using a Solid Adsorbent Trap*. DNPH-coated filters from passive badges are extracted in acetonitrile. The filter is removed and the remaining extraction solution is analyzed by HPLC-UV. QC samples include reagent blanks, matrix blanks, matrix spikes, calibration checks, and second source checks. The HPLC is calibrated using a minimum of a five-point standard curve. Chromatograms are processed using Empower2 data system and data are output as individual electronic files using an export macro. After QA review of the individual data files, data will be read electronically into the study database from the output files per data management protocol.

3.0 Definitions

3.1 HPLC – High Performance Liquid Chromatography

3.2 Stock Standard Solution - A concentrated solution containing one or more certified standard analytes or a concentrated solution of one or more analytes prepared in the laboratory with an assayed reference compound. Stock standard solutions are used to prepare primary dilution standards. The nominal standard solution is typically 15 µg/mL for the functional compounds or 100 µg/mL for the derivatized compounds.

3.3 Calibration Standards - A series of standard solutions prepared from the primary dilution standard solution and the stock standard solutions of the analytes. These calibration standards are used to calibrate the instrument response with respect to analyte concentration. Table 1 presents additional details on the calibration levels.

3.4 MW – Molecular weight

3.5 MDL – Method detection limit

4.0 Cautions

The analyst is responsible for maintaining awareness of OSHA regulations regarding the safe handling of chemicals used in this method. The toxicity and carcinogenicity of chemicals used in this method have not been precisely defined; therefore, each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Appropriate care should be exercised in handling extracts and solvents. All solvents, pure standard materials and stock standard solutions of target compounds should be handled exclusively in a chemical fume hood. Personal protective equipment (gloves, lab coat and eye protection) appropriate for handling hazardous materials should be worn.

5.0 Interferences

5.1 The extent of interferences may vary considerably from sample to sample. Interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences.

5.2 Beware of unintentional exposure of samplers and eluted samples to aldehyde and ketone sources. Laboratory air often holds high concentrations of acetone. Labeling inks, adhesives, and packaging containers (including vials with plastic caps) are all possible sources on contamination.

5.3 Contamination is most likely to occur during sample extraction. It is recommended before eluting derivatives, clean all glassware by rinsing with acetonitrile, then heating in a 60°C vacuum oven for at least 30 minutes. Eluting the samples in a nitrogen-purged glove bag further reduces the risk of contamination.

5.4 The acetonitrile used to elute the DNPH derivatives is a typical source of contamination. Formaldehyde-free acetonitrile used to elute samples should be used only for this purpose, and stored in a carbonyl free environment. A concentration of 10 µg/L of any aldehyde or ketone in the acetonitrile adds 0.05 µg of that carbonyl to sample blank values if using 5 mL extraction volumes.

5.5 Carryover contamination may occur when a sample containing low concentrations of compounds is analyzed immediately after a sample containing relatively high concentrations of similar compounds. Syringes and injectors must be thoroughly cleaned between each injection or replaced, as needed, to avoid this problem.

6.0 Apparatus & Materials

6.1 Primary analytical standards are purchased from Sigma Aldrich (St. Louis, MO), part number 47285-U

6.2 Second source analytical standards are purchased from Chem Service (West Chester, PA), part number M-DCC83152A1-1ML

6.3 Acetonitrile, LC-MS grade, Honeywell (B&J) LC015-2.5, 99.99% (VWR, Suwanee, GA) (for extractions)

6.4 Deionized water, HPLC grade

6.5 Volumetric flasks, 2 mL to 100 mL sizes

6.6 Vials, various sizes of amber glass vials with Teflon-lined screw caps, including 4-mL autosampler vials with inserts (VWR, Suwanee, GA, part numbers 46610-738, 82028-444, and 66030-396)
6.7 Waters 515 HPLC pump, Waters 717 Plus autosampler, with a UV detector or a Waters 996 photodiode array detector or equivalent capable of acquiring 360 nm (or the best response extracted from the PDA) wavelength using a data system (Empower2 or equivalent) to acquire, process, store and report data
6.8 Supelcosil™ LC-18, Supelco catalog # 58298, 25-cm x 4.6-mm, 5-µm or equivalent (Supelco, Bellefont, PA)
6.9 Pasteur pipets and bulbs
6.10 Pipettes, 1-mL to 10- mL sizes
6.11 Gastight syringes, 10- µL to 1000-µL sizes
6.12 Filter paper treated with 2,4-dinitrophenylhydrazine (DNPH), SKC, Inc. (Eighty-four, PA) part number P20084
6.13 Forceps
6.14 Acetonitrile, HPLC grade (VWR, Suwanee, GA, part number EMD AX0145 RT-34) (for mobile phase)
6.15 HPLC guard column, Supelcosil™ LC-18 Supelguard™ Cartridge 5 µm particle size, L × I.D. 2 cm × 4.0 mm, catalog # 59554 (Supelco, Bellefont, PA)

7.0 Personnel Qualifications

Personnel should read the ROP carefully and have this documented by the laboratory supervisor in their training file. All staff performing this method will have demonstrated proficiency by recovering 70% - 130% of target analytes, spiked onto filter paper treated with 2,4-dinitrophenylhydrazine (DNPH) at 1x – 5x the method lower limit of quantitation (LLOQ), for each of two duplicate samples.

8.0 Procedures

8.1 Standards:

8.1.1 Prepare calibration standards at a minimum of five different concentrations of target analytes, with a linearity of at least 0.995 R^2 over the concentration range (e.g., 45 ng/mL to 7500 ng/mL of the functional concentrations, not the derivative concentration), using the standard mixtures of compounds, purchased from Sigma. Standards should be prepared in acetonitrile. See Table 1 for specific levels.

Table 1. Calibration Curve Solutions

CAL Level	Standard Solution Used	Amount Delivered (mL)	Final Vol (mL)	Nominal Final Conc (ng/mL)
A	Stock at 15 µg/mL	1.0	2	7500
B	CAL A at 7500 ng/mL	1.0	2	3750
C	CAL A at 7500 ng/mL	0.5	2	1875
D	CAL B at 3750 ng/mL	0.5	2	938
E	CAL B at 3750 ng/mL	0.25	2	469
F	CAL B at 3750 ng/mL	0.1	2	188
G	CAL B at 3750 ng/mL	0.05	2	94
H	CAL B at 3750 ng/mL	0.025	2	47

8.1.2 Prepare a second-source check standard, using the ChemService standard mix, at a concentration near the log midpoint of the calibration curve (e.g., 420 ng/mL). See Table 2 for specific details.

Table 2. Second Source Solutions

Solution Level	Standard Solution Used	Amount Delivered (mL)	Final Vol (mL)	Nominal Final Conc (ng/mL)
Second Source A	Stock at 14 µg/mL Formaldehyde	1.0	5	2800
Second Source B	Second Source A at 2800 ng/mL Formaldehyde	0.75	5	420

8.1.3 Store all standards at -12 °C to -20 °C for a period up to six months. Vials of standards should be allowed to warm to room temperature before use.

8.2 Passive badge samplers received for analysis will be logged in using ROP CANE-CHATS-067 and stored in a freezer at (-20 °C) until extraction.

8.3 Samples will be analyzed in batches of up to 20 study samples, plus laboratory quality control samples. Analysis of each batch will be documented in a laboratory notebook.

8.4 Extraction:

8.4.1 Desorption of the DNPH-formaldehyde from the coated filter should be performed in a clean atmosphere, free of formaldehyde (working in a chemical fume hood). Remove the sampler from the pouch and the sliding cover from the sampler. Remove the coated filters from each section of the sampler using forceps (cleaned with acetonitrile). Place each section into its own properly labeled, sealed vial.

[Note: The blank/correction section has an indentation in the center of the filter paper for easy identification. The use of other badge designs will require minor procedural modification and this shall be documented.]

8.4.2 Pipette 3.0 mL of acetonitrile into each vial and mix for one minute by inversion of the vial repeatedly. Remove the coated filter from the vial and seal for analysis. The desorption efficiency should be at least 95% using this procedure.

8.4.3 If HPLC analysis is not going to be performed immediately, the DNPH-formaldehyde solution should be stored at 4 °C and analyzed within 3 days.

8.5 Analysis:

8.5.1 HPLC conditions:

Column: Supelcosil™ LC-18, 25-cm x 4.6-mm, 5-µm or equivalent with guard column LC-18 Supelguard™ Cartridge 5 µm particle size, L × I.D. 2 cm × 4.0 mm
Mobile Phase A: 45:55 Acetonitrile:Water
Mobile Phase B: 75:25 Acetonitrile:Water
Detector: ultraviolet, operating at 365 nm.
Flow Rate: 1.0 mL/min.
Sample Injection Volume: 25 µL
Gradient: 30 minute gradient from A to B, and held at B for 15 minutes
Total Run time: 45 minutes

Table 3. Peak table:

Compound Name	RT
Formaldehyde	10.41
Acetaldehyde	13.09
Acetone[1]	16.30
Propionaldehyde	17.53
Crotonaldehyde	20.18

[1] Acrolein coelutes with Acetone

8.5.2 Prepare and run an analytical sequence in Empower2. Recommended analytical sequence with sample type defined in parenthesis:

- Solvent blank (control)
- Calibration standards (if analyzed) (standard)
- Solvent blank (if calibration standards are run prior) (control)
- Calibration check standard (standard)
- 2nd source check standards (control)
- Method blank (control)
- Method control (control)
- Up to 8 unknowns (unknown)
- Duplicate injection of 1 unknown (unknown)
- Solvent blank (control)
- Calibration check standard (standard)
- Up to 10 unknowns/additional QC samples
- Solvent blank (control)
- Calibration check standard (standard)
- 2nd source check standards (control)
- Steps above repeated as necessary.

8.6 Quality control

8.6.1 The following metrics are targets:

- Solvent blanks and method blank - < 3 x MDL
- Calibration checks and 2nd source check: ± 15% of nominal value
- Method control and fortified samples – spike recovery ± 30% of nominal value

- Duplicate analyses (injections) should be performed for at least one sample in each batch. Study data quality objectives for precision (15% RSD) are as stated in the CHATS QASP.

8.6.2 Batches that meet all QC criteria described above are automatically accepted. Batch data that do not meet the criteria must be approved or rejected by the project manager or PI.

8.7 Quality Assurance

8.7.1 Export the completed analytical run data using the "Export Carbonyls to M drive" export method to the appropriate folder.

8.7.2 Compile the run data using the "ImportCARBData" macro.

8.7.3 Analyst checks the solvent blank and control recovery data to ensure acceptability.

8.7.4 Analyst saves file to CHATS folder for supervisor review.

8.7.5 Supervisor reviews file. If OK, runs "CreatCARBFlatFile" macro.

8.7.6 Supervisor checks DQIs and makes decision on forwarding file to QA.

8.7.7 QA reviews and indicated approval/disapproval.

8.7.8 Supervisor uploads approved folder to ESN per data management protocol.

9.0 Method performance including MDLs

9.1 MDLs will be created by spiking seven dnph-coated filter papers in separate vials at the lowest level of the calibration curve (e.g., 45 µL of a 3000 ng/mL standard will be spiked onto the filter resulting in a final extract concentration of 45 ng/mL). Extract and analyze the samples according to the method above in section 8.

9.2 Calculate the mean, standard deviation and RSD for all seven replicates.

9.3 The MDL will be calculated by multiplying the standard deviation by 3.143 to get the value in ng units for each analyte.

10.0 References

Compendium Method TO-11A, Determination of Formaldehyde in Ambient Air Using Adsorbent Cartridge Followed by High Performance Liquid Chromatography (HPLC) [Active Sampling Methodology], Center for Environmental Research Information, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH 45268, January 1999

SKC Update to EPA Compendium Method IP-6A, Determination of Formaldehyde and Other Aldehydes in Indoor Air Using a Solid Adsorbent Trap, www.skcinc.com, 2004.

SKC Update to EPA Compendium Method IP-6C, Determination of Formaldehyde in Indoor Air Using a Passive Diffusive Sampler, www.skcinc.com, 2004.

OSHA Method 1007, Formaldehyde (Diffusive Samplers), www.osha.gov, May 2005.

RTI SOP EAR-GLC-003 ver 1, Extraction and Analysis of Formaldehyde-DNPH from Active and Passive Media by HPLC, RTI International, Feb 2011.

Research Operating Procedure EAR-CHATS-002

Procedure for Determining Volatile Organic Compounds from Passive Samplers for Children's Health after the Storms (CHATS)

Prepared by: Jocelin Deese-Spruill Date: 3/5/2013

Reviewed by: Cynthia A. Salmons Date: 3/5/2013

Reviewed by: James H. Raymer Date: 7/30/2013

Approved by: Diane K. Wagener Date: 7/31/2013

RTI International
Exposure Analysis Research
3040 Cornwallis Road
Research Triangle Park, NC 27709
USA

List of Revisions

Revision Number	Changes	Date
0	Original from RTI	12/8/11
1	Added Naphthalene-d8 as an additional internal standard and corrected volumes and concentrations of solutions. Added definition of second source abbreviation (SS######X). Edited quantitation and qualifier ions	3/5/13

Table of Contents

1.0 Scope & Application 4
2.0 Summary of Method 4
3.0 Definitions 4
4.0 Cautions 5
5.0 Interferences 5
6.0 Apparatus and Materials 6
7.0 Personnel Qualifications 7
8.0 Procedures 7
9.0 References 14

1.0 Scope & Application

The analytical procedures described in this protocol are intended for the determination of selected volatile organic compounds (VOCs) from passive badge samples that will be collected as part of the Children's Health after the Storm (CHATS) Study. This protocol addresses:

- Extraction of passive badge samples
- Analysis of VOC sample extracts by gas chromatography (GC) with mass spectrometer detector (MSD)
- Laboratory quality control (QC) procedures
- Data processing prior to uploading to the electronically secure network (ESN) and documentation

2.0 Summary of Method

The procedure is developed for use in conjunction with methods for the analysis of VOCs extracted from 3M 3500 Organic Vapor Monitors (OVM) badges (3M, Minneapolis, MN), SOP EAR-GLC-001 and SOP EAR-GLC-002. The charcoal filters are extracted directly inside the passive badges in 2:1 acetone:carbon disulfide extraction solution. The extraction solution is analyzed by GC-MSD (Agilent Technologies 6890 gas chromatogram with a 5973N mass selective detector and ChemStation software). QC samples include reagent blanks, matrix blanks, matrix spikes, calibration checks, and second source checks. The GC-MSD is calibrated using a minimum of a five-point standard curve. Chromatograms are processed using Agilent Technologies ChemStation data system and data are output as individual electronic files. After QA review of the individual data files, data will be read electronically into the study database from the output files

3.0 Definitions

- GC/MSD – Gas Chromatograph / Mass Selective Detector
- SIM – Selective Ion Monitoring (an enhanced-sensitivity mode of GC/MSD operation in which only selected ions are monitored during the development of the chromatogram).
- Internal Standard – A pure analyte(s) in solution added in known amounts to each sample and used to measure the relative responses of other analytes and surrogates that are components of the same solution. The internal standard must be a chemical compound that is not a sample component and that will not chromatographically interfere with the analytes of interest.
- Stock Standard Solution – A concentrated solution containing one or more certified standard analytes or a concentrated solution of one or more analytes prepared in the laboratory from pure neat compounds with an assayed referenced compound. Stock standard solutions are used to prepare primary dilution standards.
-

- Calibration Standards – A series of standard solutions prepared from the primary dilution standard solution and the stock standard solution of the internal standard analytes. These calibration standards are used to calibrate the instrument response with respect to analyte concentration.
- Second Source Standard – A standard from a source different from the calibration standard suppliers. A second source standard monitors instrument drift and shall be with ± 15% of the expected value.
- MDL – Method detection limit

4.0 Cautions

The analyst is responsible for maintaining awareness of OSHA regulations regarding the safe handling of chemicals used in this method. The toxicity and carcinogenicity of chemicals used in this method have not been precisely defined; therefore, each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Appropriate care should be exercised in handling extracts and solvents. All solvents, pure standard materials and stock standard solutions of target compounds should be handled exclusively in a chemical fume hood. Personal protective equipment (gloves, lab coat and eye protection) appropriate for handling hazardous materials should be worn.

5.0 Interferences

5.1 The extent of interferences may vary considerably from sample to sample. Interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences.

5.2 OVM samples and extracts should be refrigerated. No solvent or solution containing the target compounds should be kept in the same refrigerator.

5.3 All the equipment used in the extraction procedure should be fully dedicated to that purpose and not used for any other analyses. This includes the hood in which the extractions are performed. No solvents may be stored in the hood except for the extraction solvents.

5.4 The hood space should be free of debris and dust and then lined with heavy-duty aluminum foil which should be carefully wiped with acetone and allowed to dry for approximately one to two minutes.

5.5 The foil should be replaced periodically as needed. All extraction supplies should be meticulously cleaned and dried. For example, gas-tight syringes used to prepare extraction solvents and standards should be rinsed 5 to 10 times in the appropriate solvent and dried before being used.

5.6 Avoid the use of polyethylene or other plastic materials that can contain significant residues of solvents such as toluene.

6.0 Apparatus and Materials

6.1 Standards

6.1.1 Target analytes in custom mix 1 solution (Accustandard, 2000 µg/mL, part# S-21864, New Haven, CT)

- 1,3-Butadiene
- MTBE
- Benzene
- Toluene
- Tetrachloroethene
- m,p-Xylene
- Methyl ethyl ketone
- Napthalene
- Styrene
- α-pinene
- p-Dichlorobenzene
- n-Octane

6.1.2 Target analytes in custom mix 2 solution (Accustandard, 10,000 µg/mL, part# M-603-10x)

- Acrolein
- Acrylonitrile

6.1.3 Target analyte solution (Accustandard, 2000 µg/mL, part# M-502-56-10x)

- Vinyl chloride

6.1.4 Internal standard mix (Supelco, 1000 µg/mL each component, part# 4-8835, Bellefont, PA)

- Bromochloromethane
- 1,4-Difluorobenzene
- Chlorobenzene-d5

6.1.5 Internal standard single compound (Supelco, 2000 µg/mL, part# 48715-U, Bellefont, PA)

- Naphthalene-d8

6.1.6 Second source analytical standard (Restek, part#30213)

6.1.7 Acetone, HPLC or GCMS grade(B&J, part #AH010-4)

6.1.8 Carbon disulfide (CS_2), HPLC or GCMS grade (Omnisolv EMD, part #CX0397)

6.1.9 Methanol, HPLC or GCMS grade (Honeywell B&J, part #230-4)

6.2 Bottles to contain extraction solvent fitted with a 1 mL – 10 mL bottle top dispenser (VWR, 15900-024)

6.3 Precleaned 40 mL amber vials (VWR Scientific, West Chester, PA, part # 89093-870) with septum caps (silicon/Teflon septa) shipped with the vials, to contain extraction solvent

6.4 GC autosampler vials with Teflon/silicon/Teflon lined septa and glass inserts

6.5 Glass inserts (0.3 mL, National Scientific, Rockwood, TN, part # C4010-630)

6.6 GC-MS system with Agilent J&W DB-624 column (30m length, 320 µm diameter, 0.25 µm film thickness) or equivalent

6.7 Gas-tight syringes, varying sizes (Hamilton gas-tight brand)

7.0 Personnel Qualifications

Personnel should read the ROP carefully and have this documented in their training file by the laboratory supervisor. All staff performing this method will have demonstrated proficiency by recovering 70% - 130% of target analytes, spiked onto clean OVM badges at 1x – 5x the method quantitation limit (MQL), for each of two duplicate samples.

8.0 Procedures

8.1 Solvent Mixture Preparation: The solvent mixture serves as the diluent for all internal standard solutions, calibration solutions, target analyte standard solutions, and control solutions. It is also used for preparation of the extraction solution. The solvent mixture is prepared by mixing two volumes of acetone and one volume of carbon disulfide. Prepare the solvent mixture daily.

8.1.1 Prepare sufficient solvent mixture for extraction (depending on the number of samples), preparation of standards, rinsing, etc. Solvent mixture dedicated to rinsing should be kept separate from that dedicated to extraction and that used in standard preparation.

8.2 Extraction Solution Preparation: Prepare the solution volumetrically by diluting 0.25 mL of the internal standards stock solution (1000 µg /mL) and 0.125 mL of Napthalene-d8 (2000 µg/mL) to 50 mL with acetone/carbon disulfide solvent mixture in a 50 mL volumetric flask to obtain a final concentration of 5 µg /mL. Prepare a fresh extraction solution daily. The volume prepared may be scaled up or down depending upon the number of extractions to be performed.

8.3 Intermediate Method Control Solution Preparation: Prepare the solution by diluting 100 µL of custom mix 1 (2000 µg /mL each component), 20 µL of custom mix 2 (10,000 µg/mL each component) and 100 µL of vinyl chloride (2000 µg /mL) in a 1 mL volumetric flask with acetone/carbon disulfide solvent mixture for a final concentration of 200 µg/mL. Store standard at -20 °C and discard after two weeks.

8.3.1 Prepare a working method control solution volumetrically by diluting 20 µL of intermediate method control solution (200 µg/mL) and 100 µL of internal standard solution (100 µg/mL) to 2 mL in a volumetric flask with acetone/carbon disulfide mixed solvent for a final concentration of 2 µg/mL for target compounds and 5 µg/mL for internal standard compounds. The volume prepared may be scaled up or down depending upon the number of extractions. Store intermediate standard at -20°C. Prepare daily.

8.5 Calibration Solutions Preparation: The calibration solution levels are 0.1, 0.5, 1.0, 2, 5, 10, 25 and 50 µg/mL (ppm). The internal standards have a final concentration of 5 µg/mL at each calibration level. The standards are prepared volumetrically as shown in Table 1.

8.5.1 Intermediate Internal Standard Solution Preparation: Prepare the solution by diluting 500 µL of internal standard stock (1000 µg/mL) to 5 mL in a volumetric flask with acetone/carbon disulfide solvent mixture for a final concentration of 100 µg/mL. Store the standard at -20 °C.

8.5.2 Initial Calibration: Each calibration standard (at least five levels) is analyzed and area response is tabulated against mass concentration injected. The slope of the calibration curve gives the response factor, RF. Linear response is indicated where a correlation coefficient of at least 0.999 for a linear least-squares fit of the data (detector response as a function of mass concentration) is obtained. The intercept of the calibration curve should pass through the origin. If it does not, check the reagents and standard solutions preparation procedure for possible contamination. If the calibration curve does not pass through the origin, the equation for the calibration curve should include the intercept. Note that for data exporting purposes, calibration solution concentrations should be entered in parts per billion (ng/mL), as Chemstation exports results only to two decimal places.

8.5.3 Each new calibration curve should be verified by analyzing a standard prepared from material obtained from a second source. This standard should show a recovery of 85 to 115%. If not, corrective action is required to eliminate the discrepancy between the two sources of the standard material.

8.5.4 Once linear response has been documented, a concentration standard near the anticipated levels of each target component, but at least 10 times the detection limit, should be chosen for daily calibration. The day to day response for the various components should be within 10% of the calibration value. If greater variability is observed, prepare a fresh calibration check standard. If the variability using a freshly prepared calibration check standard is greater than 15%, a new calibration curve must be developed from fresh standards. Each batch of 10 samples must be bracketed by a passing calibration check.

Table 1. Calibration Solutions Preparation

Calibration Levels	Volume (µL) of intermediate internal standard solution @ 100 µg/mL	Volume (µL) of intermediate control solution @ 200 µg/mL	Final volume of solution (mL)
50 ppm	250	1250	5
25 ppm	250	625	5
10 ppm	250	250	5
5 ppm	250	125	5
2 ppm	250	50	5
1 ppm	250	25	5
0.5 ppm	250	12.5	5
0.1 ppm	500	5	10

8.6 Sample Receipt: Samples will be logged into the laboratory notebook as received.

8.7 Sample Batch: Samples will be analyzed in batches of up to 20 study samples, plus laboratory quality control samples. Analysis of each batch will be documented in a laboratory notebook. Recommended analytical sequence:

- SB a QC
- CS hi ###a Standard
- MB ######x QC
- MC ######x ### QC
- VC####X Unknown
- VC####X Unknown
- VC####X Unknown
- VC####X Unknown
- VC####X Unknown
- VC####X Unknown
- VC####X Unknown
- VC####X Unknown
- SB b QC
- CS lo ###b Standard
- SS ######X QC

- SB = solvent blank
- CS = check standard (hi or lo, ### = conc)
- MB = method blank (######x = batch identifier)
- MC = method control (######x = batch identifier, ### = conc)
- SS = second source check standard (###### = date of aliquot)

The analyst will consult with project supervisors when selecting check standard concentrations, which will be based on expected concentrations in the sample population.

8.8 Extraction Procedure:

8.8.1 Procedure for Method Blank and Method Control using the 3M 3500 OVM Badge: Remove an unexposed badge from the can. Remove the plastic ring and white screen from the badge. Immediately, snap the plastic elution cap with plugs onto the main monitor body. Make sure that the plugs are securely closed. Label each badge appropriately. Set the can aside along with the miniature tube supplied with the badge.

8.8.2 Procedure for Unknown Samples: Open the first badge can. Place the can's lid label up in the hood. Remove the badge from the can. Place the can's cap and can in line with the badge.

8.8.3 Prepare the remaining samples in the same manner.

8.8.4 Using a 2.5 mL gas-tight syringe, withdraw 1.5 mL of the Extraction Solution. Place the tip of the syringe in the center hole of the cap. Slowly and carefully release the 1.5 mL into the badge. Close the hole with the plug.

8.8.5 Gently swirl the badge in a circular motion staying on the surface of the hood. Do not swirl the badge in the air.

8.8.6 Repeat steps 8.8.1 through 8.8.5 for the remaining unknown samples and the Method Blank.

8.8.7 Prepare the Method Control using steps 8.8.1 through 8.8.5 using 1.5 mL of the working control solution (see section 8.3.1).

8.8.8 After all badges have received the appropriate extraction solutions, swirl all of them on the hood surface for approximately one minute. Allow them to sit for 15 minutes and swirl again. Allow the badges to sit for 15 minutes and swirl again. Allow the badges sit for 15 minutes.

8.8.9 Unplug the outermost hole of the first badge. Place the miniature tube supplied with the badge in the hole.

8.8.10 Pour the extract into its correspondingly labeled autosampler vial. It may be helpful in transferring the extract from the badge to the vial to gently press the center of the cap. Cap the vial immediately after the transfer.

8.8.11 Repeat steps 8.8.8 through 8.8.10 for the remaining badges. Store all extracts at --20°C until analysis.

8.9 Analysis: Analysis is performed using an Agilent Technologies 6890 GC with a 5973N MSD and ChemStation software.

8.9.1 GC conditions:

Oven

Initial Temp: 40 ºC
Initial Time: 12.00 min
Maximum Temp: 325 ºC
Equilibration Time: 0.50 min

Ramps: 8 ºC/min
Final Temp: 200 ºC
Final Hold: 6.00 min
Total Run time: 38.0 min

Front Inlet
Mode: Splitless
Initial temp: 180 ºC
Pressure: 3.15 psi
Purge flow: 20.0 mL/min
Purge time: 0.70 min
Total flow: 24.1 mL/min
Gas saver: On
Saver flow: 15.0 mL/min
Saver time: 2.00 min
Gas type: Helium

Column
Capillary Column Model Number: J&W 123-1334 DB-624
Max temp: 260 ºC
Nominal length: 30.0 m
Nominal diameter: 320 µm
Nominal film thickness: 1.8 µm

Mode: ramped pressure
Initial pressure: 3.15 psi
Initial time: 0.50 min

#	Rate	Final pres	Final time
1	90	22.50	0.00
2	0.0 (OFF)		

Post pressure: 3.15 psi

Nominal initial flow: 1.7 mL/min
Average velocity: 48 cm/sec

Injector
Sample washes: 0
Sample pumps: 4
Injection volume: 1.0 µL
Syringe size: 10.0 µL
PostInj Solvent A washes: 4
PostInj Solvent B washes: 4
Viscosity Delay: 0 sec
Plunger speed: Fast

PreInj Dwell: 0.00 min
PostInj Dwell: 0.00 min

Thermal Auxiliary
Transfer line temp: 250 ºC

8.9.2 MS conditions:
Ionization Mode: Electron Ionization
Mode: Full scan or SIM
Quad temp: 150 ºC
Quad max temp: 200 ºC
Source temp: 230ºC
Source max temp: 250ºC

8.9.3 Table 2: Compound Retention Times (may vary) and Ions

Compound Name	Ret. Time (min)	100 % Ion	Qualifying Ion
1,3-Butadiene	0.944	39.1	54.1
MTBE	1.756	73.2	57.2
Benzene	3.358	78.1	51
Toluene	7.547	91.1	92.1
Tetrachloroethene	9.678	165.9	130.9
m,p-Xylene	15.263	91.1	106.1
Methyl ethyl ketone	2.507	72.2	57.2
Naphthalene	25.255	128.1	102.1
Styrene	16.56	104.1	78.1
αPinene	17.455	93.1	91.1
p-Dichlorobenzene	20.715	146	111.1
n-Octane	8.549	43.2	85.2
Acrylonitrile	1.801	53.1	52.1
Vinyl chloride	0.933	62.1	64.1
Bromochloromethane	2.691	49.1	129.9
Chlorobenzene d5	13.978	117.1	82.1
1,4-Difluorobenzene	3.982	114.1	63.1
Naphthalene d8	24.748	136.2	108.2

8.10 Quality Control

8.10.1 The following metrics are targets:

- Solvent blanks and method blank: < 3 x MDL
- Calibration checks and 2nd source check: ± 15% of nominal value
- Method control and fortified samples – spike recovery ± 30% of nominal value

8.10.2 Batches that meet all QC criteria described above are automatically accepted. Batch data that do not meet the criteria must be approved or rejected by the project manager or PI.

8.11 Data Management and Review

8.11.1 Chromatogram review and concentration calculations will be performed using Agilent Technologies ChemStation software. Electronic data files containing results for sample extracts as ng/sample will be created for each individual sample. The analyst will review all results for the batch in ChemStation to ensure quality criteria are being satisfied. Data will be exported by batch in .csv format using the Chemstation procedure "File > Export Data to CSV File…" procedure. The resulting .csv file will be sent to a supervisor for processing.

8.11.2 The .csv file will be processed using the macro "Transcribe VOC data" in Microsoft Excel. This macro produces a summary spreadsheet that reports the results for the entire analytical batch in a tabular format. The supervisor will review this file for accuracy of transcription, quality, and completeness. If data are acceptable the supervisor will then run the macro "CreateVOCFlatFile" which reorganizes the data into a flat format (in a new workbook) for uploading to the electronically secure network (ESN) and which assigns laboratory record quality indicators (LRQIs). The final workbook with summary and flat format worksheets will be transferred to a QA folder for final review.

8.11.3 The QA analyst will review the spreadsheet to ensure that quality indicators are consistent with QC acceptance parameters and that data are complete. On completion of review the QA analyst will either reject the spreadsheet with recommendations for corrective action, or approve the data for uploading to the ESN.

8.11.4 On approval of the batch data, the supervisor will save the flat format worksheet as a new file, named consistently with the requirements in EAR-CHATS-021, and upload it to the appropriate folder in the ESN.

8.12 Method Performance: Established MDLs are shown in Table 3. MDLs should be re-determined following corrective actions, or annually otherwise.

Table 3. Established MDLs

Target Compound	MDL (µg)
1,3-Butadiene	0.305
MTBE	0.248
Benzene	0.264
Toluene	0.132
Tetrachloroethene	0.099
m,p-Xylene	0.071
Methyl ethyl ketone	0.401
Naphthalene	0.071
Styrene	0.07
αPinene	0.051
p-Dichlorobenzene	0.07
n-Octane	0.145
Acrylonitrile	0.101
Vinyl chloride	0.231

9.0 References

Determination of Selected Organic Vapors in Air Using 3M 3500/3520 Organic Vapor Monitors, 3M Company, Occupational Health and Environmental Safety Division, May, 2002.

EAR-GLC-001; Extraction of Volatile Organic Chemicals Collected on 3M 3500/3520 Badges

EAR-GLC-002: Analysis of Volatile Organic Chemicals Extracted from 3M Organic Vapor Monitor Badges

EAR-CHATS-021: Procedure for receipt, processing, and review of analytical laboratory results from CHATS.

Morandi, M. T., Stock, T. H., *Personal Exposure to Toxic Air Pollutants,* NUATRC Research Report, No 1. Vol. 1 , 1999

Research Operating Procedure 09

Procedure for Determining Hydrogen Sulfide (H2S) from Passive Samplers for Children's Health after the Storms (CHATS)

Prepared by: Daniel K. Briggs Date: April 11, 2011

Reviewed by: Cynthia Ann Salmons Date: 2/8/2013

Reviewed by: Date: 2/26/2013

Approved by: Date: 2/27/2013

RTI International
Exposure Analysis Research
3040 Cornwallis Road
Research Triangle Park, NC 27709
USA

List of Revisions

Revision Number	Changes	Date
0	Original from RTI	

Table of Contents

1.0 Scope & Application 4
2.0 Summary of Method 4
3.0 Cautions 4
4.0 Apparatus & Materials 5
5.0 Personnel Qualifications 5
6.0 Procedures 5

1.0 Scope & Application

The analytical procedures described in this protocol are intended for the determination of hydrogen sulfide (H_2S) from passive badge samples that will be collected as part of the Children's Health after the Storms (CHATS) Study. This protocol addresses:

- Extraction of passive badge samples
- Analysis of hydrogen sulfide sample extracts by visible spectrometry (by quantitation of methylene blue)
- Laboratory quality control (QC) procedures
- Data processing and documentation

Target analyte:

- Hydrogen sulfide (H_2S)

2.0 Summary of Method

The procedure is taken from the manufacturer of the passive sampler (Radiello®, Fondazione Salvatore Maugeri, Padua, Italy). The cartridge contains zinc acetate, which adsorbs hydrogen sulfide, transforming it into stable zinc sulfide. The sulfide is recovered by extraction with water then reacted with the N,N-dimethyl-*p*-phenylendiammonium ion in a strongly acidic ferric chloride solution (an oxidizing agent) to yield methylene blue. Methylene blue is quantified by visible spectrometry. The primary QC samples are laboratory blanks, which are averaged and subtracted from the samples to correct for background. The spectrophotometer is calibrated using a minimum of a four-point standard curve, which is prepared from dilutions of a methylene blue solution obtained from the manufacturer of the passive samplers. Absorbance measurements are saved in files generated by the spectrophotometer software then processed using a spreadsheet to generate a calibration curve and quantitate sample concentrations. After QA review of the individual data files, data will be read electronically into the study database from the output files.

3.0 Cautions

This procedure involves the use of N,N-dimethyl-*p*-phenylenediammonium oxalate, which is a poison. The operator should read and understand the MSDS. The solid should be weighed only in a vented weighing enclosure and the work area should be cleaned after use. Solutions of sulfuric acid at various concentrations are to be prepared and used, and all such solutions should be handled in a fume hood, and the user should wear PPE including face protection.

4.0 Apparatus & Materials

4.1 Calibration solution for H_2S (methylene blue) #RAD171 (Supelco, Inc., Bellefonte, PA)
4.2 Laboratory deionized water
4.3 Spectrophotometer cuvettes, optical glass, matched set
4.4 UV-Visible spectrophotometer, Beckman Coulter model DU800
4.5 Screw-capped bottles to contain standard solutions and reagents, amber glass (125 mL – 1 L) with Teflon-lined caps
4.6 Volumetric flasks, glass, 50- and 100-mL
4.7 Volumetric pipettes, 2-, 5-, 10-, and 25-mL
4.8 Pasteur pipettes (glass) and bulbs
4.9 Sulfuric acid, ACS reagent grade, Sigma-Aldrich #320501-500ML
4.10 N,N-Dimethyl-*p*-phenylenediamine oxalate, 99%, Acros Organics #408490250
4.11 Iron (III) chloride hexahydrate, reagent grade, Aldrich #F2877-500G
4.12 Top loading balance, Mettler Toledo model PR1203
4.13 Chemiadsorbing cartridges, Radiello® unsampled, vacuum-sealed in plastic bags, #RAD170 (Supelco)
4.14 Multi-tube vortexer, VWR model DVX-2500

5.0 Personnel Qualifications

Testing personnel should read the ROP carefully and have this documented in their training file by the laboratory supervisor.

6.0 Procedures

6.1 Calibration

6.1.1 The stock calibration solution (4.1, above) is stable for 1 year at 25°C per manufacturer product insert. Other calibration solutions are to be prepared fresh.

6.1.2 Calibration Solution A: Dilute 2.0 mL of the stock solution to 100 mL with deionized water. The concentration of this solution is equivalent to 1.145 µg/mL of S^{-2}.

6.1.3 Calibration Solution B: Dilute Calibration Solution A 1:2 (25 mL diluted to 50 mL with deionized water). The concentration of this solution is equivalent to 0.572 µg/mL of S^{-2}.

6.1.4 Calibration Solution C: Dilute Calibration Solution A 1:5 (10 mL diluted to 50 mL with deionized water). The concentration of this solution is equivalent to 0.229 µg/mL of S^{-2}.

6.1.5 Calibration Solution D: Dilute Calibration Solution A 1:10 (5 mL to 50 mL with deionized water). The concentration of this solution is equivalent to 0.115 µg/mL of S^{-2}.

6.1.6 Measure the absorbance of each solution A-D at 665 nm. Calculate the correlation coefficient (r^2) for the four measurements using a suitable software program, e.g. Microsoft Excel. If $r^2 < 0.99$, prepare new calibration solutions, or contact your supervisor.

6.1.7 Also with a suitable software program, calculate the linear regression coefficients *m* (slope) and *b* (y-intercept) for the calibration, using the equivalent S concentrations as the *x* values, and the corresponding absorbances as the *y* values. Record all raw data and calculations in a project notebook.

6.1.8 Reagent solutions: Label, date, and initial each solution on preparation. Solution composition and nomenclature are as specified in the radiello® method instructions.

- *Sulfuric acid 70%*: Slowly add 25 mL of concentrated sulfuric acid to 10 mL water and let the solution cool.
- *Amine*: Dissolve 6.75 g of N,N-dimethyl-p-phenylendiammonium oxalate in the sulfuric acid 70% solution. Dilute this solution to 1 liter with sulfuric acid - water 1:1 v/v. Kept in a dark bottle and well capped, this solution is stable for at least four weeks.
- *Ferric chloride*: Dissolve 100 g of ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) in 40 mL water.
- *Ferric chloride-amine*: Mix 10 mL of ferric chloride solution with 50 mL of *amine* solution. This solution must be freshly prepared for each analytical batch.
- *Sulfuric acid for dilution*: slowly dissolve 40 mL of concentrated sulfuric acid in 900 mL of water, let the solution cool, and make up to 1,000 mL.

6.2 Sample analysis

6.2.1 Samples are stable for 6 months from the date of collection. Because the stability of the amine reagent is substantially shorter than this, batch analyses should be run only often enough to meet the sample holding requirements.

6.2.2 Remove the diffusive sampler from its shipping vial. Unscrew the cap and transfer the cartridge to the vial.

6.2.3 Add 10 mL of water to the plastic tube containing the cartridge, recap and vortex for 5 minutes at 2000 rpm on the multi-tube vortexer.

6.2.4 Add 0.5 mL of ferric chloride - amine solution, recap immediately and stir. The tube must be capped immediately in order to avoid escape of the developed hydrogen sulfide from the tube. Allow the sample to stand for 30 minutes at room temperature.

6.2.5 While samples are incubating, measure the absorbance of all calibration solutions.

6.2.6 Measure absorbance at 665 nm, using DI water to zero the spectrophotometer, within 24 hours. The color is stable for several weeks, per manufacturer's instructions.

6.2.7 Any sample that gives a value above the highest concentration calibration standard should be diluted serially 1:10 using the reagent *sulfuric acid for dilution*, until its absorbance falls within the range of the calibration curve. DO NOT USE WATER TO DILUTE SAMPLES.

6.2.8 Analyze two unexposed cartridges from the same lot and obtain the average blank value, then subtract it from each unknown sample absorbance. Be careful to apply the same dilution ratio to the samples and the blanks.

6.2.9 For each sample, calculate the effective sulfide concentration in the final solution using the following equation:

$$[S] = \frac{(A_{665} - b)}{m}$$

where m and b are the values determined in step 5.1.7, and A_{665} is the blank-subtracted sample absorbance. Record all raw data and calculations results in a project notebook.

6.2.10 The amount of H_2S taken up by the sampler is calculated by multiplying the value in 6.2.9 (sulfide in µg/mL) by 10.5 mL (final volume of the extract and reaction solution), then by (34.06/32.06), the ratio of the molecular weight of H_2S to the atomic weight of sulfide.

6.2.11 Following QA review, data are entered into the database by the laboratory supervisor.

Research Operating Procedures for Handling and Analysis of Passive NO2 Samplers for the Children's Health after the Storms (CHATS) Study

Environmental and Industrial Chemistry Department
Analytical Sciences Unit
RTI International, Research Triangle Park, North Carolina

Prepared by: Eva D. Hardison Date: 12/11/2011

Reviewed by: Cynthia Ann Salmons Date: 12/11/2011

Reviewed by: James W. Rayburn Date: 12/11/2011

Approved by: Dian K. Wagoner Date: 12/11/2011

List of Revisions

Revision Number	Changes	Date
0	Original from RTI	

Table of Contents

1.0 SCOPE AND APPLICATION 4
2.0 SUMMARY OF THE TEST METHOD 4
3.0 EQUIPMENT AND MATERIALS 6
4.0 PERSONNEL QUALIFICATIONS 9
5.0 PROCEDURE 9

1.0 SCOPE AND APPLICATION

1.1 Application Matrix

This Research Operation Procedure to be used for CHATS is for the extraction and analysis of atmospheric nitrogen dioxide (NO_2) that is collected and converted to nitrite ion (NO_2) on Ogawa triethanolamine (TEA)-coated pads (Ogawa & Company, Pompano Beach, FL). (See 5.9 References)

1.2 Limit of Detection and Limit of Quantitation

The minimum detectable quantity of nitrite ion using this procedure is 0.06 µg/collection pad (0.008 µg NO_2^-/ mL extract for an extraction volume of 8 mL). The limits of detection for NO_2 in air depend upon the exposure duration, and are estimated to be 2.3 ppb for a 24-hour exposure and 0.32 ppb for a 168-hour (7-day) exposure.

1.3 Interferences

A high concentration of any anion eluting close to the nitrite ion will result in an interference for measurement of NO_2 measurement. No interferences have been observed in TEA-coated NO_2 collection pads analyzed to date. If interferences are observed, several steps to increase separation can be taken, such as reducing eluent strength and/or flow rate or replacing the ion chromatography columns.

1.4 Purpose

This Standard Operating Procedure presents the procedures for

1. Aqueous extraction of nitrite ion (CAS Number 14797-65-0) from an Ogawa NO_2 collection pad, followed by ion chromatographic analysis of the extract for nitrite ion. The nitrite ion concentration can then be used to calculate the NO_2 concentration (ppb) in air.

2.0 SUMMARY OF THE TEST METHOD

Each Ogawa passive sampler consists of one TEA-coated pad (for NO_2 sampling) mounted in one end of a barrel-shaped holder that is attached to a badge. Each sampler is delivered to the sampling location inside a zip-closure plastic bag that is placed inside a screw-top, airtight storage container. At the sampling location, the passive sampler is removed from the protective shipping container and bag and exposed to ambient air for a carefully selected and documented period of time, usually one day to one week. The sampler is then returned to its bag and shipping container for storage until it is processed for analysis. Blank, unexposed TEA-

coated pads from the same lots as the study samples are provided for use as extraction Method Blanks (MBs). Results will be reported in µg NO_2^- per sample. The average NO_2 exposure concentration can then be calculated based on the nitrite content measured by ion chromatography, the exposure duration, and the appropriate collection factors.

This SOP document contains the RTI Ion Analysis Laboratory procedures for handling the exposed NO_2 pads as received from the field.

2.1 DEFINITIONS, ACRONYMS, AND ABBREVIATIONS

LOD: Limit of detection
LOQ: Limit of quantitation
NO_2: Nitrogen dioxide gas

TEA-coated pads: Sorbent pad coated with triethanolamine for collection of NO_2
NO_2^-: Nitrite ion

IC: Initial calibration
ICV: Initial calibration verification
CCV: Continuing calibration verification
MB: Method blank
MS: Matrix spike
LCS: Laboratory control sample

2.2 HEALTH AND SAFETY WARNINGS

2.2.1 Use of Equipment

The use of the ion chromatograph does not pose any danger or safety hazards.

2.2.2 Chemicals

The chemicals used include deionized water and sodium carbonate and sodium bicarbonate solutions. Concentrated sodium carbonate solution is moderately basic and requires standard laboratory eye protection.

3.0 EQUIPMENT AND MATERIALS

3.1 Equipment

The following equipment is needed for the extraction of the NO_2 collection pads and analysis of the extract:

- Calibrated automatic pipet (adjustable to 8 mL)
- Dionex Series DX-3000 Ion Chromatograph with Conductivity Detector (Dionex, Sunnyvale, CA):
  - Anion eluent flow - 1.5 mL/minute
  - Isocratic, single-flow dual- piston series pump
  - Fixed-volume sample injection loop (125-µL)
  - Electric injection (no compressed gas required)
  - Detector range - 10 µS
  - Separator Column #AS12A
  - Guard Column #AG12A
  - Anion Self-regenerating Suppressor ASRS-ULTRA (Cat # 53946)

3.2 Supplies

The following supplies are needed for extraction of the pads and analysis of the extracts:

- Extract vials (HDPE, 8-mL capacity, leak-proof with screw cap, inert), VWR Boston Round, or equivalent
- Water-resistant sample identification labels, Avery 6577™, 5/8" X 3", or equivalent, to be computer printed with sample IDs
- Forceps, blunt, for handling of pads
- Lint-free paper wipes (Kimwipes), large and small

3.3 Chemicals

The following chemicals are needed for preparation of ion chromatography reagents and standards

- Na_2CO_3, ACS reagent grade or better
- $NaHCO_3$, ACS reagent grade or better

- $NaNO_2$, ACS reagent grade or better
- NO_{2-},1000 µg/mL, NIST-traceable, purchased from GFS or CPI
- Ultrapure water (Millipore's Milli-Q 18.2MΩ-cm water or equivalent)

3.4 Glassware/Plasticware

- Five (5) 1-L volumetric flasks (HD polypropylene preferred for all flasks)
- Ten (10) 100-mL volumetric flasks
- Four (4) 200-mL volumetric flasks
- One (1) 20-L Nalgene carboy with spigot
- Graduated cylinder, 500-mL or 1-L

3.5 Reagents and Standards

3.5.1 Filter Pad Extraction

- Ultrapure water (Millipore's 18.2MΩ-cm water (MQ) or equivalent)

3.5.2 Ion Chromatographic Reagents

Use ACS reagent-grade chemicals and 18.2MΩ-cm deionized water for the preparation of all solutions.

1. Concentrated eluent (100X), 30mM $NaHCO_3$/270mM Na_2CO_3: Dissolve 2.5209 g $NaHCO_3$ and 28.6178 g Na_2CO_3 in 1 L of deionized water (Note: Do NOT dry the salts that are used to prepare the eluent.)
2. Working eluent, 0.3mM $NaHCO_3$/2.7mM Na_2CO_3: Dilute 200 mL concentrated eluent to 20 L with deionized water.

3.5.3 Calibration Standards for Initial Calibration

Use ACS reagent-grade chemicals and 18.2MΩ-cm deionized water for the preparation of all solutions. Dry the salts used for the preparation of calibration standards at 105EC for 2 hours and cool to room temperature in a desiccator immediately before use.

1. NO_2^- Stock Solution, 1000 mg/L NO_2^-: Dissolve 1.4998 g $NaNO_2$ in 1 L of deionized water
2. Standard Solution A (100 mg/L NO_2^-): Dilute 10 mL NO_2^- stock solution to 100 mL with deionized water.

3. Standard Solution B (10 mg/L NO_2^-): Dilute 10 mL Standard Solution A to 100 mL with deionized water.
4. Initial Calibration Standards: Using Standard Solutions A and B, prepare initial calibration standards with deionized water in 100 mL volumetric flasks as shown in Table 1. Prepare fresh calibration standards weekly.

Table 1. Preparation of Initial Calibration Standards

Standard	NO_2^-, (µg/mL)	mL of Standard Solution (volumetric flask volume)
Standard Solution A		
1	25.0	25.0 (100 mL)
2	10.0	20.0 (200 mL)
3	3.0	3.0 (100 mL)
Standard Solution B		
4	1.0	20.0 (200 mL)
6	0.5	5.0 (100 mL)
7	0.2	2.0 (100 mL)
1 mg/L STANDARD (Standard 4)		
8	0.1	20.0 (200 mL)
9	0.05	10.0 (200 mL)

3.5.4 Laboratory Control Samples (LCSs)

1. LCS-Intermediate Solution, 20 mg/L NO_2^-: Using NIST-traceable 1000 µg/mL solution purchased from CPI Chemicals, pipette 2 mL of 1000 µg/mL NO_2^- into a 100 mL volumetric flask and dilute to the mark with deionized water.
2. LC Samples: Using the LCS-intermediate solution, prepare LCSs with deionized water in 100 mL volumetric flasks as shown in Table 2. Prepare fresh LCSs weekly.

Table 2. Preparation of Anion Quality Control Samples

LCS Sample ID	mL LCS-Intermediate Solution	Final Volume, mL (Volumetric Flask Size)	NO_2^- Conc (µg/mL)
LCS-LOW	2.0	100	0.4
LCS-MED	5.0	100	1.0
LCS-HIGH	10.0	50	4.0

4.0 PERSONNEL QUALIFICATIONS

Personnel employed to perform ion analysis operations will have at least an associate's degree in a laboratory science and will be trained by a supervisor before being allowed to process passive samples for the CHATS Study.

5.0 PROCEDURE

5.1 Extraction of Exposed NO_2 Pads

Sample Holding Time

All NO_2 samples will be extracted within 12 days of sample collection, assuming that they are received at RTI within 2 days of collection. The extracts will be analyzed within 48 hours of extraction.

Extract Vial Preparation.

No vial preparation is required by the analysis lab.

Pad Extraction

NOTE: Since exposed pads are more stable when stored dry in the extract vials, extract only the number of samples that can be analyzed at one time.

1. Ensure that the vial is labeled with the unique sample ID as assigned by the CHATS Study.
2. Using a calibrated automatic pipette, introduce 8 mL of Milli-Q water into each extraction vial containing a NO_2 pad.
3. For each 20 exposed samples, place an unexposed, blank filter in a clean vial to be extracted and analyzed as a matrix blank (MB).
4. Check that the pad in each extract vial is completely immersed in the aqueous solution. If the pad is not completely immersed, use clean forceps, wiped with a Kimwipe moistened with Milli-Q water, to depress the pad into the aqueous solution. Be sure to wipe the forceps clean between samples.
5. Cap the vial securely and shake it manually.
6. Let the vial stand for 30 minutes, with occasional re-shaking of its contents.

5.2 Initial Calibration of the Ion Chromatograph

Steps for calibrating the ion chromatograph are as follows.

1. Fill the eluent reservoirs with the eluent.

2. Start the eluent flow, activate the self-regenerating suppressor, and allow the baseline to stabilize.

3. Inject two instrument blanks (DI H_2O) to flush the system and to ensure that the system is operating properly.

4. Using the calibration schedule, perform the daily initial calibration (IC) over the range 0.05 to 10.0 µg/mL NO_2^- (0.01 to 2.0 µg/mL Cl^-).

5. Verify that the correlation coefficient of the calibration curve (r^2) is ≥0.998 for NO_2^-. If either correlation coefficient does not meet this requirement, stop the analysis sequence, identify and correct the problem, and repeat the IC.

6. Follow the calibration with the NIST-traceable initial calibration verification (ICV) QC samples below:

   - LCS- MED, containing concentrations of Cl^- and NO_2^- typical of those found in the mid-range of actual filter extract concentrations.
   - LCS-LOW containing concentrations of Cl^- and NO_2^-, typical of those found at the lower end of actual filter extract concentrations.

     If the observed value for NO_2^- differs by more than 10 percent from the known values, identify and correct the problem before analyzing samples.

5.3 Autosampler Vial Loading

NOTE: Prior to use, autosampler vials and caps must be thoroughly cleaned by leaching with Milli-Q water, completely dried in room air while covered with Kimwipes, and stored in clean covered containers.

1. Use a calibrated automatic pipet with a clean tip to remove 2 mL extract from the extract vial.
2. Transfer the sample into an autosampler vial.
3. Repeat steps 1 and 2, for each sample extract, carefully following the sample queue to ensure that each sample is in the correct autosampler cassette and in the correct

position in the cassette. Remember to use a new, clean pipet tip for each sample extract.

5. For 5% of the samples (1 in every 20), prepare a duplicate by pipetting a second 2-mL aliquot of the sample into an autosampler vial.
6. For 5% of the samples (1 in every 20), prepare a matrix spike by pipetting a second 2-mL aliquot of the sample into an autosampler vial and adding 0.2 mL of Standard 1 (25 ppm NO_2^-). Since sample volume is limited, select a different sample than the one that was used for a duplicate analysis.
7. The samples are now ready for nitrite and/or nitrate analysis by ion chromatography.

5.4 Sample Analysis

1. Load the cassettes into the autosampler according to the sample queue for the sample batch and begin the analysis run, occasionally checking to ensure the ion chromatography system is operating correctly. Analytes are identified by comparison of retention times to those of the initial calibration (IC) standards, initial calibration verification (ICV) standards, and continuing calibration verification (CCV) standards.
2. Examine the data at the end of the run. If the concentration of any ion exceeds the upper end of the calibration curve, dilute that sample appropriately and analyze the following day. Only those samples that are found to require dilution or that require reanalysis due to instrument failure will be analyzed the day <u>after</u> extraction. All other samples will be analyzed the day that they are extracted.

5.5 Sample Extract Storage

After analysis, sample extracts will be refrigerated at 4 ˚C until disposal is requested from project management. (Is there some inventory system in place for specimen retrieval?)

5.6 Data Analysis and Calculations

5.6.1 Calculation of Nitrite Loading

Nitrite peak areas are entered into the computer where calculations are performed using a quadratic fit to the calibration data. Peak integrations are performed by the Chromeleon software (i.e., no manual integrations are performed). The quadratic fit yields the following:

$$y_i = ax_i^2 + bx_i + c$$

where:

y = the calculated nitrite concentration, mg/L
x = the instrument response

The calibration curve from 0.05 to 10.0 ppm is used for the calculation of the extract nitrite concentrations. All nitrite concentrations that exceed 10 ppm are diluted appropriately (usually 5-fold) to bring the nitrite concentration into the calibration range and reanalyzed.
The nitrite concentration calculated by the Chromeleon software, in units of ppb (ng/mL), is multiplied by the extract volume (8 mL) to obtain the collection pad loading in ng NO_2. The loading in ng NO_2^- is divided by 1000 to obtain the loading in µg NO_2^-. RTI will report the sample loading, in µg NO_2^- to the Analytical Data Coordinator. No blank corrections will be performed.

5.7 Method Performance

The performance of the passive NO_2 method is measured using matrix spikes, duplicate analyses, and quality control samples. Acceptance criteria are discussed in Section 5.8. Performance indicators for similar passive NO_2 sampling and analysis studies are summarized in Table 3.

Table 3. Method Performance Indicators from Previous NO_2 Studies

Sample Type	Performance Indicator	Target NO_2^- Conc, µg/mL	n	Minimum	Maximum	Average
Matrix Spike	% Recovery	N/A	14	98.8%	109.5%	102.3%
Duplicate Analyses	Relative % Difference	N/A	24	0.1%	9.5%	1.6%
QA_CPI-LOW	% Recovery	0.4	17	90.7%	110.2%	96.1%
QC_LOW	% Recovery	0.4	14	94.0%	104.1%	98.8%
QC_MED	% Recovery	1.0	20	95.1%	104.9%	98.8%
QC_MED-HI	% Recovery	2.0	4	95.4%	102.9%	98.2%

1. A NIST-traceable quality assurance sample.

2. RTI-prepared check standards from a source independent of the calibration standards.

3. Matrix spike % recovery $= \frac{quantity\ of\ NO_2^- added, \mu g}{quantity\ of\ NO_2^- recovered, \mu g} \times 100$

4. Relative % Difference $= \frac{|\ C_1 - C_2\ |}{(C_1 - C_2\ )/2} \times 100$

Where C = analyzed concentration of NO_2^-, $\mu g/mL$

5. % Recovery – $\frac{C\ analyzed}{C\ target} \times 100$

5.8 Data Assessment and Acceptance Criteria for Quality Control Measures

All sampling and laboratory analysis will be conducted in adherence to standard protocols for the operation of ion chromatographs. If the correlation coefficient for the calibration does not exceed 0.998, the analysis will be stopped and the problem identified before proceeding with the sample analyses.

Laboratory QC procedures include the comparison of the regression parameters for the calibration curve performed prior to each run with those obtained in the past; analysis of a QC sample at the beginning of every analytical run; analysis of a duplicate sample, spiked pad extract, and a calibration check standard every 20 samples or daily; inclusion of a commercially-prepared, NIST-traceable QC sample with each run. Additionally, five percent of the samples will be run in duplicate for the determination of analytical precision. All standards will be prepared using ACS reagent grade or better chemicals. All results from the QC checks, calibrations, and precision checks will be reported in writing with the data report.

5.8.1 QC/Technical Requirements

The CHATS Study QC elements and their acceptance limits are defined as follows:

- Initial Calibration (IC): The IC is a 7-point calibration, with a correlation coefficient ≥0.998, performed prior to sample analysis, after failure of ICV and/or CCV, and after preparation of new IC standards.
- Initial Calibration Verification (ICV): The ICV is a standard prepared from a second standard source and is performed immediately following the IC. The difference between ICV and IC must be within ±10%. The "NIST-traceable QC" sample fulfills this requirement when it is performed after the IC.
- Continuing Calibration Verification (CCV): A CCV is performed prior to sample analysis (unless IC performed), after every 10 study samples, and at the end of the analysis sequence. The difference between the CCV and ICV must be within ±10%.
- Method Blank (MB): The Method Blank (MB) consists of a blank TEA-pad, from the same lot used for NCS study samples, that is extracted as a sample. The MB is analyzed after the IC/ICV or CCV (as appropriate), and prior to analysis of the associated study samples. The quantitated analyte concentration detected in the MB is reported in the EDD. Blank corrections to sample data will not be made. A MB is required with each extraction batch (not to exceed 20 samples/batch). NOTE: DI H2O blanks will be injected prior to the IC to flush the system, but the results will not be included in the EDD.

- Duplicate Sample: A sample replicate analysis consists of a second injection of a previously extracted and analyzed sample. A duplicate sample is required for each extraction batch, and the relative percent difference (RPD) between the initial and duplicate result must be ≤ 10% when the analyte concentration is >10 times the LOQ.
- Matrix Spike: A Matrix Spike (MS) will be prepared with each extraction batch (extraction batch shall not exceed 20 samples) by spiking a known quantity of the analyte into a sample extract aliquot. Recovery must be within 90-110%; if not, data for that run must be rejected.
- Laboratory Control Sample: A Laboratory Control Sample (LCS) will be prepared with each extraction batch (extraction batch shall not exceed 20 samples) by spiking analytes into deionized water. Recovery must be within 90-110%.

5.8.2 Corrective Actions for out-of-control data.

Corrective actions may include the following:

- Replacing or remaking reagents if contaminated
- Recalibration of the ion chromatograph
- Replacing ion chromatography column bed supports
- Replacing autosampler tubing

5.8.3 Contingencies for Handling Out-of-control Data

If QC out-of-range issues are noticed, analysis will be halted and the RTI Project Leader will be notified immediately. The data will be reviewed for impact. Corrective action will be taken and all samples between the last in-control QC sample and the out- of- control QC sample will be reanalyzed. It must be noted that there is only one extract from each passive badge and if the extract is compromised, then that sample is lost. If the extract is not compromised, then corrective action will be taken with the measurement process, and another aliquot of the extract will be reanalyzed using the ion chromatographic method.

5.9 References

NO, NO_2, NO_x, and SO_2 Sampling Protocol Using the Ogawa Sampler, Edition 6.0, June 2006. (Link to procedure on Ogawa website: http://www.ogawausa.com/pdfs/prono-noxno2so206.pdf)

Research Operating Procedure CORE-HEME-1

Automated Complete Blood Count (CBC) on the Coulter LH750 for Children's Health after the Storms (CHATS)

Prepared by: Charles L. Surbeck, Jr. MT(ASCP) Date: 4/28/2010

Approved by: Dr. F. W. Brazda, MD Date: 4/28/2010

Clinical Pharmacology-Toxicology Laboratory

MCLNO Pathology Services

LSU Interim Hospital

List of Revisions

Revision Number	Changes	Date
0	Original from LSU	

This Research Operating Protocol describes the determination of Complete Blood Count (CBC) from blood samples collected for CHATS. The method will be implemented at LSU as described in Attachment A.

Attachment A.

Method from LSU Core Laboratory Hematology Manual – CORE-HEME-1

PRINCIPLE

The COULTER® LH 750 is a quantitative, automated hematology analyzer for In Vitro Diagnostic use in clinical laboratories. The LH 750 provides automated complete blood count, leukocyte differential, reticulocyte analysis, and nucleated red blood cell (NRBC) enumeration.

The purpose of the LH 750 is to separate the normal patient, with all normal system-generated parameters, from the patient who needs additional studies of any of these parameters. These studies might include further measurements of cell size and platelet distribution, biochemical investigations, manual WBC differential or any other definitive test that helps diagnose the patient's condition.

Equipment: COULTER® LH 750 System
The LH 750 System consists of three subsystems, designated as "CBC" (Complete Blood Count), "WBC (White Blood Cell) Differential" and "Retics" The CBC subsystem is based on the established Coulter principles of automated cell counting. The WBC differential subsystem and Retics are based on the Coulter principles of leukocyte differential counting as embodied in the COULTER VCS technology.

CBC Analysis:
The Coulter method counts and sizes cells by detecting and measuring changes in electrical resistance when a particle (such as a cell) in a conductive liquid goes through a small aperture.

Each cell suspended in a conductive liquid (diluent) acts as an insulator. As each cell goes through the aperture, it momentarily increases the resistance of the electrical path between two submerged electrodes, one located on each side of the aperture. This causes an electrical pulse that can be counted and sized. While the number of pulses indicates particle count, the size of the electrical pulse is proportional to the cell volume.

Reticulocyte Analysis:
A supravital dye, New Methylene Blue, is incubated with whole-blood samples. The dye precipitates the basophilic RNA network found in reticulocytes. Hemoglobin and unbound stain are removed by adding a clearing reagent, leaving clear spherical mature RBCs and darkly stained reticulocytes.
Stained reticulocytes are differentiated from mature cells and other cell populations by light scatter, direct current measurements, and opacity characteristics.

Differential Analysis:
WBC differential analysis and classification occurs in the flow cell, where:

- Low-frequency current measures volume
- High-frequency current senses cellular internal content through measuring changes in conductivity

- Light from the laser bouncing off the individual WBC cells characterizes cellular surface, shape and reflectivity

The conductive diluent must affect cells minimally, if at all. Both lytic reagents must destroy erythrocytes without significantly affecting leukocytes. They must work rapidly to satisfy the speed with which the system works. The leukocyte preservative must:

- Provide clear separation of the white blood cell populations, and
- Preserve leukocytes in their near-natural state for accurate cytometric measurement

Sampling Modes:

The LH 750 operates in two modes of sample aspiration:

- AUTOMATIC ASPIRATION MODE
  The system automatically transports, mixes, aspirates and processes specimens.
- MANUAL ASPIRATION MODE
  The Manual mode of operation is like the Automatic mode except:
  - Before you run the sample, you enter the sample identification number
  - You use an open vial and introduce the sample at the aspirator tip
  - You begin the cycle

Analyzer Parameters:

The system determines these hematologic parameters of whole-blood specimens:

WBC	White Blood Cell or leukocyte count
RBC	Red Blood Cell or erythrocyte count
Hgb	Hemoglobin concentration
Hct	Hematocrit (relative volume of erythrocytes)
MCV	Mean Corpuscular (erythrocyte) Volume
MCH	Mean Corpuscular (erythrocyte) Hemoglobin
MCHC	Mean Corpuscular (erythrocyte) Hemoglobin Concentration
RDW	Red Cell (erythrocyte volume) Distribution Width
Plt	Platelet or thrombocyte count
MPV	Mean Platelet (thrombocyte) Volume
LY%	Lymphocyte percent
MO%	Monocyte percent
NE%	Neutrophil percent
EO%	Eosinophil percent
BA%	Basophil percent
LY#	Lymphocyte number
MO#	Monocyte number
NE#	Neutrophil number
EO#	Eosinophil number
BA#	Basophil number
NRBC%	Nucleated Red Blood Cell percent
NRBC#	Nucleated Red Blood Cell number

RET%	Reticulocyte percent
RET#	Reticulocyte number
*HLR%	High Light scatter Reticulocytes %
*HLR#	High Light scatter Reticulocytes #
IRF	Immature Reticulocyte Fraction
MRV	Mean Reticulocyte Volume
*MSCV	Mean Sphered Cell Volume
*Pct	Plateletcrit
*PDW	Platelet Distribution Width

**For Research Use Only. Not For Use In Diagnostic Procedures.*

Unless otherwise stated, all parameter results are shown in a US unit format throughout the manuals.

The following parameters are measured **directly**:

WBC: Coulter principle
RBC: Coulter principle
PLT: Coulter principle
HGB: Photometric measurement
Diff% VCS Technology NE%, LY%, MO%, EO%, BA%, RET %

The following parameters are **derived from RBC or PLT Histograms**:

MCV: RBC histogram
RDW: RBC
histogram PLT: PLT
histogram MPV: PLT
histogram

The histograms are developed using the Coulter principle. Therefore, indirectly, these parameters are based on the Coulter principle.

The following parameters are **computed**:

HCT = RBC x MCV /l0
MCH = HGB x l0/ RBC
MCHC = HGB x l00/HCT
DIFF # = DIFF % x
WBC/l00

SPECIMEN COLLECTION AND HANDLING

Detailed generic specimen collection and handling procedures are located in the second section of this CWC Manual. The following are specimen collection and handling procedures specific to the LH 750 Series analyzer.

Conditions for patient preparation:
No patient preparation is necessary.
Specimen type:
Anticoagulated human whole blood

Venous blood collection:
Collect venous samples via syringe or collection tube using a salt of EDTA (K_2, K_3) with the proper proportion of blood to anticoagulant. Beckman Coulter recommends you use a dipotassium or tripotassium salt of EDTA. Although the LH 750 analyzer only needs 1.0 mL of sample to aspirate in Automatic mode, collect enough venous blood required for the type of vacuum tube system used. Follow the directions on the manufacturer's package insert to ensure correct fill volume. Do not test samples that are incorrectly filled or that are clotted.

Microtainer tubes:
Microtainer samples are collected in a salt of EDTA. Follow the manufacturer's recommendations for the micro-collection device. Do not test samples that are incorrectly filled or that are clotted.

Specimen handling, venous blood:
Mix venous blood sample at least 8 times by hand inversion. Gently turn capped sample upside down then back straight up. Alternatively, use a mechanical mixer for at least 5 minutes.

Analyze specimens as soon as possible for optimum accuracy. Analyze venous blood samples within 24 hours of collection if stored at room temperature (23.9°C or 75°F). Run blood samples within 48 hours after collection if refrigerated at 2 to 8°C (35.6 to 46.4°F). For reticulocyte counts, run blood samples within 24 hours of collection if stored at room temperature (23.9°C or 75°F). Reticulocyte counts may be done within 72 hours of collection if the blood sample has been refrigerated at 2 to 8°C (35.6 to 46.4°F). Warm samples to room temperature (15.5° to 32°C or 60° to 90°F) before testing.

Specimen handling, Microtainer tubes:
Mix Microtainers by flicking the plastic sample vial with finger several times. Check sample for clots before testing. CAUTION: Do not flick the specimen too hard; vigorous mixing can destroy fragile RBCs.

Follow the manufacturer's recommendations for specimen stability and storage when collected in micro-collection devices.

Specimen labeling:
Coulter recommends the use of bar-code labels for specimen identification. The bar-code labels used with this system must conform to the Coulter specifications.

Sample tube:
It is important to affix the labels carefully so that the scanner reads them correctly.

1. Place the bar-code label so that the first bar of the bar-code symbol is at least ½ inch from the tube cap.

2. Press the label down securely, including edges and corners, so that it is flat and smooth against the tube.
3. Ensure the bars on the label are parallel to the stopper. If the label is skewed 12° or more, the scanner may not read it.

Specimen rejection criteria:
Clotted samples, QNS samples, and samples received in improper containers should not be processed. Physician or licensed caregiver should be notified. Document this in the LIS with physician or licensed caregiver name and time of notification.

EQUIPMENT AND MATERIALS

Equipment:
The LH 750 is a modular system that consists of a Power Supply, Diluter, Analyzer, LH 750 Workstation, Printer and Handheld Scanner. Hardware Options include the integrated LH 750 SlideMaker, and LH 750 SlideStainer.

CAUTION *System integrity can be compromised and operational failures can occur if:*
- *This equipment is used in a manner other than specified*
- *You introduce software that is not authorized by Beckman Coulter into your computer*
- *You install software that is not an original copyrighted version*

Operate the instrument as instructed in your product documentation. Only operate your system's computer with software authorized by Beckman Coulter. Only use software that is an original copyrighted version to prevent virus contamination.

Equipment performance parameters:
Operate the system in a room with a temperature of 15.5° to 32°C (60° to 90°F) and humidity up to 95% without condensation. If the average room ambient temperature changes more than 5.5°C (10°F) from the calibrating temperature, verify calibration and recalibrate if necessary to ensure conformance to specifications.

Materials, Reagents:
Beckman Coulter recommends these reagents or their equivalents. All stated performance characteristics are based on the use of the LH 750 with these reagents. Refer to the container's label for detailed information before using the reagent.

Diluent
LH 700 series diluent is an isotonic electrolyte solution that:
- Dilutes the whole-blood samples
- Stabilizes cell membranes for accurate counting and sizing
- Conducts aperture current
- Rinses instrument components between analyses

- Carries and focuses the sample stream in the flow cell to direct the blood cells through the aperture

Since cell size (volume) is measured, the effect of diluent on osmosis or other phenomena must be tightly controlled. The diluent must not contain particles and must not support growth of bacteria or molds. LH Series diluent is azide free, bacteriostatic, and fungistatic.

CBC Lytic Reagent

LYSE S® III Diff lytic reagent:

- Rapidly lyses erythrocytes (RBCs), freeing hemoglobin (Hgb) and reducing the size of cellular debris to a level that does not interfere with leukocyte (WBC) count
- Causes a substantial conversion of the Hgb to a stable cyanide-containing pigment, the absorbance of which is directly proportional to the Hgb concentration over the clinical range

LH 700 Series PAK Reagent System

The LH 700 Series PAK reagent system contains Erythrolyse II (PAK LYSE) and StabiLyse (PAK PRESERVE).

Erythrolyse II erythrocyte lytic reagent:

- Dilutes the blood samples
- Rapidly lyses erythrocytes (RBCs)
- Reduces cellular debris to an insignificant level

StabiLyse leukocyte preservative:

- Maintains leukocytes (WBCs) in their near-natural state
- Allows the leukocytes to be differentiated into their subpopulations through the volume, conductivity and light-scatter measurements

LH 700 Series RETIC PAK Reagent System

The LH 700 Series RETIC PAK contains Reagent A and Reagent B.

Reagent A, Retic Stain:

- Reticulocyte staining solution is a specially formulated, New Methylene Blue (NMB) dye that stains the reticulum

Reagent B, Retic Clearing Solution:

- Reticulocyte clearing solution is a clearing reagent that removes hemoglobin from the erythrocytes (RBCs) without removing the precipitated dye-RNA complex, keeping the cell and its membranes intact

Cleaning Agent

COULTER CLENZ® cleaning agent cleans and rinses the internal surfaces of the instrument components. Daily use prevents protein buildup and eliminates the need for routine aperture bleaching.

Reagent preparation:
No reagent preparation is required. Appropriate safety precautions for handling reagents are contained on the respective Material Safety Data Sheets located in the MSDS binder.

Reagent storage:
Store reagents at ambient room temperature (23.9°C or 75°F). Keep containers closed. Discard reagents at the expiration date. Replace reagents when the level sensors detect low reagent volume. The screen prompt appears:

C Cleaner is low
D Diluent is low
L CBC lytic reagent is low
P DIFF PAK is low
R RETICPAK is low
V Vacuum overflow is full
W Waste container is full.

Replacing Reagents:

1. Remove any cardboard cutouts.
2. Remove the cap and seal from the new reagent container. Be sure to completely remove the foil seal.
3. Remove the plastic collar that secures the pickup tube assembly.
4. Unscrew the pickup tube assembly from the old container.
5. Lift the assembly straight up and out.
6. I**MPORTANT:** Incorrect results can occur if the tubes become contaminated. Do not touch the tubes or let them touch any laboratory surfaces. If the tubes touch anything, rinse them with distilled water and then wipe them with a lint-free tissue.
7. Inspect the pickup tube for damage, and replace it if necessary.
8. Carefully insert the pickup tube assembly straight into the new container.
9. Tighten the cap. Insert the plastic collar that secures the pickup tubes.
10. Record the new container's information in Quality Assurance set up and date and initial the reagent package.
11. Ensure the pneumatics are on.
12. Press **STARTUP** on the Numeric Keypad to prime the reagents.
13. Print the System Setup page, print the StartUp page, and place with the daily QC.

Reagent tracking:
Reagent Log is printed daily and when LH Series diluent, Lyse S, LH Series PAK, StabiLyse (PAK PRESERVE) Diff Preservative**,** and/or the LH Series RETIC PAK are changed. This log includes reagent lot #, open expiration, date opened, and shelf life expiration.

Supplier: Reagents, controls, and calibrators are supplied by Beckman Coulter.

Waste Container:
Be sure the waste line is connected to either a chemically-resistant open drain less than 30 inches above the floor, or a waste container with a minimum capacity of 20 L (5 gallons). In either case, the maximum waste line length is 12 ft. (3.7 m). Incomplete waste chamber drainage and eventual waste chamber overflow into the vacuum system can occur if the waste line is too long. ***WARNING**: Possible biohazardous condition. The contents of the old waste container and its associated tubing can include residual biological material and must be handled with care. Check the tubing connection and container location periodically. Avoid skin contact and clean up spills immediately. Dispose of the contents of the waste container in accordance with your local regulations and acceptable laboratory procedures.*

Draining Overflow Chamber:
Drain the overflow chamber if it has liquid in it because the baths overflowed.

1. Ensure pneumatics are on.
2. Use FO5 to activate solenoid 12 to drain the overflow chamber.

***WARNING** Possible biohazardous condition. The overflow chamber can contain residual biological materials and must be handled with care. Follow your laboratory's protocol for safety measures. This may include, but is not limited to, wearing protective eyewear, gloves, and suitable laboratory attire when operating or maintaining this analyzer.*

Waste Disposal:
Waste can contain biohazardous material. Avoid skin contact and clean up spills immediately. All instrument waste is disposed of through drain lines.

Controls:
Use stable reference controls to monitor the instrument performance as part of your quality control and to verify calibration. Refer to the package insert for detailed information before using a control.

- 5C®-ES Control, three levels in pierceable tubes, monitors the CBC and differential (Diff) parameters.
- LATRON Primer, used immediately prior to running LATRON control, prepares the tubing and the instrument components for the LATRON control process.
- LATRON Control monitors the performance of the volume, conductivity and light scatter measurements.
- Retic-C Cell control monitors the performance of reticulocyte (Retic) parameters.

Calibrator:
The S-CAL® calibrator kit is an acceptable alternative to the whole-blood reference method of calibration. S-CAL calibrator is traceable to reference methods and materials. Use S-CAL

calibrator to ensure accurate instrument measurements. Refer to the package insert for detailed information before use.

The differential and reticulocyte measurement devices are set for optimum performance at the factory.

Control/calibrator handling:
Handle controls and calibrators using universal precautions. Controls and calibrator contain stabilized human erythrocytes and no test method can offer complete assurance that Human Immunodeficiency Virus (HIV), Hepatitis C Virus (HCV), Hepatitis B Virus (HBV) or other infectious agents are absent. Take appropriate safety measures to avoid contact from aerosols when removing the cap/stopper assembly.

Use absorbent material such as gauze when removing the rubber stopper from the vial. This prevents accidental contact with the product.

Allow refrigerated controls/calibrators to remain at ambient room temperature for 15 minutes before use. Mix by hand according to directions in the technical insert accompanying the product.

Control/Calibrator storage:
Store at 2 to 8°C (35.6 to 46.4°F). Sealed control and calibrator vials are stable until the expiration date. Opened calibrator vials are stable for 1 hour. Discard expired control/calibration materials.

Before use, inspect controls/calibrators for indications of instability or deterioration. Gross hemolysis (darkly colored supernatant) is indicative of product deterioration. Do not use deteriorated product.

Supplier: Reagents, controls, and calibrators are supplied by Beckman Coulter.

DAILY STARTUP AND SHUTDOWN

Press **POWER ON** to turn the instrument on.

LASER WARNING: *Possible harm to operator. Do not use any controls, make any adjustments, or perform any procedures other than those specified herein. To do so may result in hazardous radiation exposure.*

The Triple Transducer Module and Bar-Code Reader contain lasers. A laser is a unique light source that exhibits characteristics different from conventional light sources. The safe use of the

laser depends upon familiarity with the instrument and the properties of coherent, intense beams of light. The beam can cause eye damage and instrument damage. There is enough power from the laser to ignite substances placed in the beam path, even at some distance. The beam might also cause damage if contacted indirectly from reflective surfaces (specular reflection). The lasers on the LH 750 are covered by protective housings that are held in place by tamper-proof screws.

Do not attempt to remove the laser or to open it. Failure to comply can result in hazardous radiation exposure. If removal is required, a Beckman Coulter Representative must do it.

Trained personnel at the Beckman Coulter factory must do all service and maintenance of the laser. If removal is required, a Beckman Coulter Representative must do it.

Logging Off:

1. Select on the Command Center to display the Log Off window.
2. Select to confirm that you want to log off the current user name and display the Log On window.

Logging On:

1. Type your user name that was defined by your laboratory administrator.
2. Type your password that was defined by your laboratory administrator. If you forget your password, contact your laboratory administrator to reset your password.
3. Select **OK**. The Workstation checks your password and starts the appropriate application.

Performing Daily Startup:

1. Check to see if the instrument is running properly.
   a. Verify that the instrument has been turned o. If the power is off, press **POWER ON** on the Diluter Keypad.
   b. Ensure the pneumatics are on. (Pneumatics are OFF if the Power Supply pneumatic light is red or not on, or if the Analyzer screen is blank.) If necessary, press **PRIME APERT** on the Diluter Keypad to activate the pneumatics.
   c. Check the Power Supply and vacuum level.

CAUTION: System damage can occur if operating the instrument when any indicator is outside the following limits. DO NOT operate the instrument if any indicator is outside these limits!

On the Power Supply, check the following statys and function levels:

Status or Function Level	*Should Appear*
Input Power (AC) Status	Green indicates the function is within normal range; red indicates outside normal range.
Output Voltage (DC) Status	
Pneumatic (PNEU) Status	
Temperature (TEMP) Status	
60 PSI	60 ± 5 psi
30 PSI	30 ± 1 psi
5 PSI	5 ± 0.1 psi
VACUUM	22 in. Hg minimum at sea level
AC LINE	Above 90 V

Turn adjustment controls clockwise to increase pressure; counterclockwise to reduce pressure. If the vacuum is out of range, call the Coulter Representative.

d. Check the baths to ensure proper function
   - Open the aperature compartment door
   - Press **DRAIN** on the Diluter Keypad
   - Verify that both aperture baths drain completely
   - Press **RINSE** on the Diluter Keypad
   - Verify that both baths fill with liquid
   - Verify that the waster chamber drains
   - Close the aperture compartment door

2. Press **START UP** on the Numeric Keypad to begin the automatic startup cycles.
   a. Watch the cycles for normal reagent flow. When "COUNT" appears on the Analyzer screen ensure:
      - No bubbles appear in the three lines from each aperture bath
      - Diluent drips steadily from the three lines into the vacuum isolator
      - All traces of cleaning agent are removed from diluent dispensers, baths, and associated tubing

3. If you have system options, such as the LH 750 SlideMaker, startup the system option.
4. Check the startup test results.
5. If necessary, select to display the daily startup test results.
6. To see past startup test results:
   a. Select .
   b. Select a row indicating the date, time and type of test result you want to see. The results appear on the window.

7. Check the reagent status, background status, and subsystem status for any items that failed
8. Refer to LH 750 System Help for the appropriate action to resolve any failed items.

9. Select on the Command Center to verify the Workstation run configuration.
10. Verify the following settings at the Analyzer
    - Test Mode
    - Alarm
11. Run controls.

Performing Daily Shutdown:

1. At least once every 24 hours perform the following procedure.
2. Go to the Numeric Keypad.
3. Press **SHUTDOWN**.
4. Log off the workstation.
5. Let the instrument sit in cleaning agent for at least 30 minutes.

CALIBRATION

Calibration fine-tunes the LH 750System so it provides the most accurate results possible. Your laboratory is responsible for the final calibration of the CBC parameters and for recording the calibration factors. Beckman Coulter recommends S-CAL® calibrator, or an exact equivalent, as an acceptable alternative to whole-blood calibration. In the normal process of tracking data for an extended period of time, your laboratory can make a specific decision to recalibrate a given parameter. Never adjust to a specific value for an individual sample.
For best performance, calibrate all the CBC parameters. The WBC differential and Retic parameters are calibrated at the factory; they do not require calibration in the laboratory.

Calibration frequency:
You should calibrate your instrument:

- At installation
- After the replacement of any component that involves dilution characteristics (such as the BSV) or the primary measurements (such as the apertures)
- Twice a year with a minimum required frequency of once every 6 months
- When advised to do so by your Beckman Coulter Representative.

Calibration verification:
You should verify the calibration of your instrument:

- Twice a year with a minimum required frequency of once every 6 months
- When controls begin to show evidence of unusual trends
- When controls exceed the manufacturer's defined acceptable limits.
- If the average ambient room temperature changes more than 10°F from the calibrating temperature.

Calibrating CBC Parameters with S-CAL Calibrator

1. Ensure the apertures are clean.
2. Ensure the instrument is functioning properly.
3. Perform a 10-sample reproducibility study on the CBC parameters using Automatic aspiration mode.
4. Perform carryover.
5. Prepare the instrument for calibration.
6. Set up the CBC calibration information at the Workstation.
7. Run S-CAL calibrator.
8. Review results on the Calibration window.
9. Adjust parameters as needed.
10. On the Command Center, select **AUTO ANALYSIS** as the process type.
11. Verify calibration by cycling each level of COULTER 5C®-ES cell control in Automatic aspiration mode.

Ensure the apertures are clean:
If the instrument is shut down for at least 30 minutes every 24 hours in COULTER CLENZ cleaning agent, cleaning is unnecessary.

If COULTER CLENZ cleaning agent is routinely used but you are beginning calibration after processing patient samples, shut down the instrument in the cleaning agent for 30 minutes before proceeding.

CAUTION: DO NOT aspirate bleach! Possible flow cell damage could occur if you aspirate bleach.

Ensure the instrument is functioning properly:

1. Check the reagent containers for:
   - Sufficient quantity
   - Not beyond expiration date
   - No precipitates, turbidity, particulate matter, or unusual color.
2. Proper connections between the Diluter and the reagent containers.
3. Perform daily startup.
4. In addition to verifying daily startup results, verify acceptable:
   - Reproducibility
   - Carryover
   - Control results.

Perform Reproducibility Check:

Sample Requirements

Collect whole blood from a single donor who:

- Is receiving no medication.
- Has normal hematologic parameters, with a WBC count of 10,000 ± 1,000.
- Has normal erythrocyte, leukocyte and platelet morphology and, if checking the Diff parameters, with Diff values:

Neutrophils	40 to 72%
Lymphocytes	17 to 45%
Monocytes	4 to 12%
Eosinophils	0 to 10%
Basophils	0 to 1%

Procedure

1. Ensure the whole blood volume from a single donor is enough for 11 cycles.
2. Ensure pneumatics are on.
3. Ensure the blood detector is enabled.
4. Ensure the number of aspirations per tube is set to 1.
5. If necessary, select the instrument name on the Command Center.
6. Select REPRODUCIBILITY as the processing control on the Command Center.
7. If necessary, clear out the values that appear on the results table.
8. Cycle one sample of normal whole blood in Automatic aspiration mode.

WARNING: Needle damage can occur if you pierce a specimen tube more than five times.

9. Set the number of aspirations per tube to 5.
10. Separate the well-mixed normal whole blood sample into two tubes.
11. Place the tubes into consecutive positions in a cassette and place the cassette in the loading bay. The system automatically begins processing the cassette. It pierces, aspirates, and analyzes the samples.
12. Review the reproducibility results.

Reviewing Reproducibility Results

1. Select **QA** on the Command Center to display the Quality Assurance application.
2. Select to display the Reproducibility window.
3. If necessary, select the instrument to review.
4. Review the results table and statistics table on the window. Use the scroll bars to review results that do not appear on the window.

5. Verify that the CV (Coefficient of Variation) does not exceed the established limits. If any results exceed these limits, an instrument problem may exist. Call the Coulter Representative.

Reproducibility Limits for CBC:

Parameter	*%CV*
WBC	*≤ 2.5*
RBC	*≤ 0.8*
Hgb	*≤ 0.8*
MCV	*≤ 0.8*
Plt	*≤ 3.2*
MPV	*≤ 5.0*

Perform Carryover Check:

Procedure

1. Ensure pneumatics are on.
2. Ensure the blood detector is disabled.
3. Ensure the mode of operation is set to CBC.
4. Ensure the number of aspirations per tube is set to 1.
5. If necessary, select the instrument name on the Command Center.
6. Select CARRYOVER as the processing control on the Command Center.
7. If necessary, clear out the values that appear on the results table.
8. Obtain a normal whole blood specimen.
9. Separate the well-mixed whole blood specimen into two 5 mL tubes.
10. Place the tubes into consecutive positions in a cassette.
11. Dispense 2 to 3 mL of diluent into three separate 5 mL tubes.
12. Place the three diluent tubes in the same cassette. Place the tubes after the whole blood specimens.
13. Place the cassette in the loading bay. The instrument begins processing the cassette automatically.
14. Verify the carryover results.

Carryover Formula

The LH 750 software calculates the percent carryover by using:

(1st diluent - 3rd diluent) ÷ 2nd sample x 100 = % carryover

Reviewing Carryover Results

1. Select QA on the Command Center to display the Quality Assurance application.
2. Select to display the Carryover window.
3. If necessary, select the instrument to review.

4. Verify that the message "CARRYOVER ACCEPTABLE" appears on the Carryover window. If Carryover is unacceptable, the unacceptable values appear flagged. Review the results table and the limits table.

   NOTE: If negative carryover values persist, and the third diluent is higher than the first two, check background. If the problem persists, an instrument problem may exist; call the Coulter Representative.
5. Print the carryover results for the logbook. Carryover results are kept in the Calibration Log Book.

Prepare the instrument for Calibration:

1. Ensure the room temperature is stable and within the normal ambient temperature range. If the average ambient room temperature changes more than 10°F from the calibrating temperature, verify calibration and recalibrate (if necessary).
2. Ensure the blood detector is enabled.
3. Ensure the test mode is CBC.
4. Go to the Diluter Keypad.
5. Press **PRIME APERT** if necessary to activate the pneumatics.
6. Cycle a sample of normal whole blood in Automatic aspiration mode as a prime.

Set up CBC Calibration Information at the Workstation:

1. On the Command Center, select the instrument to be calibrated.
2. On the Command Center, select **CALIBRATION** as the processing control.
3. Select **QA** to display the Quality Assurance application. Select to display the Calibration window.
4. If necessary, select the instrument to be calibrated.
5. If necessary, select to record the old calibration information in its history log and delete the information from the database. The values on the window change to 0.00.
6. If you have already set up calibration information, check that the appropriate lot number for calibration is selected; otherwise, set up new calibration information.

Run S-CAL Calibrator:

1. Prepare the S-CAL calibrator according to the instructions in the package insert.

IMPORTANT: Misleading results could occur if the calibration procedure is not performed within 1 hour of opening the S-CAL calibrator vials. Follow the instructions in the S-CAL calibrator package insert.

2. Place the vial of S-CAL calibrator in position 1 of a cassette, and place the cassette in the loading bay. This automatically begins processing the cassette. The system pierces, aspirates and analyzes the S-CAL calibrator 11 times. The

Workstation automatically deletes the results from the first sample because the results are used as a prime.

Reviewing calibration results:

1. On the Calibration window, inspect the calibration results table for trending; the parameter results must not show a trend. If the results show trending, there could be an instrument problem; call the Coulter Representative. DO NOT CONTINUE. If the calibrator is expired, the expiration date is displayed in black text with a red background, and the calibration run is discarded.
2. Use the calibration statistics to determine whether to transmit the calibration factors to the Analyzer. (See "Calibration Criteria" on the next page.)

   * The Workstation checks the results to ensure the FAC % DIFF numbers are less than or equal to established limits, and for precision (%CV within the established limits). The Workstation flags results outside the limits with a red background. If calibration factors that are outside the limits are transmitted, a message appears. Confirm the transmission of the calibration factors. Once confirmed, the Workstation transmits the calibration factors and posts a message to the Calibration history log.

IMPORTANT: Misleading results could occur if calibration factors that are outside the established limits are transmitted. If results are outside the limits, call the Coulter Representative.

   * The Workstation checks the FAC % DIFF and DELTA DIFF. The Workstation automatically selects (marks with √) the parameters that need adjustment and flags results that meet the calibration criteria with a yellow background. If you do not want to adjust a marked parameter, unmark it. If all parameters are within limits, a message appears indicating that all calibration factors are set correctly.

IMPORTANT: Misleading results could occur if MCV is calibrated when the RBC FAC % DIFF is out of range because MCV depends on RBC. DO NOT calibrate MCV if the RBC FAC % DIFF is out of range.

3. Print a copy of the calibration results for the logbook. Calibration results are kept in the Calibration Log Book.
4. Ensure the calibration factors to be transmitted to the Analyzer are selected with √

Calibration Criteria:

PARAMETER	PRECISION (%CV)	ACCEPTABLE FAC % DIFF	CALIBRATE IF FAC % DIFF IS:	CALIBRATE IF DELTA DIFF

				IS:
WBC	CV≤2.5%	≤ 5.0%	>1.25% but ≤5.0%	>0.1 but ≤0.4
RBC	CV≤0.8%	≤ 2.0%	>0.7 % but ≤2.0%	>0.03 but ≤0.09
HGB	CV≤0.8%	≤ 3.0%	>0.78% but ≤3.0%	>0.1 but ≤0.4
MCV	CV≤0.8%	≤ 2.5%	>1.18% but ≤2.5%	>1.0 but ≤2.0
PLT	CV≤3.2%	≤ 9.0%	>2.70% but ≤9.0%	>6.0 but ≤20.0
MPV	CV≤5.0%	≤ 20.0%	>5.0 % but ≤20.0%	>0.5 but ≤2.0

Adjust Parameters as needed:

1. Ensure the Analyzer is ready to receive the new calibration factors.
2. On the Workstation Calibration window, select to transmit the calibration factors (for the selected parameters) to the Analyzer.
3. Print the new calibration factors for the logbook. All calibration records are kept in the Calibration folder.
4. Verify calibration by cycling each level of COULTER 5C cell control in Automatic aspiration mode.

MIXER VERIFICATION

Specimens collected in anticoagulant for hematologic studies must be mixed thoroughly prior to analysis. Proper mixing of samples on each tube rocker/mixer and automated analyzer should be verified. Verification should occur upon installation of a mixer or automated analyzer and when repairs are made that would alter mixing capabilities of the equipment.

Verification specimen:
5 settled EDTA anticoagulated whole blood samples.

Verification procedure for rotary tube rocker/mixer:

1. Mix 5 samples by manual inversion 60 times to ensure proper mixing of the specimen.
2. Run the well mixed samples on the LH750 analyzer by selecting REPRODUCIBILITY as the process type and using the SECONDARY or MANUAL mode. The mean value of the 5 samples is placed in row 1 (designated as "Run 1") of the Mixer Verification Sheet.
3. Allow the 5 samples to settle until separation of plasma and cells is clearly visible.
4. Place the 5 samples on the tube rocker/mixer to be verified and allow the specimens to mix for 5 minutes.
5. Rerun the samples as described in #2 by selecting REPRODUCIBILITY as the process type and using the MANUAL mode.
6. Record the mean values in row 2 (designated as "Run 2") on the Mixer Verification Sheet.
7. The difference between the values in row 1 and row 2 are recorded in row 3 of the sheet. The maximum allowable difference for each parameter
   are: WBC ±0.4 NE% ±5.0%

RBC ±0.12	LY% ±3.0%
HGB ±0.3	MONO%
±3.0% MCV ±2.0	EOS% ±2.0%
PLT ±25	BASO% ±1.0%

Verification procedure for automated analyzer:

1. Mix 5 samples by manual inversion 60 times to ensure proper mixing of the specimen.
2. Run the well mixed samples on the LH750 analyzer by selecting REPRODUCIBILITY as the process type and using the PRIMARY or AUTOMATIC mode. The mean value of the 5 samples is placed in row 1 (designated as "Run 1") of the Mixer Verification Sheet.
3. Allow the 5 samples to settle until separation of plasma and cells is clearly visible.
4. Place the 5 settled samples in an LH750 cassette and allow the analyzer to transport and mix the specimens on the rocker bed and process in the AUTOMATIC mode via REPRODUCIBILITY process type.
5. Record the mean values in Row 2 of the Mixer Verification Sheet.
6. The difference between the values in row 1 and row 2 are recorded in row 3 of the sheet and compared to the acceptable ranges stated above.

Procedural notes:

1. If mixing by manual inversion, 60 complete inversions must be performed.
2. It is recommended that rotary tube mixers be used for at least 5 minutes to mix samples properly. Some rocking platforms may mix adequately to maintain even cellular distribution of previously well-mixed specimens but cannot fully mix settled specimens.
3. Analyzers with automated samplers should allow adequate mixing time to homogeneously disperse cells in a settled specimen prior to sampling.

QUALITY CONTROL

Quality control includes monitoring routine performance and service in conjunction with the use of controls and calibrators. The combination of these methods provides the assurance of complete quality control. The LH 750 incorporates multiple quality control techniques. For the CBC, CBC/DIFF and RETIC parameters, the LH 750 uses the established technique of commercial controls. The LH 750 uses a stabilized particle suspension, such as LATRON, to verify flow cell alignment, gains, and CVs for flow cell volume, conductivity and light scatter. The Workstation stores information about the control setup and control results in the DataBase.

QC frequency:

Test QC samples at scheduled intervals throughout the day using Coulter 5C cell control and test every 24 hours with Coulter Latron primer and control. Coulter 5C is tested once per shift (every 12 hours) and Coulter RETIC C is analyzed once per 8 hours.

Warm Coulter 5C and RETIC C cell controls to room temperature. Vials are slowly rolled between the palms of the hands eight times in an upright position; invert vial and again roll it eight times. Repeat this mixing procedure. Controls are assayed in the same manner as patient samples. Verify that the open expiration date is not exceeded. Open control vials should not be used after 13 days.

The bar coded Coulter cell controls are placed in a cassette and run. The Workstation automatically identifies the control by level, stores data in proper control file, compares results to expected ranges, and flags out of range results by H (high) or L (low). If the Control is OUT and is between 2SD and 3SD, rerun to determine if it is a statistical outlier. A statistical outlier is a value that is between 2SD and 3SD and occurs only 5% of the time (approximately 1 out of 20). If it is a statistical outlier, the second set of results should be in control. Do not delete statistical outliers. If the same parameter is out on another control level, delete controls and troubleshoot. DO NOT RUN SPECIMENS until all controls are acceptable. If the Control is OUT of 3SD, delete and rerun the control. If it is still out of 2SD, delete results, troubleshoot and do not run specimens. Document all troubleshooting in the instrument Service Log. Document each out of range QC at the analyzer Workstation QC Results Table comment column. Notify supervisor immediately.

Note: After troubleshooting, if a problem has been found in the analytical process which would affect patient results, a random reassay of patients must be performed from the previous run. Refer to QC Overview for details.

Latex Primer and Control can be run three ways: Diff only, Diff & Retic, or Retic only. All three methods are shown below.

Running Latex Control – *Diff only:*

1. Check that the instrument process type on the Command Center is set to AUTO ANALYSIS.
2. Ensure the latex primer and control are within the correct temperature range. For COULTER LATRON primer and control the correct temperature range is 18-30°C/64-86°F.
3. Verify the lot number of the primer and control. If you must use a new lot number, ensure that it has been set up properly. Refer to "Setting Up Controls" section of LH750 System Help.

CAUTION: Possible system damage could occur if you aspirate anything except latex control or primer using this function. Do not aspirate any other materials with this function.

1. Go to the Numeric Keypad.
2. Press **F55 ENTER** to aspirate latex for the Diff test mode. The Numeric Keypad displays PRESS MANUAL OR CLEAR APERATURE.

3. Press **CLEAR APERT**. The Numeric Keypad displays PRESS MANUAL OR PRESENT SAMPLE.
4. Remove the cap of the latex primer vial.
5. Immerse the aspirator tip in the latex primer vial. The instrument automatically aspirates the primer.
6. Remove the vial from the aspirator tip when you hear a beep and the Analyzer Status line displays PREPARING SAMPLE. The probe cleaner retracts the aspirator and automatically cleans it.
7. At the Workstation, check the results from the primer.
8. If the results in the PRIMER column are less than or equal to 500, proceed to step 12. Otherwise, if the results in the PRIMER column are greater than 500:
   a. At the Numeric Keypad, press **ENTER** to reactivate the function for the control. The Numeric Keypad displays PRESS MANUAL OR CLEAR APERTURE. b. Perform steps 6 through 9 up to three more times.
   c. If you do not get a result below 500, cycle a new vial of primer.
   d. If you still do not get a result below 500, call your Beckman Coulter Representative.
9. At the Numeric Keypad, press **ENTER** to reactivate the function for the latex control. The Numeric Keypad displays PRESS MANUAL OR CLEAR APERTURE.
10. Gently mix the latex control according to the directions in the package insert.
11. Immerse the aspirator tip in the latex control vial. The instrument automatically aspirates the control.
12. At the Workstation, verify the results from the control.
13. At the Numeric Keypad, press **STOP** to exit this function. The Numeric Keypad displays READY.

Running Latex Control – *Diff and Retic:*

1. Check that the instrument process type on the Command Center is set to AUTO ANALYSIS.
2. Ensure the latex primer and control are within the correct temperature range. For COULTER LATRON primer and control the correct temperature range is 18-30°C/64-86°F.
3. Verify the lot number of the primer and control. If you must use a new lot number, ensure that it has been set up properly. Refer to "Setting Up Controls" section of LH750 System Help.

CAUTION: *Possible system damage could occur if you aspirate anything except latex control or primer using this function. Do not aspirate any other materials with this function.*

1. Go to the Numeric Keypad.
2. Press **F57 ENTER** to aspirate latex primer and control for the Diff and Retic test modes. The Numeric Keypad displays PRESS MANUAL OR CLEAR APERATURE.

3. Press **CLEAR APERT**. The Numeric Keypad displays PRESS MANUAL OR PRESENT SAMPLE.
4. Remove the cap of the latex primer vial.
5. Immerse the aspirator tip in the latex primer vial. The instrument automatically aspirates the RETIC+DIFF primer.
6. Remove the vial from the aspirator tip when you hear a beep and the Analyzer Status line displays PREPARING SAMPLE. The probe cleaner retracts the aspirator and automatically cleans it.
7. At the Workstation, check the results from the primer.
8. If the results in the PRIMER column are less than or equal to 500, proceed to step 12. Otherwise, if the results in the PRIMER column are greater than 500:
   a. At the Numeric Keypad, press **ENTER** to reactivate the function for the control. The Numeric Keypad displays PRESS MANUAL OR CLEAR APERTURE. b. Perform steps 6 through 9 up to three more times.
   c. If you do not get a result below 500, cycle a new vial of primer.
   d. If you still do not get a result below 500, call your Beckman Coulter Representative.
9. At the Numeric Keypad, press **ENTER** to reactivate the function for the latex control. The Numeric Keypad displays PRESS MANUAL OR CLEAR APERTURE.
10. Gently mix the latex control according to the directions in the package insert.
11. Immerse the aspirator tip in the latex control vial. The instrument automatically aspirates the control.
12. At the Workstation, verify the results from the control.
13. At the Numeric Keypad, press **STOP** to exit this function. The Numeric Keypad displays READY.

Running Latex Control – *Retic only:*

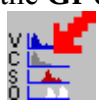
1. Check that the instrument process type on the Command Center is set to AUTO ANALYSIS.
2. Ensure the latex primer and control are within the correct temperature range. For COULTER LATRON primer and control the correct temperature range is 18-30°C/64-86°F.
3. Verify the lot number of the primer and control. If you must use a new lot number, ensure that it has been set up properly. Refer to "Setting Up Controls" section of LH750 System Help.

CAUTION: Possible system damage could occur if you aspirate anything except latex control or primer using this function. Do not aspirate any other materials with this function.

1. Go to the Numeric Keypad.
2. Press **F56 ENTER** to aspirate latex for the Retic test mode. The Numeric Keypad displays PRESS MANUAL OR CLEAR APERATURE.

3. Press **CLEAR APERT**. The Numeric Keypad displays PRESS MANUAL OR PRESENT SAMPLE.
4. Remove the cap of the latex primer vial.
5. Immerse the aspirator tip in the latex primer vial. The instrument automatically aspirates the primer.
6. Remove the vial from the aspirator tip when you hear a beep and the Analyzer Status line displays PREPARING SAMPLE. The probe cleaner retracts the aspirator and automatically cleans it.
7. At the Workstation, check the results from the primer.
8. If the results in the PRIMER column are less than or equal to 500, proceed to step 12. Otherwise, if the results in the PRIMER column are greater than 500:
   a. At the Numeric Keypad, press **ENTER** to reactivate the function for the control. The Numeric Keypad displays PRESS MANUAL OR CLEAR APERTURE. b. Perform steps 6 through 9 up to three more times.
   c. If you do not get a result below 500, cycle a new vial of primer.
   d. If you still do not get a result below 500, call your Beckman Coulter Representative.
9. At the Numeric Keypad, press **ENTER** to reactivate the function for the latex control. The Numeric Keypad displays PRESS MANUAL OR CLEAR APERTURE.
10. Gently mix the latex control according to the directions in the package insert.
11. Immerse the aspirator tip in the latex control vial. The instrument automatically aspirates the control.
12. At the Workstation, verify the results from the control.
13. At the Numeric Keypad, press **STOP** to exit this function. The Numeric Keypad displays READY.

Reviewing LATRON Control Results:

1. Select **QA** on the Command Center to display the Quality Assurance application.
2. Select **QC** to display the Controls window.
3. If necessary, select the instrument to review.
4. Select the specific control to be reviewed. The Control Results table, statistics and graphs appear on the window. Use the scroll bars on the window to view other parameter results and graphs.
5. To view the results and graphics for a specific latex run:
   a. Select the **Gr** column of the run in the table to be viewed.
   b. Select  to see the Latex Graphs window.

When a Latex Control is Outside its Expected Ranges:

1. Ensure the control setup information (assigned values and ranges) matches those on the package insert. If they do not, change the control information to match the package insert, and then rerun the control.

2. Ensure no bubbles exist in the flow cell by rerunning the primer and the control. If the control is still outside the expected ranges:
   a. Go to the Numeric Keypad.
   b. Use **F13** to purge the flow cell.
   c. Run primer and control again.
3. Check the control:
   a. Ensure the control is not contaminated, properly mixed and not expired.
   b. Ensure the aspirator tip is clean and dry.
   c. If necessary, use a new vial of latex control. Be sure to mix it according to the directions on the package insert.
4. Ensure the flow cell is clear by performing the procedure for clearing a clogged flow cell, found in the LH 750 System Help.
5. Rerun the control. If the control is still outside the expected ranges, call your Beckman Coulter
   Representative.

Cycling Cell Controls in the Automatic Mode:

1. Ensure the instrument is set up for the appropriate control.
2. Prepare the controls according to the directions in the package insert.
3. Ensure the controls are properly set up on the Workstation.

   NOTE: If you run a Beckman Coulter control without setting it up, the Workstation automatically creates control set up information for you, identifying the lot number, source, type and level of the control.

4. Load the cassette with the control material.

   Loading the Cassette: Slide each sample firmly into the cassette and ensure that the bar codes are facing up.

5. Place the cassette firmly and securely into the loading bay. The instrument begins to cycle the controls.
6. On the Command Center, Select **AUTO ANALYSIS** as the process type for control tubes with bar-code labels.
7. Review the control results.

Cycling Cell Controls in Manual Mode:

1. Ensure the instrument is set up for the appropriate control.
2. Prepare the control(s) according to the directions on the package insert.
3. Ensure the controls are properly set up on the Workstation.
4. If you are entering the sample ID manually,
   a. Press **ID** and enter the sample ID at the Numeric Keypad.
   b. If you are entering the sample ID using the handheld scanner, put the cursor in the barcode field at the workstation Command Center, scan the sample ID using the handheld scanner, and then press **ID**.
5. Press **ENTER.**

6. Remove the stopper from the specimen tube, using the proper precautions.
7. Immerse the aspirator tip in the tube. The instrument automatically aspirates the sample.
8. When you hear a beep, remove the tube from the aspirator tip. The probe cleaner retracts the aspirator and automatically cleans it.
9. Review the control results.

Reviewing Control Results:

1. Select **QA** on the Command Center to display the Quality Assurance application.
2. Select **QC** to display the Controls window.
3. Select the specific control for which you want to review results. The Control Results table, statistics and graphs appear on the window. Use the scroll bars on the window to view other parameter results and graphs.

When a Control is Outside its Expected Ranges:

1. Ensure the control:
   - Material was mixed properly. If not, mix it according to the package insert.
   - Identification information was entered correctly. If using a bar-code reader, ensure the bar-code labels are clean and positioned correctly. If using the Numeric Keypad, ensure you typed the correct information.
   - Setup information (assigned values and expected ranges) matches the control package insert. If they do not, change the control's information to match the package insert.
2. If any of the problems existed, rerun the control; otherwise, proceed to the next step.
3. Rerun the control to ensure the problem was not a statistical outlier.
4. Ensure the control material was not contaminated by running another vial or level of control.
5. Watch for normal sample flow as part of troubleshooting the instrument. If necessary, call your Beckman Coulter Representative.
6. DO NOT REPORT PATIENT TEST RESULTS UNTIL CONTROL VALUES ARE ACCEPTABLE.

Automatic to Manual Mode Comparison:

Daily, a specimen is run in the automatic mode and manual mode. Results should agree within established limits and recorded on the *Auto to Manual Mode Comparison Sheet*. Limits established by comparing 30 specimens over a period of days.

Differences between the two modes should not exceed the following limits:

WBC	0.4×10^3 cells or 5 %, whichever is greater.	NEUTROPHILS	±5.0
RBC	0.2×10^6 cells or 2 %, whichever is greater.	LYMPHOCYTES	±5.0
HGB	0.3 g/dL or 2 %, whichever is greater.	MONOCYTES	±3.0
PLT	20×10^3 cells/uL or 7 %, whichever is greater.	EOSINOPHILS	±4.0
		BASOPHILS	±0.5

Twice Yearly Analyzer Comparison:

Twice yearly, three specimens are run on each LH750 analyzer to check for correlation of patient results using CBC parameters (WBC, RBC, Hgb, and PLT) within normal range. Values from each instrument on each parameter should be within 10% of the instrument mean for CBC and RETIC C.

Levy Jennings:
The laboratory has determined the Historic SD for Coulter 5C and RETIC C cell control values from 6 months of IQAP summary data and Mysis Levy Jennings charts. The working SD is applied to the Quality Control files in the Workstation, as well as in Mysis. Data is collected for 10 runs over a period of 5 days for each new lot number. Each Workstation QC file calculates the new mean and applies the Historic SD to determine the 2SD range. Running mean, SD, and CV calculations are stored in the Workstation QC Files for each parameter. At the end of each lot, the results should be printed out. The individual file data may then be deleted. Control files may be created for new lot numbers alongside current files.

Bull-algorithm:
The XB is monitored 0700-1530. A high incidence of abnormal specimens at the stated time limits the usefulness of this tool. XB is used as a troubleshooting tool and as an early indicator of clinically insignificant calibration drift. It does not prevent result reporting, provided process controls (Coulter 5C and RETIC C) are acceptable.
The stability of the red cell indices (MCV, MCH, and MCHC) is the basis of a quality control technique called XB Analysis. The XB Analysis used in the Workstation does the calculating automatically. Target Values are established by calculating the means of 1,000 specimens run for MCV, MCH and MCHC (50 batches of 20). Once the Target Values are established, the XB Analysis can be applied using 20 patient sample batches. The XB formula both trims the data by giving less weight to outliers and smoothes it by incorporating information from the previous patient batch in the analysis of the current batch. As each sample is processed, the mean of the previous set of samples is subtracted from each of the red cell indices. The square root of this deviation (difference between the means) is stored. After 20 samples have been processed, the sum of the square roots is divided by 20. The result is squared to recover the mean deviation. The individual deviations carry a positive or negative sign, so then it can be added to or subtracted from the corresponding previous means. The resulting new mean is then used for the succeeding batch of 20 samples.

If the batch means are not within the target values limits, the XB Analysis OUT will appear on the Workstation and the XB batch values should be reviewed for a very abnormal patient population (AIDS patients - high MCVs) or for data that does not represent patient results. These results can be deleted. If the XB is still out, investigate the instrument and Coulter LH 750 systems associated with the directly-measured parameters. If the MCV is out, check Diluent and/or troubleshoot MCV problem. If the MCH is out, review results for Hgb and RBC to isolate the problem parameter. If the MCHC is out, review 5C results for Hgb, RBC and MCV. Troubleshooting individual parameters includes cleaning the BSV and bleaching apertures. After troubleshooting, run three levels of 5C and verify that the parameter has been corrected.

Notify supervisor. If corrected, specimens may be processed and the XB monitored. If not corrected, call Service and document all actions taken in the Service Log. XB should be printed out at the end of 20 batches.

Interlaboratory Quality Assurance Program:
Interlaboratory Quality Assurance Program is offered by Beckman Coulter to provide a summary of monthly quality control and comparison with peer group analysis. Data is submitted from the Workstation via upload from disk or manually entered to the Beckman Coulter IQAP website. The data is analyzed and a report of the data and peer group information is available on the website within 2-3 weeks. The information is reviewed by the supervisor. SDI and CVI should be less than 2.0. The SDI is an expression of the number of standard deviations our results are from the pool mean. The CVI is the ratio of our CV to the pooled CV. The CV should not change >50% from month to month. If so, investigation is warranted. The SDI should be <1.5 for two QAP reports in a row or investigation is warranted.

MAINTENANCE AND TROUBLESHOOTING

Maintenance
A LH 750 maintenance log is located in the *Hematology Maintenance Log* book for each LH750S. Document all Daily, Weekly, Monthly, Twice-Yearly, and As Needed maintenance procedures on the maintenance log. Record all Coulter service problems in the Service Log. See Coulter LH750 System Help for maintenance procedures.

Troubleshooting

1. Remove and clean BSV - only performed when instructed by Coulter
2. Clean and/or bleach apertures - performed when decreased cell counts are observed, increased MCV is noted, increased vote-outs experienced, or debris is seen in the aperture (see Workstation Help for instruction)
3. XB Analysis: see Bull Algorithm above

SAFETY

1. Gloves and lab coats must be worn.
2. Blood and materials contaminated with blood should be disposed of in a special container lined with Biohazard bags.
3. For a blood spill, decontaminate spill with a 10% bleach solution. Cover the area with paper towels to absorb spill. The spill is cleaned and towels discarded in contaminated containers lined with Biohazard bags. Gloves must be worn while cleaning.

4. Open stoppers on EDTA tubes using gauze to prevent aerosol contamination when utilizing secondary mode.

RUNNING PATIENT SAMPLES

Automatic Aspiration Mode:
The Workstation identifies a sample by:

- Reading the cassette number and cassette position of each sample at the time it is cycled.
- Reading the tube's bar-code label automatically.
- Allowing you to provide sample demographic information that includes optional identifiers, such as a patient identifier.
- Time-stamping sample results with the date and time they were analyzed.

Using Bar-Code Labels:
Coulter recommends the use of bar-code labels for specimen identification. The LH 750 System comes with cassette and cassette position numbers 1 to 100. Two labels provide identification:

- Cassette ID label provides a 4-digit cassette number.
- Cassette bar-code label provides the cassette ID number and the 2-digit position number.

Specimen tube bar-code labels provide sample identification.

LIS Downtime:
When LIS is down, refer to the manual downtime procedure located in the Core Lab Policy Manual. To manually transmit data to the LIS, select the **PC to PC** icon after selecting the samples to transmit.

Labeling Requirements:
Ensure the bar-code labels are undamaged; the use of poor quality, dirty, improperly placed or damaged bar-code labels could keep the instrument from reading them. Place the bar-code label so that the first bar of the bar-code symbol is at least ½ inch from the tube cap. Place each label so that it does not cover the bottom of the tube and is flat and smooth against the tube. Do not skew the bar-code label more than 12°.

Using cassette carriers:
The cassette is the carrier for the sample tubes (patient, control, or special test) used in Automatic aspiration mode where automatic loading, mixing, and sampling occurs. Tubes should be pushed into the cassette with the tube bar-code label facing up. Always hold the cassette firmly by its edges. Do not try to hold or lift a cassette by grabbing a tube. The weight of the remaining tubes could cause the cassette to fall.

IMPORTANT: *Use of poor quality, dirty, improperly placed or damaged bar-code labels could keep the instrument from reading the bar-code labels. Ensure the bar-code labels are undamaged and are placed straightly, flush with the tube cap. Place each label flat and smooth against the tube.*

Two sizes (2 mL and 3 mL) of gray sleeve adaptors are available to accommodate narrow and short tubes. Place the tube inside the adapter before placing it into a 13-mm cassette. You must position the gray sleeve adaptors in a cassette so that their keys (located on top of the bar-code read window) fit into the top openings of the cassette. Make sure the bar-code label appears within the read window.

CAUTION: Possible specimen leakage or clogging of the aspiration system can occur. Excessive piercing of the sample tubes causes significant coring of the stopper. The number of pierces without problems can vary slightly among sample tube types and manufacturers. Do not pierce a blood collection tube more than five times.

WARNING: Forcing a tube into the cassette improperly could cause it to break. Do not force a tube into a cassette. If a tube should break, use the laboratory's safety procedure for cleaning the broken glass and your work area.

Cycling Samples in Automatic Aspiration Mode:

1. Ensure the instrument is set up for the appropriate test.

   IMPORTANT: *Misleading results can occur if specimens contain clots. Inspect specimens for clots and use good laboratory practices for verifying results to ensure you do not receive misleading results.*

2. Ensure your specimens have been collected, stored, and mixed properly.
3. Load the cassette by sliding each tube firmly into the cassette, with bar codes facing up.
4. Place the cassette firmly and securely into the loading bay on the right side of the Diluter. The instrument automatically begins cycling the cassette.
5. After the instrument cycles the samples, review the sample results on the Workstation.

Manual Aspiration Mode:

The workstation identifies a sample by:

- Reading the tube's bar-code label when you use the handheld scanner.
- Reading a sample identifier you provided by using the Numeric Keypad.
- Allowing you to provide sample demographic information that includes optional identifiers, such as a patient identifier.
- Time-stamping sample results with the date and time they were analyzed.

Using the Handheld Bar-Code Scanner:

1. Ensure the cursor is in the field you want to fill with the scanned information.
2. Aim the scanner at the barcode and press the trigger. If necessary, adjust the scanner position so the red scan beam is centered on the bar code and overlaps it on both sides.
3. When the scanner has read the symbol, you will hear a beep. If you do not hear a beep:
   - Ensure the scanner is properly connected to your Workstation.
   - Make sure the scanner is properly configured for your labels. Refer to the LH 750 System Help for more information.

   IMPORTANT: *Risk of missing identifier. If you fail to send the sample ID to the instrument within 60 seconds of data entry in the Barcode ID field, the sample ID provided is cleared. This minimizes the risk of sample misidentification.*

4. Press **ID**, then • to send the bar-code ID to the Analyzer. After the bar-code ID appears on the Analyzer, ENTER ACCEPT/STOP REJECT appears on the Numeric Keypad.
5. Press ENTER to accept the bar-code ID or STOP to reject the bar-code ID.

Entering the ID Manually:

1. Press **ID** and enter the sample ID at the Numeric Keypad.
2. Press **ENTER**.

Cycling Samples in Manual Mode:

1. Ensure the instrument is set up for the appropriate test.

   IMPORTANT: *Misleading results can occur if specimens contain clots. Inspect specimens for clots and use good laboratory practices for verifying results to ensure you do not receive misleading results.*

2. Ensure your specimens have been collected, stored, and mixed properly.
3. Remove the stopper from the specimen tube, using the proper precautions.
4. Immerse the aspirator tip in the tube. The instrument automatically aspirates the sample.
5. When you hear a beep, remove the tube from the aspirator tip. The probe cleaner retracts the aspirator and automatically cleans it.
6. After the instrument cycles the samples, review the sample results on the Workstation.

Reviewing Results:

As appropriate, the LH 750 applies instrument-generated and/or laboratory-defined flags, codes and/or messages to each set of patient results. Flags, codes and suspect or definitive messages are used to alert you of an instrument malfunction, specimen abnormality, abnormal data pattern, or abnormal results. Beckman Coulter recommends review, appropriate to the requirements of the patient population, of all results displaying a flag, code or message. More information about Flags and Codes can be found in your LH 750 System Help.

1. Select on the Command Center to display the Patient Tests application.
2. If necessary, select to display the Results & Graphics window that contains:
   - Parameters
   - Flags and codes
   - Suspect/definitive messages
   - Histograms
   - DataPlots
   - Identification information.
3. If necessary, find the sample results you want to review.
4. Specify the way you want the window updated:
   - Keep the current sample displayed. You can view the graphs, demographics and detailed parameter results (including research data) for the sample results as needed.
   - The Workstation continues to receive, process and store analysis data from the Analyze r.
   - Automatically updates the window as the Workstation receives patient sample analysis data from the Analyzer.

Reference Ranges: Normal Values for Males and Females:

.

Age	WBC ($x10^3$/uL)	RBC ($x10^6$/uL)	HGB (gm%)	HCT (%)	MCV (FL)	MCH (PG)	MCHC (gm/dL)
Birth-2 weeks	9.0-30.0	3.9-6.3			95-121	31-37	30-36
Birth-2 month			14-20				
Birth-3 month				42-63			
2 weeks-1 mo	5.0-21.0	3.0-5.4			86-124		
2 weeks-2 mo						23-34	29-37
1 mo-2 mo		2.7-4.9					
1 mo-3 mo					77-115		
1 mo-6 mo	5.0-19.5						
2 mo-6 mo		3.1-4.5				25-35	
2 mo-2 years			9-14				30-36

3 mo-6 mo				31-43	74-108		
6 mo-2 years	6.0-17.5	3.7-5.3		33-43	70-86	23-31	
2 years-4yrs	6.0-17.0						
2 -6 years		3.9-5.3	9-14	34-42	75-87	24-30	
2 years-Adult							31-37
4 -6 years	5.0-15.5						
6-8 years	5.0-14.5						
6-12 years		4.0-5.2	11.5-15.5	36-51 (male) 35-45 (female)	77-95	25-33	
8-16 years	4.5-13.5						
12-18 years		4.4-5.3 (male) 4.1-5.2 (female)	13-16 (male) 12-16 (female)		78-98	25-35	
12 years-Adult				40-51 (male) 35-46 (female)			
16 years-Adult	4.5-11.0						
18 years-Adult		4.5-5.9 (male) 4.0-5.2 (female)	13.5-17.5 (male) 12-16 (female)		80-100	26-34	

Age	RDW (%)	PLTC ($x10^3$/uL)	MPV (fL)	RETIC (%)	RETIC ABS (#)	Differentials
Adult		130-400				
All Ages	11.5-14.5		7.4-10.4	0.5-1.5	0.02-0.08 (male) 0.02-0.09 (female)	Refer to WBC Differential procedure

Reportable Ranges: The operating ranges reflect the range of values over which the instrument displays, prints and transmits results. Values that are between the linear range and the operating range, and values outside the reportable range, are displayed, printed and transmitted with an over linear range flag (+). Values that are above the operating range are inhibited, and the value is replaced by pluses (+++++). The reportable range, or Analytic Measyrement Range (AMR) identifies the values where the instrument is accurate.

Parameter	Operating Range	Analytic Measurement Range/ Reportable Range	Units
WBC	0.0 - 900	0 - 400	x 10^3 cells/ µL

RBC	0.00 - 20.00	0.00 - 8.0	x 10^6 cells/µ L
Hgb	0.0 – 99.9	0.0 -25.0	g/dL
Hct	0.0 – 99.9	N/A	%
MCV	0.0 - 300.0	0.0 – 150.0	fL
MCH	0.0 – 99.9	N/A	pg
MCHC	0.0 - 99.9	N/A	g/dL
RDW	0.0 – 99.9	N/A	%
Pltc	0.00 – 5000	0.00 - 3000	x 10^3 cells/ µL
Pct	0.0 – 9.999	N/A	%
MPV	0.0 – 99.9	N/A	fL
PDW	0.0 – 99.9	N/A	%
NE%	0 – 100	0 – 100	%
LY%	0 – 100	0 – 100	%
MO%	0 – 100	0 – 100	%
EO%	0 – 100	0 – 100	%
BA%	0 – 100	0 – 100	%
NE#	0 – 500.00	0.00 – 100.00	x 10^3 cells/ µL
LY#	0 – 500.00	0.00 – 100.00	x 10^3 cells/ µL
MO#	0 – 500.00	0.00 – 100.00	x 10^3 cells/ µL
EO#	0 – 500.00	0.00 – 100.00	x 10^3 cells/ µL
BA#	0 – 500.00	0.00 – 100.00	x 10^3 cells/ µL
RET%	0.00 – 100	0.00 – 30.0	%
RET#	0.00 – 999.9	0.0000 – 0.7500	x 10^6 cells/µ L

Reporting format: The Sample Results screen shows sample identification information, sample mode, sample results, and gives messages.

Panic/Alert values:

Parameter	Panic/Alert Values	
WBC >6 months	<1.0	>35.0
WBC <6 months	<3.5	>35.0
Hgb >2 months	<7.0	>20.0
Hgb <2 months	<8.5	NA
Hct	<22	
Plt >1 year	<30	>1000
Plt <1 year	<30	NA
Blasts, Malaria, Babesiosis, or other organisms	First occasion or undiagnosed Leukemia must be reviewed by Pathologist	

Alarm values are called upon each occurrence to the physician or licensed caregiver. Add comment 'Confirmed results released and critical value notification call initiated at' (RCF), time of initial call, 'Phoned, confirmed patient identification, receiver read back' (RB), time of receipt, and caregiver name/credentials to the alarm value. Name and credentials of the caregiver are documented along with the critical value when resulted in the computer.

REVIEW OF RESULTS

On completion of any keyboard entry task, the results must be verified before they are accessible by anyone other than laboratory personnel. At this verification step all results are displayed. Previous results can be displayed if selected. All abnormal results, delta check failures, and verify (panic values) are flagged at this time. The results are reviewed for clerical or gross technical error as well as inconsistencies with previous results (e.g. changes in MCV) which would indicate technical error or patient ID error. Any suspected patient ID error should be followed up. Gross technical error must be followed up by appropriate troubleshooting, QC review (see test procedure), and examining companion analyses to check for systematic error or data ID error. Document errors as you would a QC problem. Patient assays are to be repeated once the problem is resolved.

Delta check prior to notification of physician in cases of abnormal results:
The Values for Notification protocol include checking patient history for change from the previous result. Extraordinary changes from previous results are investigated before reporting.
To find a sample: Refer to Coulter Workstation Help. Select a folder icon to retrieve results. A sample list is displayed. Scroll through the list and select the sample you need. Sample data will be displayed. Select the printer icon to print.

Review and Check Abnormal results listed below:

NOTE: *The specimen is initially rerun to check the abnormal result.*

WBC

1. **WBC above 100,000**
   a. Blank HGB and indices in the computer. Append comment INWBC (HGB and indices N.A., WBC elevated).
   b. Review smear for WBC, PLTC, MPV, and DIFF.

2. **WBC above 140,000**
   a. Blank HGB and indices in the computer. Append comment INWBC (HGB and indices N.A., WBC elevated).
   b. Perform microhematocrit and report. Attach comment ESTHCT (estimated micro HCT) to the result.
   c. Report the corrected RBC value: RBC – WBC = corrected RBC value.
   d. Review smear for WBC, PLTC, MPV, and DIFF.

3. WBC above 400,000

a. Blank HGB and indices in the computer. Append comment INWBC (HGB and indices N.A., WBC elevated).
b. Prepare an appropriate WBC dilution using LH Series Diluent and run on the LH 750 in the secondary mode. Multiply the WBC by the dilution factor and report. Append comment RCKDIL (Results checked by dilution).
c. Perform microhematocrit and report. Attach comment ESTHCT (estimated micro HCT) to the result.
d. Report the corrected RBC value: RBC – WBC = corrected RBC value.
e. Review smear for WBC, PLTC, MPV, and DIFF.

4. WBC above 30,000 and below 2500

If values do not Delta check, repeat.

5. WBC 'R' flag or Cellular Interference suspect message

A peripheral smear is reviewed to verify WBC count. If the slide estimation does not correlate with the automated WBC count or if giant platelets or platelet clumps are present (2 or more per field per 10 consecutive 50x fields), the WBC count must be reported by peripheral smear estimate using the following guidelines:

WBC Estimate (Reported answer)	**WBC Criteria** (events per 50x)
WBC count DECREASED	<1 WBC's per field
WBC count NORMAL	1 – 3 WBC's per field
WBC count INCREASED	>3 WBC's per field

NOTE: In the case of Cellular Interference suspect flag, correlate the WBC smear estimate with the LH 750 Uncorrected WBC count. If it does not correlate, then perform WBC estimate by peripheral smear and report using the guidelines above.

WARNING: In the case of 'LY BLASTS' suspect message, the WBC count must be corrected for nRBCs using the formula.

Corrected WBC = WBC (from printout) x 100 / #NRBCs + 100

RBC

1. RBC above 8 million

Prepare an appropriate RBC dilution using LH Series Diluent and run on the LH 750 in the secondary mode. Multiply RBC, HGB, and HCT by the dilution factor, calculate the indices, and report. Append comment RCKDIL (Results checked by dilution).

2. RBC below 1 million

Check for clots; then rerun.

HBG above 18.0 and below 7.0
Repeat if values do not delta check.

HCT below 22
Check for clots. Repeat if values do not delta check.

MC
V
l. MCV above 120
a. In the case of cold agglutinin, warm blood in heating block. Rerun and append appropriate comment. Refer to MCHC section for more details on agglutination. Check smear for agglutination.
b. Check for high glucose, sodium or BUN values. Report microhematocrit; report indices ONLY if microhematocrit agrees with the automated value.
c. Check smear for macrocytosis. Some medications cause macrocytosis (Ex. AIDS).

2. MCV below 50 and above 200
Check HCT by micromethod.

MCHC
1. MCHC below
31.4
Repeat.

2. MCHC above 37.0 (Note: MCHC >37.0 can be found in sickle cell disease) **and above 38.0 in neonates**.
a. Check for clots, then rerun.
b. Check for lipemia, hemolysis, and/or icteria by observing the plasma after the specimen has been allowed to stand undisturbed.

On lipemic specimens: Report only WBC, RBC, HCT, MCV and RDW. Append comment LIPIND (Lipemic - HGB, MCH, MCHC invalid).

On severely hemolyzed specimens: Report only the WBC and HGB. Append comment HEML (Hemolyzed specimen received). If hemolyzed, correlate PLT count with smear estimate to check for RBC fragments which may cause erroneously high PLT count.

On icteric specimens: Report only WBC, RBC, HCT, MCV, & RDW. Append comment ICTER (Specimen too icteric for testing).

a. Check for agglutination: Warm blood in heating block. Rerun on LH 750 and append comment CAGG (Cold agglutinin - may affect RBC, MCV, indices). If results do not change, specimen could still be a cold agglutinin even though there is no response after heating or it could be a warm agglutinin. Perform a micro HCT and check smear for agglutination. If present, report only the WBC and micro HCT

(and LH 750 HGB as appropriate on a case-by-case basis).
Notify the unit of the agglutinin. If further information is requested, refer the inquiry to the Blood Bank.

b. Check smear for spherocytes.

IMPORTANT: The specimen can have more than one abnormality! For example, cold agglutinin AND lipemia. For additional information on abnormal patient blood, refer to LH 750 Training Guide or to the Hematology Bench Notes.

NOTE: Specimens repeated on the LH 750 should check within the following limits:
WBC ± 0.4 RBC ± 0.1 HGB ± 0.3 HCT ± 1.0 MCV ± 2.0 PLAT ± 20,000
If results check, enter comment CKD (Results checked).

PLATELET

l. Platelet above 2,500,000

a. Repeat on LH 750.
b. Prepare an appropriate dilution possible using LH Series Diluent and run on the LH 750 in the manual mode. Multiply PLT count by the dilution factor. Enter result in the computer and append the comment RCKDIL (Results checked by dilution). Do not verify the result until correlated with smear or Delta check.

2. Platelet 700,000 – 1,000,000

a. Check patient history for previous platelet count. If one is shown and the change from the previous count is less than 100,000, verify the result. No further action is necessary. If change is greater than +100,000 or no previous result is noted, proceed as follows.
b. Rerun on the LH 750; check must fall within ± 50,000/uL.
c. Check smear for small RBCs, WBCs, or cell fragments that may produce false elevations. If correlation check fails, perform platelet estimate by peripheral smear evaluation using the following guidelines:

Platelet Estimate (Reported answer)	**Platelet Criteria** (events per 100x)
Platelet count MARKEDLY DECREASED	<5 platelets/100x
Platelet count SLIGHTLY DECREASED	5 – 7 platelets/100x
Platelet count ADEQUATE	7 – 20 platelets/100x
Platelet count SLIGHTLY INCREASED	20 – 40 platelets/100x
Platelet count MARKEDLY INCREASED	>40 platelets/100x

3. Platelet below 130,000

a. Check patient history for previous platelet count. If the change from the previous to the current count shows less than a 20,000/uL drop, verify the result. If no previous result is displayed or if the count has dropped more than 20,000/uL, proceed as follows.

b. Check by repeat analysis those counts below 50,000/uL; check must fall within ± 20,000. Check for clots.
c. Review the slide for giant platelets, platelet clumps, fibrin clots, and platelet satellitism. If a discrepancy is noted, perform a platelet estimate by peripheral smear evaluation using the guidelines stated in the table above. Report platelet satellitism to the pathologist for review and appropriate action.
d. Check smear for correlation. If a discrepancy is noted, perform platelet estimate by peripheral smear evaluation using the guidelines stated in the table above.

4. R flag

a. Prepare and review a peripheral smear to verify the PLT count and MPV, checking for platelet clumps, giant platelets, very small RBCs or WBCs, and cell fragments. If a discrepancy is noted, perform platelet estimate by peripheral smear evaluation using the guidelines stated in the table above.
b. If a significant number of microcytic erythrocytes and/or small cell fragments are seen on the smear, the PLT count may be inaccurate. If correlation check fails, perform platelet estimate by peripheral smear evaluation using the guidelines stated in the table above.

5. Repeated vote out

Perform platelet estimate by peripheral smear evaluation using the guidelines stated in the table above.

For patients with excessive platelet clumping (possibly due to EDTA):

The specimen can be collected in a blue top citrated tube. The final PLT count is multiplied by 1.1. ONLY the corrected PLT count may be reported. DO NOT report other CBC parameters on these samples.

RETIC

1. Retic above 30%

Repeat on LH 750. If the repeat checks, perform a manual Retic count. See manual retic procedure.

2. Retic Interference or Verify Retic Suspect Message

Repeat on LH 750. If the repeat checks, perform a manual Retic count. See manual retic procedure.

CRITERIA FOR AUTOMATED DIFFERENTIAL REVIEW

Suspect messages flag an abnormal cell distribution or population. Definitive messages flag results based on numeric limits entered by the laboratory for certain parameters. If the results exceed the limits, a message is generated by the LH750.

Review Criteria for a slide review:
If the slide does not agree with the automated differential, override the differential and perform a manual differential. Do not verify any results that must be reviewed with slide.

1. First time Alarm Value

2. All suspect flags:
   - Imm NE l and l1
   - LY Blasts (review WBC, PLTC, MPV, and diff for CBC and CBCND orders)
   - MO Blasts and NE Blasts
   - Variant Lymphs
   - Review Slide
   - Dimorphic RBC only when RDW >20
   - nRBCs (review WBC, PLTC, and MPV; perform manual diff for CBC and CBCND orders)
   - Platelet clumps
   - Verify Diff

3. WBC Definitive flags (if diff is requested):
   - Neutrophilia % (NE% ≥80%)
   - Lymphocytosis % (LY% ≥75%)
   - Lymphopenia % (LY% ≤10%)
   - Monocytosis % (MO% ≥20%)
   - Eosinophilia % (EO% ≥15%)
   - Basophilia % (BA% ≥5%)
   - Neutropenia % (NE% ≤43.3%)
   - Eosinophilia % (EO% ≥7.8%)

4. RBC Definitive flags:
   3+ morphology, if diff is requested.

Exception: AIDS patients DO NOT require 3+ Macrocytosis review if a previous diff has been done.

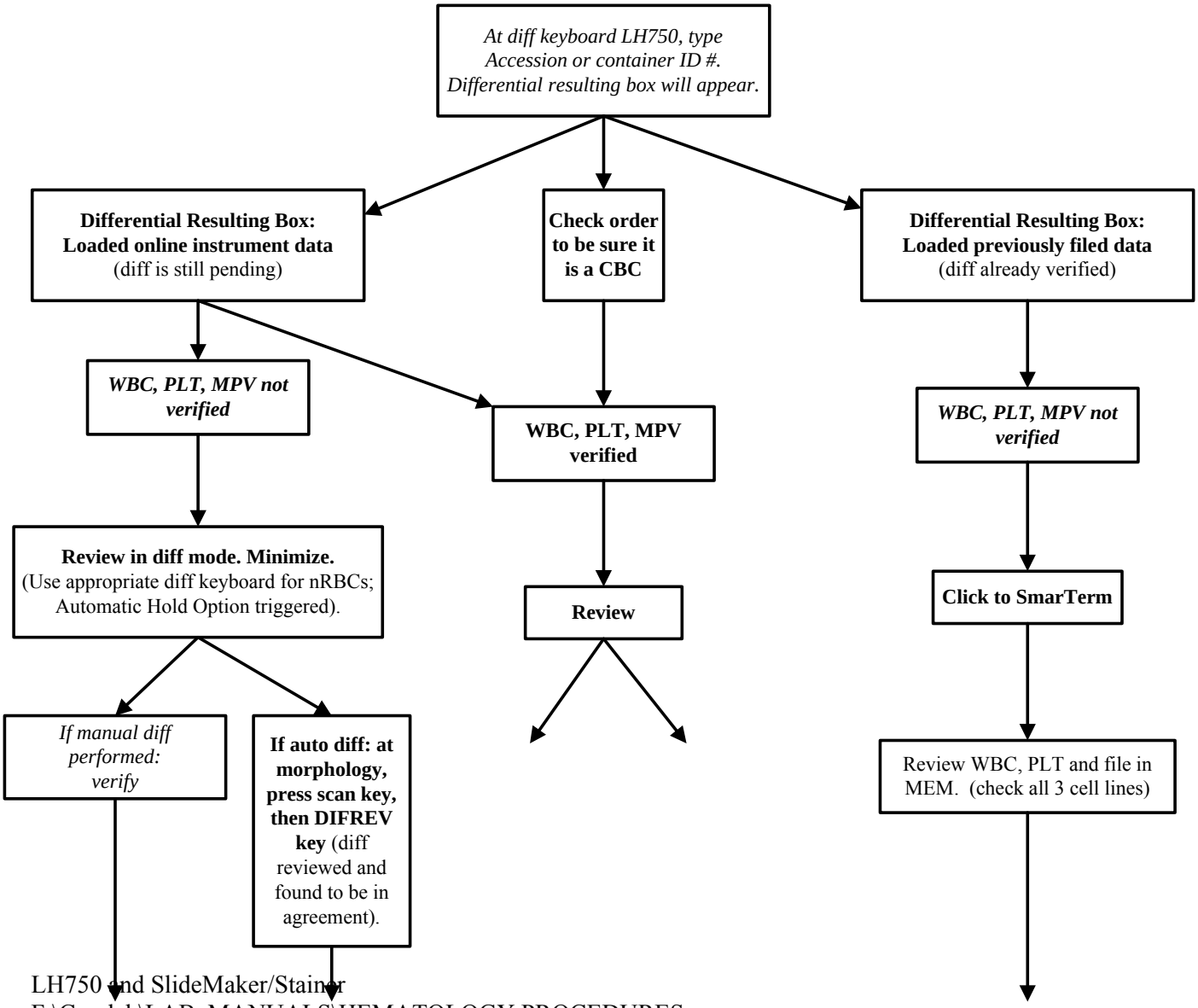
5. Dimorphic morphology if RDW >20 and diff is requested.
6. RDW >20, if diff is requested.
7. MCV <65, if no previous diff has been performed when MCV <65.
8. Review WBC, PLT count, MPV, and DIFF for all Cellular Interference flags.
9. All NURSERY differentials

Samples requiring a differential (if not initially requested):

1. Specimens with any first time alarm value.
2. Nucleated RBC seen when reviewing WBC or PLT flags.
3. Blasts seen on smear when reviewing WBC or PLT flags.
4. MCV < 65, if no previous manual differential was performed for the same reason.
5. Requested by physician.

Specimens requiring a PLT count verified by smear:

1. First time alarm value.
2. PLT count > 700 with no previous PLT count > 700 and <130 with no previous <130.
3. Review PLT count and MPV by smear for all "R" flags next to the PLT count.



If manual diff performed: verify

If auto diff is accepted: at morphology, press scan key, then DIFREV key. (diff reviewed and found to be in agreement).

If any abnormal cells seen: a corrected report on the diff is required (override the auto diff and perform manual diff). Call ward with new results.

Go to MEM and verify WBC, PLT, MPV. Add comment HEMREV (hemogram verified by slide review)

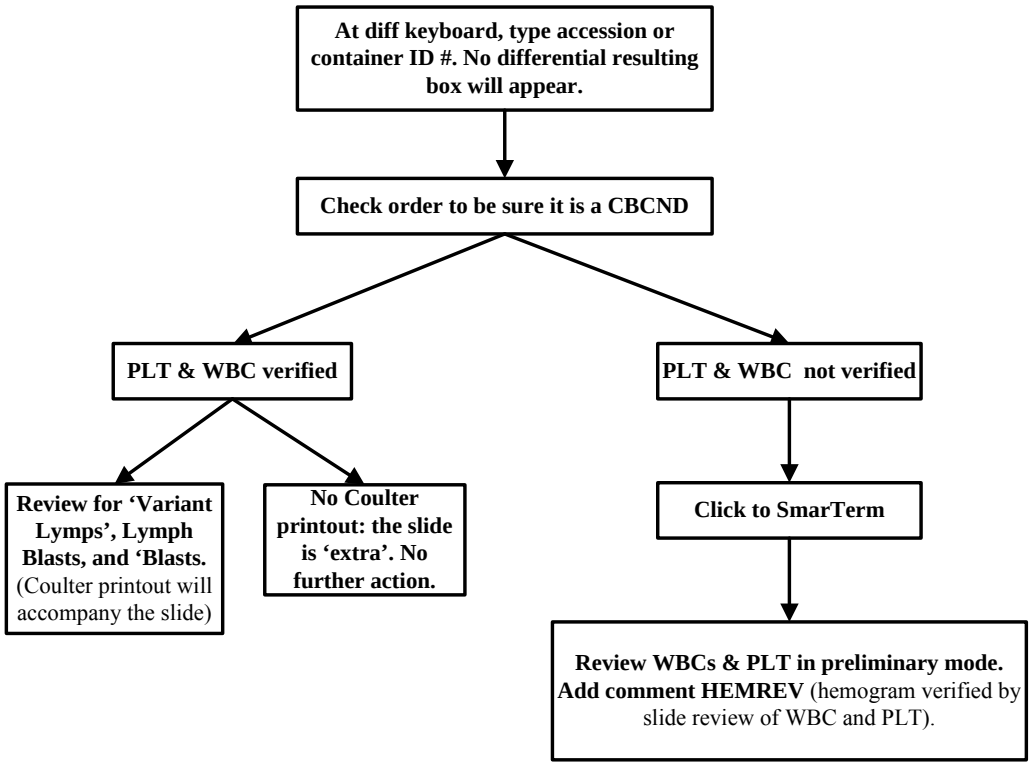
↓

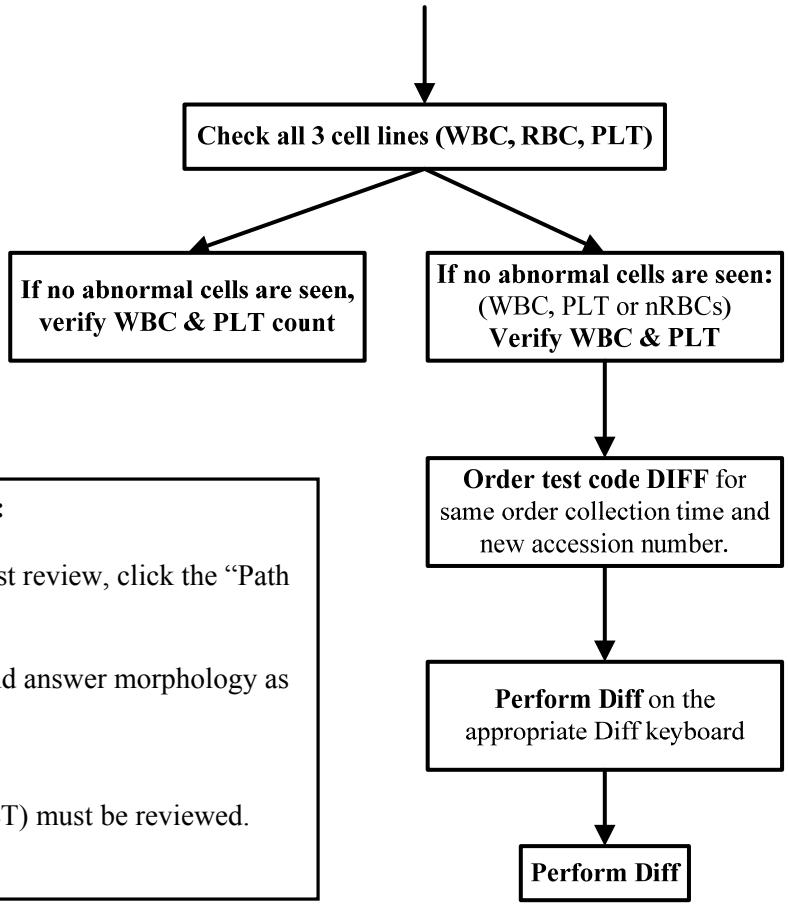
Go back to diff mode. Type acc#. At "Load previous data from hold file" click OK.

↓

WBC & PLTC now appears. Click on morphology, QA Review, & Save. *(WBC will be corrected in DIFF14 keyboard ONLY if nRBCs are present for LY BLAST flag)*

CBCND Slide Review Flow Chart:





REMEMBER:

1. When a slide requires pathologist review, click the "Path Review for diff" comment key.
2. Always review RBC cell line and answer morphology as indicated or "RBCs normal".
3. All 3 cell lines (WBC, RBC, PLT) must be reviewed.

PROCEDURE NOTES

The LH 750 system counts the individual cells and provides cell size distribution. The number of cells counted per sample is approximately 100 times greater than the usual microscope count to reduce the statistical error by a factor of approximately 10 times.

The LH 750 system confirms results prior to reporting. For a detailed explanation of counting, sizing, Hgb measurement, and derivation of calculated parameters, refer to the LH 750 Operator's Guide.

LIMITATIONS OF THE PROCEDURE

Recommended anticoagulants are K_3EDTA or K_2EDTA . Use of other anticoagulants can yield misleading results.

Known Interfering Substances
Misleading results can occur if the specimen is not properly collected, stored or transported. Beckman Coulter recommends that you follow NCCLS or equivalent procedures to ensure

proper specimen collection, storage and transport. Always follow manufacturer's recommendations when using microcollection devices for capillary specimen collection. These can also yield misleading results for the parameters listed:

- All: Misleading results can occur if specimens contain clots. Always use good laboratory practices for inspecting specimens for clots and verifying results.
- All: Misleading results can occur if the specimen is not properly mixed. Always use good laboratory practices to ensure specimens are appropriately mixed. Do not bypass or circumvent the automated mixing process used on the LH 750.
- WBC: Certain unusual RBC abnormalities that resist lysing, malarial parasites, giant platelets, platelet clumps, NRBCs, fragmented white cells, agglutinated white cells, lyse-resistant red cells, cryoglobulin, some extremely elevated proteins, unlysed particles greater than 35 fL in size.
- RBC: Very high WBC count, high concentration of very large platelets, auto-agglutination .
- Hgb: Very high WBC count, severe lipemia, heparin, certain unusual RBC abnormalities that resist lysing.
- MCV: Very high WBC count, high concentration of very large platelets, auto-agglutination.
- RDW: Very high WBC count, high concentration of very large platelets, auto-agglutination.
- Plt: Giant platelets, platelet clumps, white cell fragments, electronic noise, very small red cells, red cell fragments
- Hct: Known interferences related to RBC and MCV.
- MCH: Known interferences related to Hgb and RBC.
- MCHC: Known interferences related to Hgb, RBC and MCV.
- Differential: Hypogranular granulocytes, agranular granulocytes, extremely elevated triglycerides, lyse-resistant red cells, multi-population lymphocytes, cryoglobulin.
- Reticulocytes: Erythrocyte inclusions stained by New Methylene Blue, if sufficiently numerous within a sample, and some hemoglobinopathies (SS, SC) might affect the accuracy of the reticulocyte enumeration.

Precautions:

System integrity might be compromised and operational failure might occur if:

- The equipment is used in a manner other than specified. Operate the instrument as instructed in the LH 750 System Help.
- Software that is not authorized by Beckman Coulter is introduced into your computer. Only operate your system's computer with the software card authorized by Beckman Coulter. Observe the copyright statement on the card.
- If there is a power failure or brownout, turn the instrument off. When the power returns, turn the instrument back on. It automatically reboots. If you are processing a sample when you turn the instrument off, you lose the sample's results. You must rerun the sample when you turn the instrument back on.

Alternative Method:
When the LH750 system is inoperable, pending specimens are stored at room temperature (23.9°C or 75°F) in the first 24 hrs after collection. For specimens that are not analyzed within 24 hours after collection, store them at. 2 to 8°C (35.6 to 46.4°F). These specimens must be analyzed within 48 hours after collection when the Coulter LH750 is operable. Call the specimen area of origin when delays in turn-around-time occur to inform them of the delay.

SLIDEMAKER

The SlideMaker makes blood smears based on the criteria and rules set up for the sample on the Workstation. You, the operator, can also request a slide for a particular sample prior to running the sample by using the Make Slide field on the Add Test window.

Slide Preparation: Load at least one slide cassette containing clean slides into the Slide Ejector module. A mechanism pushes the cassette to the front of this module. Two Prongs, called pawls, push a slide out of the cassette. Grippers hold the slide on the smear truck that carries the slide to the shuttle. The LH 750 aspirates sample from the specimen tube. The LH 750 uses the first sample aspiration for analysis. The LH 750 makes a second aspiration of 250 µL of sample from the vent side of the needle for the SlideMaker. Blood smears can be made only on samples processed in the Automatic mode. The slide on the shuttle moves to the label printer and receives a label with the user-defined sample information sent from the LH 750.

Setting Up the SlideMaker:

1. Ensure that your Beckman Coulter Representative has enabled your SlideMaker.
2. Select to display the System Setup window.
3. Select **SlideMaker/SlideStainer** to display the SlideMaker Setup window.
4. Specify the smear dispense mode.
5. Specify your laboratory ID.
6. Specify if you want to print bar-code tube ID.
7. Specify the print layout for the slide label.
8. Select to close the SlideMaker Setup window and return to the System Setup window. The next time the SlideMaker makes a slide it will use the current setup.

Loading Baskets:

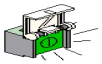
WARNING: *Risk of injury from movable part. Be careful when working with movable parts.*

1. Open the plastic basket cover on the SlideMaker.

2. Hold the needed basket with two fingers, one finger on each end of the basket.
3. Place the empty basket in one of the empty positions on the conveyer belt. Position the basket in between two white uprights on the belt.
4. Load up to six baskets in the allowable positions on the conveyer belt.
5. Close the plastic basket cover on the SlideMaker.

Starting Up the SlideMaker:

Perform this procedure once every 24 hours after the daily SlideMaker Shutdown.

1. Check that the LH 750 has successfully completed startup and that the LH 750 status is READY.
2. Verify that the SlideMaker power is ON. If the power is OFF, press  to turn on the SlideMaker.
3. On the SlideMaker screen, press EXIT until you reach the MAIN MENU screen.
4. Press ROUTINE FUNCTIONS.
5. Press ROUTINE FLUIDICS.
6. Press RUN START UP. The SlideMaker drains and rinses the lines in preparation for making slides.
7. When startup is complete, verify that a PASS message is displayed on the LH 750 Daily Checks Workstation screen in the SlideMaker (SM) field.

   NOTE: If a FAIL message is displayed, rerun the SlideMaker startup. If the SlideMaker startup fails again, call your Beckman Coulter Representative.
8. Press RUN MODE to return to the RUN MODE screen.

Loading Slides into a Cassette:

1. Open the box of slides and move the wrapping out of the way, then carefully place the box on a level surface.

   NOTE: Do not use dirty or stuck-together slides.
2. Hold the cassette in the palm of your hand with the arrow pointing forward.
3. Hold the cover with two fingers and move it back until it locks.
4. Place the cassette on the slide loading stand.
5. Remove any broken slides from the box and pick up the slides.
6. Place the slides in the cassette with the frosted band facing the front opening of the cassette.
7. Slide the cover closed.
8. Tilt the cassette forward so the slides move into place at the front of the cassette and load the cassette into the SlideMaker.

Loading Slide Cassettes:

WARNING: Risk of personal injury if you try to load or unload a slide cassette when it is moving. Load or unload a slide cassette only when the slide cassette is not moving.

1. Position the cassette with the raised arrow pointing up and facing you.
2. Place up to four filled slide cassettes, one at a time, into the input queue in front of the cassette pusher.

Checking Smear Quality:

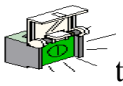
Smear quality may be affected by several factors, including the quality of the glass slide, the condition of the blood specimen, and the instrument's performance. A good smear should show a gradual transition (or feathering) of the blood from the thick to the thin areas. There should be no streaks, troughs, or ridges in the feathered end. Beckman Coulter slides have been manufactured to maximize compatibility with your SlideMaker. They are double-washed to ensure cleanliness and specially packaged to reduce humidity and debris. However, because excessive humidity could cause even these slides to stick together, you should load the cassettes with only those slides needed for one day. Old or inadequately mixed blood specimens could cause morphological artifacts on the smear. Daily performance of the "Shutting Down the SlideMaker" procedure, on the next page, and visual inspection of the dispense probe and rinse cup will enhance slide quality.

Unlocking A Slide Cassette:

1. Ensure:
    a. SlideMaker is ready to make smears.
    b. LH 750 is either processing or ready to process samples.
2. Press **EXIT** until you reach the MAIN MENU screen.
3. Press **ROUTINE FUNCTIONS**.
4. Press **UNLOCK CASETTE**. The SlideMaker status line displays *UNLOCKING CASETTE* while it releases the cassette or as it releases the cassette. When the SlideMaker completes this processing, *READY* appears on the SlideMaker status line.
5. Press **EXIT** until you reach the MAIN MENU screen.
6. Press **RUN MODE** to return to the RUN MODE screen.

Removing Slide Cassettes:

WARNING: Risk of personal injury if you try to load or unload a slide cassette when it is moving. Load or unload a slide cassette only when the slide cassette is not moving.

1. If necessary, press  to turn off the SlideMaker and release the current slide cassette.
2. Move the cassette backward and up from the output queue.
3. If necessary, check the cassette to make sure the slides in it are not stuck together.
4. If a slide is jammed inside the shuttle area, remove and dispose of the slide.

Unloading Baskets:

1. Open the plastic basket cover.
2. Hold the needed basket with your thumb and two fingers on each end of the basket.
3. Lift the basket up and out.
4. Close the plastic basket cover.
5. You can now stain these slides.

Shutting Down the SlideMaker:

Perform this procedure once every 24 hours, before shutting down the Analytical station. A Message will be posted to the SlideMaker event log. Use the extended shutdown procedure if you are not going to operate the SlideMaker for 48 hours or more. This will prevent the reagents from drying out and forming precipitate.

1. Check that the LH 7650 status is *READY*.
2. On the SlideMaker screen, press **EXIT** until you reach the MAIN MENU screen.
3. Press **ROUTINE FUNCTIONS**.
4. Press **ROUTINE FLUIDICS**.
5. Press **RUN SHUT DOWN**. The SlideMaker fills the lines with cleaning agent.
6. When the Numeric Keypad displays *READY*, press **SHUT DOWN**.
7. Allow the cleaning agent to remain in the instrument for at least 30 minutes.
8. Visually inspect the rinse block and dispense probe for debris or dried blood. If blood is present, perform the Cleaning the Dispense Probe and Rinse Cup procedure, found in the LH 750's System Help.
9. Perform the SlideMaker Startup procedure prior to processing samples.

SLIDESTAINER

Daily Operation:

Use the daily operation procedure once every 24 hours. This will prevent the reagents from drying out and forming precipitate, which may in turn clog reagent lines. Use the procedures found below:

1. Drain all the baths using the "Draining Baths" procedure.
2. Add new stain/buffer solution according to the "Manual Stain/Buffer Bath Introduction" procedure.
3. Fill the stain baths using the "Filling Baths" procedure.

Draining Baths:

The primary reason for draining baths is to replenish the baths with fresh reagents. Baths may also be drained to prevent spillage during maintenance.

1. Select from the Command Center on the Workstation to display the System setup screen.
2. Select **SlideMaker/SlideStainer** from the System setup screen to display the System Setup – (Slide Preparation) screen and access the SlideMaker/SlideStainer Options.
3. Select **SlideStainer** to display SlideStainer options.

   IMPORTANT: Make sure that all slide baskets have been removed from the baths prior to performing this procedure to prevent stain precipitate from adhering to the slides.

4. Select **Standby Mode** to place the instrument in Standby Mode. If you attempt to perform a drain during Auto mode or when the instrument is busy, the following message will display:

   FILL/DRAIN OPERATIONS CANNOT BE PERFORMED BECAUSE SLIDESTAINER IS NOT IN STAND BY MODE.

5. Under the SlideStainer Maintenance section, select the Bath or All Baths to be drained by clicking the appropriate box.
6. A dialog box displays with one of the following messages:
   - All baths: Drain All Baths Request sent to Stainer!
     NOTE: Baths will be drained one at a time until all baths are empty.
   - Bath *n* (1–5): Drain Bath *n* Request sent to Stainer!
     NOTE: Baths will be drained one at a time until all baths are empty
7. The SlideStainer will then drain the selected baths until the liquid level sensor determines that the bath has been fully drained.

Filling Baths:

The primary purpose of this procedure is to replenish baths with fresh reagents. This procedure may also be used as part of a cleaning procedure using methanol.

1. Select from the Command Center on the Workstation to display the System Setup screen.
2. Select **SlideMaker/SlideStainer** from the System setup screen to display the System Setup – (Slide Preparation) screen.
3. Select **SlideStainer** to display SlideStainer options.

IMPORTANT: Make sure that all slide baskets have been removed from the baths prior to performing this procedure to prevent stain precipitate from adhering to the slides

4. Select **Standby Mode** to place the instrument in Standby Mode. If you attempt to perform a fill during Auto mode or when the instrument is busy, the following message will display:

   FILL/DRAIN OPERATIONS CAN NOT BE PERFORMED BECAUSE SLIDESTAINER IS NOT IN STAND BY MODE.

5. Under the SlideStainer Maintenance section, select the Bath or All Baths to be filled by clicking the appropriate box.
6. A dialog box displays with one of the following messages:
   - All baths: Fill All Baths Request sent to Stainer!
     NOTE: Baths will be filled one at a time.
   - Bath *n* (1–5): Fill Bath *n* Request sent to SlideStainer!
     NOTE: Baths will be filled one at a time until all baths are filled.

7. The SlideStainer will then fill the selected bath(s) until the liquid level sensor determines that the bath has been fully filled. The bath volume is 300mL.

   NOTE: Baths 1-5 will automatically fill simultaneously is they are defined in the protocol (time set to a number other than zero) and Auto mode is selected.

Drain Reagent Lines:

This procedure drains the reagent lines for the purpose of shutting down the SlideStainer or preventing spillage during maintenance procedures.

1. Select from the Command Center on the Workstation to Display the System Setup screen.
2. Select **SlideMaker/SlideStainer** from the System Setup screen to display the System Setup – (Slide Preparation) screen.
3. Select **Slide Stainer** to display the Slide Stainer options.
4. Select **Standby Mode** to place the instrument in Standby mode.

5. Select to display the Drain Reagent Lines dialog box.

   NOTE: You can select baths 1-4 individually or all at once to drain the baths and reagent lines. The baths will drain first and then the reagent line.

6. Select to return to the System Setup – (Slide Preparation) screen.

Loading Empty Baskets:

Ensure the SlideStainer basket tray drawer light appears green.

1. Open the basket tray drawer.
2. Fill the input tray with empty slide baskets.
3. Close the basket tray drawer.

Manually Loading a Basket Tray:

Ensure the SlideStainer basket tray drawer light appears green.

1. Open the basket tray drawer.
2. Fill the input tray with the slide basket to be processed.
3. Close the basket tray drawer.

Removing Slide Baskets from a Tray:

1. Ensure the SlideStainer basket tray drawer light appears green.
2. Open the basket tray drawer.
3. Hold the slide basket with the thumb and forefinger on each end of the basket.
4. Life the basket up and out.
5. Close the basket tray drawer.

REFERENCES

Use the **LH 750 System Help** for:

- Getting started and running the instrument day-to-day
- Verifying screen icon definitions
- Reviewing unusual results (how to read a result report and what flags mean)
- Performing special procedures such as cleaning, replacing, or adjusting a component of the instrument
- Troubleshooting problems with your instrument
- What the instrument does and methods it uses
- Instrument specifications and requirements
- How to interface your analyzer to your laboratory's host computer
- How to safely use the instrument
- Initially setting up the instrument and printer
- Powering up the instrument
- Customizing the software

5C-ES, BECKMAN COULTER, COULTER, COULTER CLENZ, COULTER COUNTER, LYSE S, S-CAL, are trademarks of Beckman Coulter Incorporated.

Research Operating Procedure for Determination of Specific IgEs using the IMMULITE® 2000 3gAllergy™ Specific IgE Universal Kit

for

Children's Health after the Storms (CHATS)

Prepared by: Phyllis Carlson MT (ASCP) Date: 2/07/2012

Approved by: Dr. F. W. Brazda, MD Date: 2/07/2012

Clinical Pharmacology-Toxicology Laboratory
Department of Pathology
LSU Interim Hospital

List of Revisions

Revision Number	Changes	Date
0	Original from LSU	

Table of Contents

Summary and Explanation 4
Principle of the Procedure 4
Specimen Collection 4
Warnings and Precautions 5
Materials Supplied 5
Kit Components Supplied Separately 6
Assay Procedure 7
Interpretation of Results 8
Limitations 8
Performance Data 9
References 10
Sourced From 10

IMMULITE® 2000 3gAllergy™ Specific IgE Universal Kit
Intended Use: For in vitro diagnostic use with the IMMULITE 2000 Analyzer — for the quantitative measurement of allergen-specific IgE in human serum, as an aid in the clinical diagnosis of IgE-mediated allergic disorders.
Catalog Number: **L2KUN6** (600 tests)
Test Code: **SPE** Color: **Light Gray**

Summary and Explanation

Many allergies are mediated by immunoglobulins of the IgE class. In sensitized individuals suffering from this immediate (atopic or anaphylactic) type of allergy, IgE molecules act as points of contact between the allergen and specialized cells that release histamine and other agents upon exposure to the allergen; this initiates the events which we recognize as allergic reactions.[5,9] When evaluated in the light of other clinical and laboratory findings, *in vitro* allergen-specific IgE tests can help the physician identify the allergen (or allergens) to which an individual is sensitive.

Principle of the Procedure

IMMULITE 2000 3gAllergy™ Specific IgE is a solid-phase, two-step, chemiluminescent immunoassay that exploits liquid phase kinetics in a bead format.[10,11] (U.S. Patent No. 4,778,751) It represents a significant advance over conventional methods relying on allergens attached to a solid-phase support, such as a paper disk.

The allergens are covalently bound to a soluble polymer/co-polymer matrix, which in turn is labeled with a ligand. The use of an amino acid co-polymer amplifies the amount of allergen that the matrix can support.

Incubation Cycles: 2 × 30 minutes.

Specimen Collection

The use of an ultracentrifuge is recommended to clear lipemic samples.

Hemolyzed samples may indicate mistreatment of a specimen before receipt by the laboratory; hence the results should be interpreted with caution.

Centrifuging serum samples before a complete clot forms may result in the presence of fibrin. To prevent erroneous results due to the presence of fibrin, ensure that complete clot formation has taken place prior to centrifugation of samples. Some samples, particularly those from patients receiving anticoagulant therapy, may require increased clotting time.

Blood collection tubes from different manufacturers may yield differing values, depending on materials and additives, including gel or physical barriers, clot activators and/or anticoagulants. IMMULITE 2000 3gAllergy™ Specific IgE has not been tested with all possible variations of tube types. Consult the section on Alternate Sample Types for details on tubes that have been tested.

Volume Required: 50 µL serum.

Storage: 7 days at 2–8°C, or
6 months at –20°C.[13]

Warnings and Precautions

For *in vitro* diagnostic use.

Reagents: Store at 2–8°C. Dispose of in accordance with applicable laws.

Follow universal precautions, and handle all components as if capable of transmitting infectious agents. Source materials derived from human blood were tested and found nonreactive for syphilis; for antibodies to HIV 1 and 2; for hepatitis B surface antigen; and for antibodies to hepatitis C.

Sodium azide, at concentrations less than 0.1 g/dL, has been added as a preservative. On disposal, flush with large volumes of water to prevent the buildup of potentially explosive metal azides in lead and copper plumbing.

Chemiluminescent Substrate: Avoid contamination and exposure to direct sunlight. (See insert.)

Water: Use distilled or deionized water.

Materials Supplied

Components are a matched set. Labels on the inside box are needed for the assay.

3gAllergy™ Specific IgE Bead Pack (L2UN12)

With barcode. 200 beads, coated with anti-ligand. Stable at 2–8°C until expiration date.
L2KUN6: 3 packs.

3gAllergy™ Specific IgE Reagent Wedge (L2UNA6)

With barcode. 30 mL alkaline phosphatase (bovine calf intestine) conjugated to monoclonal murine anti-human IgE antibody in a human/nonhuman serum buffer matrix, dispensed equally into chambers B and C. Stable at 2–8°C until expiration date.
L2KUN6: 1 wedge.

Before use, tear off the top of the label at the perforations, without damaging the barcode. Remove the foil seal from the top of wedge; snap the sliding cover down into the ramps on the reagent lid.

3gAllergy™ Specific IgE Adjustors (L2UNJ3, L2UNJ4)

Two vials (Low and High), 2.0 mL each, of human IgE in a nonhuman serum matrix, with preservative. Stable at 2–8°C for 30 days after opening, or for 6 months (aliquotted) at –20°C.
L2KUN6: 2 sets.

Before making an adjustment, place the appropriate Aliquot Labels (supplied with the kit) on test tubes so that the barcodes can be read by the on-board reader.

3gAllergy™ Specific IgE Adjustor Antibody (L2UNS1)

Two tubes, 2.75 mL each of liquid, ready-to-use ligand-labeled polyclonal goat anti-human IgE antibody, with preservative. Stable at 2–8°C until expiration date. This reagent is placed in the Allergen Holder Wedge when running the Specific IgE Adjustors.
L2KUN6: 2 sets.

3gAllergy™ Specific IgE (SPE) Universal Kit Controls (L2UNC1, L2UNC2)
Two vials, 2 mL each of human IgE in a nonhuman serum matrix, with preservative. Stable at 2–8°C for 30 days after opening, or for 6 months (aliquotted) at –20°C.
L2KUN6: 2 sets.

Refer to the control insert for concentration levels.

Before use, place the appropriate Aliquot Labels (supplied with the kit) on test tubes so that the barcodes can be read by the on-board reader.

3gAllergy™ Specific IgE Control Antibody (L2UNS2)
Two tubes, 2.75 mL each of liquid, ready-to-use ligand-labeled polyclonal goat anti-human IgE antibody, with preservative. Stable at 2–8°C until expiration date. This reagent is placed in the Allergen Holder Wedge when running the IMMULITE 2000 IgE Controls.
L2KUN6: 2 sets.

Kit Components Supplied Separately

3gAllergy™ Specific IgE Sample Diluent (L2UNZ)
For on-board dilution of samples. One vial, concentrated (ready-to-use), human serum albumin matrix, with preservative. Storage: 30 days (after opening) at 2–8°C or 6 months (aliquotted) at –20°C. Dispose of in accordance with applicable laws.
L2UNZ: 25 mL

Barcode labels are provided for use with the diluent. Before use, place an appropriate label on a 16 × 100 mm test tube, so that the barcodes can be read by the on-board reader.
L2UNZ: 3 labels

L2SUBM: Chemiluminescent Substrate
L2PWSM: Probe Wash
L2KPM: Probe Cleaning Kit
LRXT: Reaction Tubes (disposable)
L2AW1-3: Allergen Holder Wedges (barcoded)
L2AW1: serially coded 1-33
L2AW2: serially coded 34-66
L2AW3: serially coded 67-99
L2ATC: Allergen Tube Caps
L2ATS: Allergen Tube Septa

Also Available
MC6L, DC1L, DC2L, L2SNC: Human serum-based allergen-specific IgE controls

Also Required
Distilled or deionized water; test tubes; controls.

3gAllergy ™Specific Allergens and
Mixed Allergen Panels
Individual allergens and mixed allergen panels in liquid phase, with preservative, are packaged and sold in 40 and 20 test modules, and are intended for use with both the IMMULITE 2000 and IMMULITE 2500 platforms. (For catalog numbers, see the 3gAllergy™ Product Catalog). Store refrigerated: stable at 2–8°C until the expiration date marked on the label. Do *not* use if signs of microbial contamination such as a cloudy appearance have been observed.

Assay Procedure

Note that for optimal performance, it is important to perform all routine maintenance procedures as defined in the IMMULITE 2000 Operator's Manual.

See the IMMULITE 2000 Operator's Manual for: preparation, setup, dilutions, adjustment, assay and quality control procedures.

Allergen Loading

1 Select an open position on the Reagent Carousel through the software.

2 Replace caps on Allergen Tubes with septa. Do not invert the Allergen Tube once the septum is installed.

3 Place Allergen Tubes containing IMMULITE 2000 specific allergens, specific allergen panels, Specific IgE Adjustor Antibody, and/or Specific IgE Control Antibody in the Allergen Holder Wedge, with the barcodes facing the open side of the wedge.

4 Close the wedge and scan the allergen barcodes with the hand-held imaging scanner.

5 Once scanning is complete, load the Allergen Holder Wedge into the reagent carousel.

6 Repeat this procedure to load subsequent Allergen Holder Wedges.

The Allergen Holder Wedge **must** be scanned prior to installation into the instrument to ensure correct instrument operation. Removing or replacing any vials within the allergen wedge will require rescanning of the wedge with the barcode scanner to update the allergen information.

Recommended Adjustment Interval: 2 weeks. The following controls are run after an adjustment is performed: Universal Kit Controls (L2UNC1, L2UNC2) and specific allergen controls ((DC1L, DC2L, MC6L and L2SNC).

MCL Daily Quality Control: Controls supplied with the kit (3gAllergy™ Specific IgE (SPE) Universal Kit Controls (L2UNC1, L2UNC2),one of the specific allergen controls ((DC1L, DC2L, MC6L) and L2SNC (negative IgE control).

Interpretation of Results

Individual Allergen Results:

The class number is an indication of the amount of endogenous IgE specific for the selected allergen. Quantitative values (kU/L) and interpretation of class results for two scoring systems (standard and extended) are provided in the tables below.

* Class 0 in the standard system signifies: not detectable by second-generation assays.

† ND: not detectable by IMMULITE 2000 3gAllergy.

The **extended** classification system utilizes the following class cutoffs.

Class	kU/L	Reactivity for Individual/Component Allergen(s)
0	< 0.1	Absent or ND†
0/1	0.11 – 0.24	Very Low
I	0.25 – 0.39	Low
II	0.40 – 1.29	Moderate
III	1.30 – 3.89	High
IV	3.90–14.99	Very High
V	15.00– 24.99	
VI	≥ 25	

† ND: not detectable by IMMULITE 2000 3gAllergy.

Mixed Allergen Panel Results

A positive result (Class I or greater) with a Mixed Allergen Panel indicates that antibodies to one or more of the component allergens in the panel are present in elevated amounts in the patient serum sample. To identify the allergen-specific IgE, the sample should be retested with individual allergens corresponding to components of the panel.

A negative result (Class 0) indicates the absence or very low levels of IgE specific for the panel's components.

Panel results cannot be compared with results based on testing for individual allergens, nor can they be considered the cumulative total of individual allergen results.

Limitations

A definitive clinical diagnosis should not be made solely on the basis of an *in vitro* allergen-specific IgE result. Diagnosis should be made by the physician only after all clinical and laboratory findings have been evaluated.[2,4]

In vitro allergen-specific IgE results should not be used as a definitive guide to selecting an initial dose for immunotherapy. A skin test with the proposed initial dilution of the allergenic extract should be performed first to demonstrate the patient's ability to tolerate the dose.

In food allergy, circulating IgE antibodies may remain undetectable if directed towards allergens which are revealed or altered during processing or digestion and which therefore do not exist in the original food for which the patient is tested.[1,3]

Identical results for different allergens may not be associated with clinically equivalent manifestations, due to differences in IgE-binding capacity.[6]

The user should be aware of the possibility of clinical crossreactivity within an allergen family.[7,8]

The following special considerations apply to latex allergy testing:

- The possibility of clinical crossreactivity exists between latex and certain foods including avocado, banana, chestnut, and kiwi.[12]
- Since the latex assay measures allergen-specific IgE, type IV delayed reaction or irritation from latex will not be detected.

Class 0 results for insect venoms indicate absent or very low levels of circulating venom-specific IgE antibodies. Such results do not preclude existence of current or future clinical hypersensitivity to insect sting.

Heterophilic antibodies in human serum can react with the immunoglobulins included in the assay components causing interference with *in vitro* immunoassays. [See Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988:34:27-33.] Samples from patients routinely exposed to animals or animal serum products can demonstrate this type of interference potentially causing an anomalous result. These reagents have been formulated to minimize the risk of interference; however, potential interactions between rare sera and test components can occur. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

Performance Data

See Tables and Graphs for data representative of the assay's performance. Results are expressed in kU/L. (Unless otherwise noted, all were generated on serum samples collected in tubes without gel barriers or clot-promoting additives.)

IMMULITE 2000 3gAllergy Specific IgE has been the subject of a number of published studies.[14,15]

Working Range: 0.1 – 100 kU/L
(WHO 2nd IRP 75/502).

Analytical Sensitivity: 0.1 kU/L.

Functional Sensitivity: 0.2 kU/L

Precision: Samples were assayed in duplicate over the course of 20 days, two runs per day, for a total of 40 runs and 80 replicates. (See “Precision” table.)

Linearity: Samples were assayed under various dilutions. (See "Linearity" table for representative data.)

Specificity: The antibodies are highly specific for human IgE and exhibit no crossreactivity to other human Immunoglobulin classes.

Bilirubin: Presence of conjugated and unconjugated bilirubin in concentrations up to 200 mg/L has no effect on results, within the precision of the assay.

Hemolysis: Presence of hemoglobin in concentrations up to 500 mg/dL has no effect on results, within the precision of the assay.

Lipemia: Presence of triglycerides in concentrations up to 3,000 mg/dL has no effect on results, within the precision of the assay.

Alternate Sample Type: To assess the effect of alternate sample types, blood was collected from 18 volunteers into plain, heparinized, EDTA and Becton Dickinson SST® vacutainer tubes. All samples were assayed by the IMMULITE 2000 3gAllergy™ Specific IgE procedure, with the following results.

(Heparin) = 1.10 (Serum) + 0.16 kU/L
r = 0.986

(EDTA) = 0.81 (Serum) + 0.01 kU/L
r = 0.996

(SST) = 0.91 (Plain Tubes) + 0.15 kU/L
r = 0.992

Means:
7.1 kU/L (Serum)
8.0 kU/L (Heparin)
5.8 kU/L (EDTA)
6.6 kU/L (SST)

References

1) Aas K. The diagnosis of hypersensitivity to ingested foods. Clin Allergy 1978;8:39–50. 2) Barbee RA, et al. Longitudinal changes in allergen skin test reactivity in a community population sample. J Allergy Clin Immunol 1987;79:16–24. 3) Bleumink E. Food allergy: the chemical nature of the substance eliciting symptoms. World Rev Nutr Diet 1970;12:505–70. 4) Bloch K, Salvaggio J. Use and interpretation of diagnostic immunologic laboratory tests. JAMA 1982; 246:2734–58. 5) Halpern GM. Markers of human allergic disease. J Clin Immunoassay 1983;6:131–8. 6) Lichtenstein LM, et al. IgE antibody measurements in ragweed hay fever; relationship to clinical severity and the results of immunotherapy. J Clin Invest 1973; 52:472–82. 7) Lowenstein H. Cross reactions among pollen antigens. Allergy 1980;35:198–200. 8) Weber RW, Nelson HS. Pollen allergens and their interrelationships. Clin Rev Allergy 1985;3:291–318. 9) Wide L, Bennich H, Johansson SGO. Diagnosis of allergy by an *in vitro* test for allergen antibodies. Lancet 1967;2:1105–7. 10) El Shami AS, Alaba O. Liquid-phase *in vitro* allergen-specific IgE assay with in situ immobilization. Adv Biosci 1989;74:191–201. 11) Alaba O, El Shami AS. Evaluation of non-specific IgE binding: comparison of two *in vitro* allergen assays. Adv Biosci 1989;74:203–14. 12) Pecquet C. IgE-mediated allergy to latex in 80 patients. Presented at the XVth European Congress of Allergology and Clinical Immunology, Paris, 12 May 1992. 13) Tietz NW, editor. Clinical guide to laboratory tests. 3rd ed. Philadelphia: WB Saunders, 1995:358. 14) Li TM, Chuang T, Tse S, Hovanec-Burns D, El Shami AS. Development and validation of a third generation allergen-specific IgE assay on the continuous random access IMMULITE 2000 analyzer. Ann Clin Lab Sci 2004;34(1):67-74. Available via www.AnnClinLabSci.org. 15) Guilloux L, Hamberger C. Évaluation du dosage des IgE spécifiques sur l'IMMULITE® 2000 DPC. Immuno-analyse & Biologie Spécialisée. 2004;19(1):71-80. Available via www.sciencedirect.com.

Sourced From

IMMULITE 2000 3gAllergy™ Specific IgE Universal Kit (PIL2KUN-16, 2006-04-20)

Research Operating Procedure Total IGE for Determination of Total IgE in CHATS using the Beckman Coulter Access Immunoassay System

for

Children's Health after the Storms (CHATS)

Prepared by: _____ Date: _____

Approved by:_____ Date: _____

Clinical Pharmacology-Toxicology Laboratory

MCLNO Pathology Services

LSU Interim Hospital

List of Revisions

Revision Number	Changes	Date
0	Original from LSU	

This Research Operating Protocol describes the determination of total IgE from blood samples collected for CHATS. The method will be implemented at LSU as described in Attachment A.

Attachment A.

Method from Beckman Coulter Manual

Access®
Immunoassay Systems

TOTAL IgE

REF 35000

Intended Use

The Access Total IgE assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of total E (IgE) levels in human serum and plasma (heparin, EDTA) using the Access Immunoassay Systems.

Summary and Explanation

Immunoglobulin E (IgE) was first isolated and defined as a new immunoglobulin class by Ishizaka, et al. and Johansson and Bennich in the 1960's.[1,2] IgE has a molecular weight of approximately 188,000 daltons, making it slightly larger than the monomers of the other immunoglobulins. The epsilon (ε) heavy chain contains five domains (VH, Cε1, Cε2, Cε3, and Cε4) with the IgE receptor binding region believed to be located near the Cε2–Cε3 region. High affinity IgE specific receptors are found on the surface membranes of mast cells and basophils. Once IgE has been bound by these receptors, it plays a key role in the generation of immediate hypersensitivity reactions.

The World Health Organization (WHO) has recognized IgE as a unique immunoglobulin and has established calibration standards for it.[3,4] One international unit (IU) of IgE has been defined as equal to 2.4 ng.

The levels of circulating IgE in serum are extremely low compared to the other immunoglobulins. Levels at birth are almost non-detectable, but increase with age to approximately 20 IU/mL (48 µg/L) in normal adults.[5] IgE has been linked to atopic disease and there is a strong correlation between increased total serum IgE levels and allergy.[6] The determination of total IgE levels has been found to be useful in the assessment of atopic diseases such as allergic rhinitis, extrinsic asthma, urticaria, and atopic eczema.[7,8] Several investigators have also shown that increased IgE levels in cord blood and infants may have a predictive value for the early onset of allergic disease.[9,10,11,12] Patients with pulmonary aspergillosis, parasitic infestations, and some immunodeficiencies have also been found to have increased amounts of IgE.[13,14,15]

Total IgE levels may vary due to a variety of different factors, including genetic disposition and allergen exposure. Low levels of circulating IgE do not necessarily indicate the absence of allergic disease as certain individuals may have low total IgE levels but have a high concentration of allergen-specific IgE.

The Access Total IgE assay is based on the two-site immunoradiometric assay (IRMA) described by Addison, et al., but utilizes an enzyme-labeled antibody in place of the radio-labeled tracer.[16]

Principles of the Procedure

The Access Total IgE assay is a sequential two-step immunoenzymatic ("sandwich") assay. A sample is added to a reaction vessel along with paramagnetic particles coated with goat anti-mouse: mouse anti-IgE complexes. The IgE in the sample binds to the mouse anti-IgE on the particles. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Equine anti-IgE conjugated to alkaline phosphatase is then added and binds to the previously bound IgE on the particles. A second separation and wash step removes unbound conjugate. Then, the chemiluminescent substrate Lumi-Phos* 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of IgE in

© 2006 Beckman Coulter, Inc.

the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

Product Information

Access Total IgE Reagent Pack

Cat. No. 35000: 100 determinations, 2 packs, 50 tests/pack

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.
- Stable at 2 to 10°C for 28 days after initial use.
- Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range.
- If the reagent pack is damaged (i.e., broken elastomer), discard the pack.
- All antisera are polyclonal unless otherwise indicated.

R1a:	Paramagnetic particles coated with goat anti-mouse IgG: mouse monoclonal anti-IgE complexes suspended in TRIS buffered saline, with surfactant, BSA matrix, protein (goat), < 0.1% sodium azide, and 0.1% ProClin** 300.
R1b:	Equine anti-IgE-alkaline phosphatase (bovine) conjugate in TRIS buffered saline, with surfactant, BSA matrix, protein (equine), < 0.1% sodium azide, and 0.1% ProClin 300.

Warnings and Precautions

- For *in vitro* diagnostic use.
- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.[17]
- ProClin 300 is a potential skin sensitizer. Avoid spilling or splashing this reagent on skin or clothing. In case of contact with the reagent, flush thoroughly with soap and water.
- The Material Safety Data Sheet (MSDS) is available upon request.

Specimen Collection and Preparation

1. Serum and plasma (heparin, EDTA) are the recommended samples.
2. Observe the following recommendations for handling, processing, and storing blood samples:[18]
   - Collect all blood samples observing routine precautions for venipuncture.
   - Allow serum samples to clot completely before centrifugation.
   - Keep tubes stoppered at all times.
   - Within two hours after centrifugation, transfer at least 500 µL of cell-free sample to a storage tube. Tightly stopper the tube immediately.
   - Store samples tightly stoppered at room temperature (15 to 30°C) for no longer than eight hours.
   - If the assay will not be completed within eight hours, refrigerate the samples at 2 to 8°C.
   - If the assay will not be completed within 48 hours, or for shipment of samples, freeze at -20°C or colder.
   - Thaw samples only once.

 © 2006 Beckman Coulter, Inc.

3. Use the following guidelines when preparing specimens:
   - Ensure residual fibrin and cellular matter has been removed prior to analysis.
   - Follow blood collection tube manufacturer's recommendations for centrifugation.
4. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot-to-lot.
5. Do not use heat-inactivated samples for this assay.

Materials Provided

R1 Access Total IgE Reagent Packs

Materials Required But Not Provided

1. Access Total IgE Calibrators
   Provided at zero and approximately 3, 15, 60, 240, 1000, and 3000 IU/mL
   (7, 36, 144, 576, 2400, and 7200 µg/L).
   Cat. No. 35005
2. Quality Control (QC) materials: commercial control material.
3. Access Substrate
   Cat. No. 81906
4. **Access, Access 2, SYNCHRON LX®i:**
   Access Wash Buffer II, Cat. No. A16792
   UniCel® DxI:
   UniCel DxI Wash Buffer II, Cat. No. A16793

Procedural Comments

1. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.
2. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.
3. Use ten (10) µL of sample for each determination in addition to the sample container and system dead volumes. Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.
4. The system default unit of measure for sample results is IU/mL. To change sample reporting units to the International System of Units (SI units), µg/L, refer to the appropriate system manuals and/or Help system. To manually convert concentrations to the International System, multiply IU/mL by multiplication factor 2.4.

Procedure

Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.

Calibration Details

An active calibration curve is required for all tests. For the Access Total IgE assay, calibration is required every 28 days. Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

Quality Control

Quality control materials simulate the characteristics of patient samples and are essential for monitoring the system performance of immunochemical assays. Because samples can be processed at any time in a "random access" format rather than a "batch" format, quality control materials should be included in each 24-hour time period.[19] Include commercially available quality control materials that cover at least two levels of analyte. Follow manufacturer's instructions for reconstitution and storage. Each laboratory should establish mean values and acceptable ranges to assure proper performance. Quality control results that do not fall within

© 2006 Beckman Coulter, Inc.

acceptable ranges may indicate invalid test results. Examine all test results generated since obtaining the last acceptable quality control test point for this analyte. Refer to the appropriate system manuals and/or Help system for information about reviewing quality control results.

Results

Patient test results are determined automatically by the system software using a weighted four parameter logistic curve (4PLC) math model. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration data. Patient test results can be reviewed using the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions on reviewing sample results.

Limitations of the Procedure

1. Samples can be accurately measured within the analytic range of the lower limit of detection and the highest calibrator value (approximately 0.25–3000 IU/mL [0.6–7200 µg/L]).
   - If a sample contains less than the lower limit of detection for the assay, report the results as less than that value (i.e., < 0.25 IU/mL [< 0.6 µg/L]).
   - If a sample contains more than the stated value of the highest Access Total IgE Calibrator (S6), report the result as greater than that value (i.e., > 3000 IU/mL [> 7200 µg/L]). Alternatively, dilute one volume of sample with nine volumes of Access Total IgE Calibrator S0 (zero), which is also available as Access Total IgE Calibrator S0 Cat. No. 35006. Refer to the appropriate system manuals and/or Help system for instructions on entering a sample dilution in a test request. The system reports the results adjusted for the dilution.
2. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples.[20,21]

   Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
3. The Access Total IgE results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests and other appropriate information.

Expected Values

1. Each laboratory should establish its own reference ranges to assure proper representation of specific populations.
2. Total IgE concentrations were measured in serum samples from 134 adult subjects (over 21 years of age) having no known history of allergy, using the Access Total IgE assay. The results were as follows.

n	Geometric Mean* (IU/mL)	2 SD Range* (IU/mL)	Arithmetic Mean (IU/mL)	95% Range** (IU/mL)
134	17.48	1.27–241.3	35.0	1.31–165.3

n	Geometric Mean* (µg/L)	2 SD Range* (µg/L)	Arithmetic Mean (µg/L)	95% Range** (µg/L)
134	42	3–579	84	3–397

* Logarithmic transformations

** Non-parametric estimate of 95% confidence interval

 © 2006 Beckman Coulter, Inc.

Specific Performance Characteristics

Methods Comparison

A comparison of 105 values using the Access Total IgE assay on the Access Immunoassay system and a commercially available enzyme immunoassay kit gave the following statistical data:

n	Range of Observations (IU/mL)	Intercept (IU/mL)	Slope	Correlation Coefficient (r)
105	1.42–2439.0	9.23	0.97	0.995

A comparison of 47 values obtained by assaying clinical samples of serum and plasma (heparin) using the Access Total IgE assay kit on the Access Immunoassay system gave the following statistical data:

n	Range of Observations (IU/mL)	Intercept (IU/mL)	Slope	Correlation Coefficient (r)
47	2.17–365.52	1.048	0.99	0.998

A comparison of 46 values obtained by assaying clinical samples of serum and plasma (EDTA) using the Access Total IgE assay kit on the Access Immunoassay system gave the following statistical data:

n	Range of Observations (IU/mL)	Intercept (IU/mL)	Slope	Correlation Coefficient (r)
46	2.17–365.52	-0.012	0.985	0.998

Dilution Recovery (Linearity)

Multiple gravimetric dilutions of two samples containing various IgE levels with Access Total IgE Calibrator S0 (zero) resulted in the following data:

Sample 1	Expected Concentration (IU/mL)	Determined Concentration (IU/mL)	Recovery (%)
Neat	N/A	2383.0	N/A
1/1.26	1891.3	1800.4	95.2
1/1.66	1435.5	1472.3	102.6
1/2.50	953.2	952.9	100.0
1/4.98	478.5	507.2	106.0
1/19.57	121.8	133.5	109.6
		Mean % Recovery	**102.7**

Sample 2	Expected Concentration (IU/mL)	Determined Concentration (IU/mL)	Recovery (%)
Neat	N/A	1417.9	N/A
1/1.25	1134.3	1089.0	96.0
1/1.68	844.0	847.0	100.4
1/2.54	558.2	540.0	96.7
1/4.98	284.7	284.7	100.0
1/19.96	71.0	74.1	104.4
		Mean % Recovery	**99.5**

© 2006 Beckman Coulter, Inc.

Spiking Recovery

Addition of five different levels of IgE to two patient samples with low total IgE resulted in the following data:

Sample 1	Expected Concentration (IU/mL)	Determined Concentration (IU/mL)	Recovery (%)
Neat	N/A	9.5	N/A
Level 1	54.2	54.6	100.7
Level 2	187.6	195.4	104.2
Level 3	480.5	467.9	97.4
Level 4	1078.2	1159.0	107.5
Level 5	1694.3	1744.8	103.0
		Mean % Recovery	**102.6**

Sample 2	Expected Concentration (IU/mL)	Determined Concentration (IU/mL)	Recovery (%)
Neat	N/A	11.5	N/A
Level 1	91.2	95.1	104.3
Level 2	328.0	319.6	97.4
Level 3	638.3	585.8	91.8
Level 4	1240.9	1233.0	99.4
Level 5	1820.9	1945.0	106.8
		Mean % Recovery	**99.9**

Imprecision

This assay exhibits total imprecision of less than 10% across the assay range. One study, using commercially available human serum based control material generating two assays per day, two replicates per assay, for 10 days, provides the following data, analyzed via analysis of variance. (ANOVA).[22,23]

Sample	Grand Mean (n=40) (IU/mL)	Within Run (%CV)	Total Imprecision (%CV)
Low	21.2	3.3	3.6
Medium	57.8	3.7	4.5
High	229.0	4.2	4.7

Analytical Specificity/Interferences

Samples containing up to 10 mg/dL (171 µmol/L) bilirubin, lipemic samples containing the equivalent of 1800 mg/dL (20.32 mmol/L) triolein, and hemolyzed samples containing up to 1000 mg/dL (10 g/L) hemoglobin do not affect the concentration of IgE assayed. In addition, samples ranging from 5–9 g/dL (50–90 g/L) albumin do not affect the concentration of IgE assayed.

Cross-reactivity was evaluated by testing neat IgA, IgG, IgM, and IgD myeloma sera in the Access Total IgE assay. When available, both kappa and lambda light chain myelomas were utilized. IgE concentration and percent cross-reactivity columns describe data obtained from the samples with the maximum concentration of myeloma protein tested.

 © 2006 Beckman Coulter, Inc.

Immunoglobulin Class	Number of Samples Tested	Maximum Concentration of Myeloma Protein Tested (mg/dL)	IgE Concentration (IU/mL)	Cross-reactivity (%)
IgA	2	4750	1.13	5.8×10^{-6}
IgG	3	6747	0.22	8.0×10^{-7}
IgM	2	2432	6.48	6.5×10^{-5}
IgD	1	1020	3.08	7.3×10^{-5}

Analytical Sensitivity

The lowest detectable level of IgE distinguishable from zero (Access Total IgE Calibrator S0) with 95% confidence is 0.25 IU/mL (0.6 µg/L). This value is determined by processing a complete seven point calibration curve, controls, and ten replicates of the zero calibrator in multiple assays. The analytical sensitivity value is interpolated from the curve at the point that is two standard deviations from the mean measured zero calibrator signal.

© 2006 Beckman Coulter, Inc.

Access®
Immunoassay Systems

BECKMAN COULTER®

TOTAL IgE
REF 35000

Intended Use

The Access Total IgE Calibrators are intended to calibrate the Access Total IgE assay for the quantitative determination of total IgE levels in human serum and plasma (heparin, EDTA) using the Access Immunoassay Systems.

Summary and Explanation

Quantitative assay calibration is the process by which samples with known analyte concentrations (i.e., assay calibrators) are tested like patient samples to measure the response. The mathematical relationship between the measured responses and the known analyte concentrations establishes the calibration curve. This mathematical relationship, or calibration curve, is used to convert RLU (Relative Light Unit) measurements of patient samples to specific quantitative analyte concentrations.

Traceability

The measurand (analyte) in the Access Total IgE Calibrators is traceable to the WHO 2nd International Reference Preparation for Immunoglobulin E (IgE) 75/502.[4] Traceability process is based on EN ISO 17511.

The assigned values were established using representative samples from this lot of calibrator and are specific to the assay methodologies of the Access reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Product Information

Access Total IgE Calibrators
Cat. No. 35005: S0, 6.0 mL/vial; S1–S6, 4.0 mL/vial

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Mix contents by gently inverting before use. Avoid bubble formation.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.
- Signs of possible deterioration are control values out of range.
- Refer to calibration card and or vial labels for exact concentrations.

S0:	Equine serum with < 0.1% sodium azide, and 0.5% ProClin** 300. Contains 0.0 IU/mL (µg/L) IgE.
S1, S2, S3, S4, S5, S6:	Human IgE in equine serum at levels of approximately 3, 15, 60, 240, 1000, and 3000 IU/mL (7, 36, 144, 576, 2400 and 7200 µg/L), respectively, with < 0.1% sodium azide, and 0.5% ProClin 300.
Calibration Card:	1

 © 2006 Beckman Coulter, Inc.

Warnings and Precautions

- For *in vitro* diagnostic use.
- Human source material used in the preparation of the reagent has been tested and found negative or non-reactive for Hepatitis B, Hepatitis C (HCV), and Human Immunodeficiency Virus (HIV-1 and HIV-2). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease.[24]
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.[17]
- Xi. Irritant: 0.5% ProClin 300.

R 43: May cause sensitization by skin contact.

S 28–37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- The Material Safety Data Sheet (MSDS) is available upon request.

Procedure

Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

Calibration Details

The Access Total IgE Calibrators are provided at seven levels - zero and approximately 3, 15, 60, 240, 1000, and 3000 IU/mL. The calibrators are prepared gravimetrically from human IgE and normal equine serum. Assay calibration data are valid up to 28 days.

Calibrators run in duplicate.

Limitations of the Procedure

If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial.

References

1 Ishizaka K, Ishizaka T, Hombrook MM. Physico-chemical properties of human reaginic antibody. IV. Presence of a unique immunoglobulin as a carrier of reaginic activity. J Immunol 1966; 97: 75.

2 Johansson SGO, Bennich H, Wide L. A new class of immunoglobulin in human serum. Immunology 1968; 14: 265.

3 Bennich HH, et al. Immunoglobulin E, a new class of human immunoglobulin. Bulletin World Health Organization 1968; 38: 151.

4 Seagroatt V, Anderson SG. The second international reference preparation of human serum immunoglobulin E and the first British standard for human serum immunoglobulin E. J Biological Standards 1981; 9: 431.

5 Berg T, Johansson SGO. Immunoglobulin levels during childhood, with special regard to IgE. Acta Paediatr Scand 1969; 58: 513.

6 Halonen M, Barbee RA, Lebowitz MD, Burrows B. An epidemiologic study of the interrelationships of total serum immunoglobulin E, allergy skin-test reactivity, and eosinophilia. J Allergy Clin Immunol 1982; 69: 221-228.

7 Johansson SGO. Raised levels of a new immunoglobulin class (IgND) in asthma. Lancet 1967; 2: 951-953.

8 Burrows B, et al. Association of asthma with serum IgE levels and skin-test reactivity to allergens. New Engl J Med 1989; 320: 271-277.

9 Michel FB, et al. Comparison of cord blood immunoglobulin E concentrations and maternal allergy for prediction of atopic disease in infancy. J Allergy Clin Immunol 1980; 65: 422.

10 Bousquet J, Menardo JL, Viala JL, Michel FB. Predictive value of cord serum IgE determination in the development of "early onset" atopy Ann Allergy 1983; 51: 291.

11 Kjellman NIM, Croner S. Cord blood IgE determination for allergy prediction – a follow-up to seven years of age in 1,651 children. Ann Allergy 1984; 53: 167.

12 Kjellman NIM. Predictive value of high IgE levels in children. Acta Paediatr Scand 1976; 65: 465-471.

13 Patterson R, et al. Serum immunoglobulin E in pulmonary allergic aspergillosis. J Allergy Clin Immunol 1972; 49: 98-99.

14 Savanat T, Thammapalerd N, Jaroonvesma N, Bunnag D. Total serum IgE level in patients with amoebic liver abscess and other parasitic infections. Southeast Asian J Trop Med Public Health 1977; 8: 149-154.

15 Waldmann TA, et al. Immunoglobulin E in immunologic deficiency diseases. II. Serum IgE concentration of patients with acquired hypogammaglobulinemia and Wiskott-Aldrich Syndrome. J Immunol 1972; 109: 304-310.

16 Addison GM, et al. An immunoradiometric assay for ferritin in the serum of normal subjects and patients with iron deficiency and iron overload. J Clin Path 1972; 25: 326-329.

17 DHHS (NIOSH) Publication No. 78-127, August 1976. Current Intelligence Bulletin 13 - Explosive Azide Hazard. Available http://www.cdc.gov/niosh.

18 Approved Guideline – Procedures for the Handling and Processing of Blood Specimens, H18-A2. 1999. National Committee for Clinical Laboratory Standards.

19 Cembrowski GS, Carey RN. Laboratory quality management: QC & QA. ASCP Press, Chicago, IL, 1989.

20 Kricka, L. Interferences in immunoassays–still a threat. Clin Chem 2000; 46: 1037.

21 Bjerner J, et al. Immunometric assay interference: incidence and prevention. Clin Chem 2002; 48: 613–621.

22 Tentative Guideline – User evaluation of precision performance of clinical chemistry devices, EP5-T. 1984. National Committee for Clinical Laboratory Standards, 4: No. 8.

23 Krouwer JS, Rabinowitz R. How to improve estimates of imprecision. Clinical Chemistry 1984; 30: 290-292

24 HHS Publication, 4th ed., May 1999. Biosafety in Microbiological and Biomedical Laboratories. Available http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

Access, SYNCHRON LX, UniCel, DxI and the Beckman Coulter logo are trademarks of Beckman Coulter, Inc.

*Lumi-Phos is a trademark of Lumigen, Inc.

**ProClin is a trademark of Rohm and Haas Company or of its subsidiaries or affiliates.

Manufactured by:
Beckman Coulter, Inc.
4300 N. Harbor Blvd.
Fullerton, CA 92835 USA

EC REP
Beckman Coulter Ireland Inc.
Mervue Business Park,
Mervue, Galway,
Ireland 353 91 774068

Printed in U.S.A.
Made in U.S.A.
Revised October 2006

Research Operating Procedure CORE-CHEM-DXC-24 for Quantitative Determination of Creatinine in Human Plasma, Serum Using the SYNCHRON® System(s) for Children's Health after the Storms (CHATS)

Prepared by: Patricia Harrison MT(ASCP) Date: 2/8/2010

Approved by: Dr. F. W. Brazda, MD Date: 7/14/2013

Clinical Pharmacology-Toxicology Laboratory
MCLNO Pathology Services
LSU Interim Hospital

List of Revisions

Revision Number	Changes	Date
0	Original from RTI	7/14/2011

Table of Contents

PRINCIPLE 5
INTENDED USE 5
CLINICAL SIGNIFICANCE 5
METHODOLOGY 5
CHEMICAL REACTION SCHEME 5
SPECIMEN 5
TYPE OF SPECIMEN 5
SPECIMEN STORAGE AND STABILITY 5
SAMPLE VOLUME 6
REAGENTS – Creatinine (Part no. A40920) 6
CONTENTS 6
VOLUMES PER TEST 6
REACTIVE INGREDIENTS 6
EUROPEAN HAZARD CLASSIFICATION 7
MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT 7
REAGENT PREPARATION 7
ACCEPTABLE REAGENT PERFORMANCE 7
REAGENT STORAGE AND STABILITY 7
CALIBRATION 7
CALIBRATOR REQUIRED 7
CALIBRATOR PREPARATION 7
CALIBRATOR STORAGE AND STABILITY 7
CALIBRATION INFORMATION 8
TRACEABILITY 8
QUALITY CONTROL 8
TESTING PROCEDURE(S) 8
CALCULATIONS 8
REPORTING RESULTS 9
REFERENCE INTERVALS 9
PROCEDURAL NOTES 9

ANTICOAGULANT TEST RESULTS 9
LIMITATIONS 9
INTERFERENCES 9
PERFORMANCE CHARACTERISTICS 10
MCLNO ANALYTICAL MEASUREMENT RANGE: 10
EQUIVALENCY 10
PRECISION 11
ADDITIONAL INFORMATION 12
SHIPPING DAMAGE 12
REFERENCES 12

SYNCHRON® System(s)
Chemistry Information Sheet

CR-S
Creatinine
Kit Reorder # A40920

For *In Vitro* Diagnostic Use

PRINCIPLE

INTENDED USE

CR-S reagent, when used in conjunction with UniCel® DxC 600/800 System(s) and SYNCHRON® Systems AQUA CAL 1 and 2, is intended for the quantitative determination of Creatinine concentration in human serum, plasma or urine.

CLINICAL SIGNIFICANCE

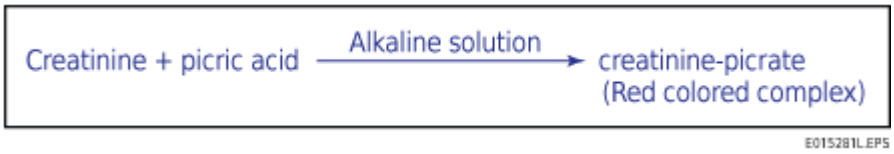
Creatinine measurements are used in the diagnosis and treatment of renal diseases, in monitoring renal dialysis, and as a calculation basis for measuring other urine analytes.

METHODOLOGY

CR-S reagent is used to measure the creatinine concentration by a modified rate Jaffé method.[1,2,3] In the reaction, creatinine combines with picrate in an alkaline solution to form a creatinine-picrate complex.

The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into the cuvette. The ratio used is one part sample to 11 parts reagent for serum and one part sample to 73 parts reagent for urine. The System monitors the change in absorbance at 520 nanometers. This change in absorbance is directly proportional to the concentration of CR-S in the sample and is used by the System to calculate and express CR-S concentration.

CHEMICAL REACTION SCHEME



SPECIMEN

TYPE OF SPECIMEN

Freshly drawn serum or lithium heparinized plasma or freshly collected urine (random/timed) are the specimens of choice. Acceptable anticoagulants are listed in the PROCEDURAL NOTES section of this chemistry information sheet. Whole blood is not recommended for use as a sample.

SPECIMEN STORAGE AND STABILITY

1. Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the serum or plasma be physically separated from contact with cells within two hours from the time of collection.[5]

2. Separated serum or plasma should not remain at room temperature longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2°C to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.[5]

3. It is recommended that urine assays be performed within 2 hours of collection. For timed specimens, the collection container is to be kept in the refrigerator or on ice during the timed period. If a special preservative is required, it should be added to the container before urine collection begins.[6]

SAMPLE VOLUME

A filled 0.5 mL sample cup is the optimum volume. For optimum primary sample tube volumes in primary tube samples and minimum volumes, refer to the Primary Tube Sample Template for your system.

REAGENTS – Creatinine (Part no. A40920)

CONTENTS

Each kit contains the following items:

Two CR-S Reagent Cartridges (2 x 300 tests)

VOLUMES PER TEST

Sample Volume	
Serum/Plasma	20 µL
Urine	3 µL
Total Reagent Volume	219 µL
Cartridge Volumes	
A	175 µL
B	44 µL
C	– –

REACTIVE INGREDIENTS

REAGENT CONSTITUENTS

Picric Acid	8.1 mmol/L
Buffered to pH	> 13.3

Also non-reactive chemicals necessary for optimal system performance.

EUROPEAN HAZARD CLASSIFICATION

Creatinine Reagent (Compartment A)	T+;R27	Very toxic in contact with skin.
	R34	Causes burns.
	S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
	S27	Take off immediately all contaminated clothing.
	S36/37/39	Wear suitable protective clothing, gloves and eye/face protection.
	S9	Keep container in a well-ventilated place.

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

SYNCHRON® Systems AQUA CAL 1 and 2 (Part no. 471288 & 471291)
Antifoam (Part no. 445967)
At least two levels of control material
Saline

REAGENT PREPARATION

Add 1 drop of Antifoam to reagent compartment A. Mix gently. Do not use more than the recommended volume of Antifoam.

ACCEPTABLE REAGENT PERFORMANCE

The acceptability of a reagent is determined by successful calibration and by ensuring that quality control results are within acceptance criteria.

REAGENT STORAGE AND STABILITY

CR-S reagent, when stored unopened at room temperature, will obtain the shelf-life indicated on the cartridge label. Once opened, the reagent is stable for 15 days at +2°C to +8°C unless the expiration date is exceeded. DO NOT FREEZE.

CALIBRATION

CALIBRATOR REQUIRED

SYNCHRON® Systems AQUA CAL 1 and 2 (Part no. 471288 & 471291)

CALIBRATOR PREPARATION

No preparation is required.

CALIBRATOR STORAGE AND STABILITY

1. If unopened, the calibrators should be stored at +2°C to +8°C until the expiration date printed on the calibrator bottle. Once opened, the calibrators are stable at room temperature for 30 days.

2. Repetitive refrigeration of the aqueous calibrators may facilitate crystal formation. Once removed from refrigerated storage, these calibrators should remain at room temperature.

CALIBRATION INFORMATION

1. The system must have a valid calibration factor in memory before control or patient samples can be run.

2. Under typical operating conditions the Creatinine assay must be calibrated every 5 days or with each new cartridge of reagent and also with certain parts replacements or maintenance procedures, as defined in the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

3. This assay has within-lot calibration available. For detailed calibration instructions, refer to the UniCel DxC 600/800 Systems *Instructions for Use* (IFU) manual.

4. The system will automatically perform checks on the calibration and produce data at the end of calibration. In the event of a failed calibration, the data will print out with error codes and the system will alert the operator of the failure. An explanation of these error codes can be found in the UniCel DxC 600/800 Systems *Instructions For Use* (IFU) manual.

TRACEABILITY

For Traceability information refer to the Calibrator instructions for use.

QUALITY CONTROL

At least two levels of control material should be analyzed daily. In addition, these controls should be run with each new calibration, with each new reagent cartridge, and after specific maintenance or troubleshooting procedures as detailed in the appropriate system manual.

Refer to the Core Laboratory Policy Manual for Quality Control material and frequency.

TESTING PROCEDURE(S)

7 1. If necessary, prepare reagent cartridge as described in the Reagent Preparation section of this chemistry information sheet and load the reagent onto the system.

8 2. After reagent load is completed, calibration may be required.

9 3. Program samples and controls for analysis.

10 4. After loading samples and controls onto the system, follow the protocols for system operation.

For detailed testing procedures, refer to the UniCel DxC 600/800 Systems *Instructions For Use* (IFU) manual.

CALCULATIONS

The system performs all calculations internally to produce the final reported result. The system will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming.

If calculation of creatinine clearance is desired, refer to References (4).

REPORTING RESULTS

REFERENCE INTERVALS

INTERVALS	SAMPLE TYPE	CONVENTIONAL UNITS
Laboratory	Serum or plasma (Male)	18Y 0.70 – 1.40 mg/dL 12y 0.50 – 1.10 mg/dL 0 0.40 – 0.90 mg/dL
	Serum or plasma (Female)	18Y 0.50 – 1.10 mg/dL 12Y 0.50 – 1.00 mg/dL 0 0.40 – 0.90 mg/dL
	Urine (Male)	0.7 – 2.6 g/24hr
	Urine (Female)	0.6 – 2.1 g/24hr

PROCEDURAL NOTES

ANTICOAGULANT TEST RESULTS

If plasma is the sample of choice, the following anticoagulants were found to be compatible with this method based on a study of 20 healthy volunteers:

TABLE 3 ACCEPTABLE ANTICOAGULANTS

ANTICOAGULANT	LEVEL TESTED FOR IN VITRO INTERFERENCE	DEMING REGRESSION ANALYSIS
Lithium Heparin	14 Units/mL	Y= 0.985X + 0.02; r = 0.999
Sodium Heparin	14 Units/mL	Y= 1.006X - 0.02; r = 0.999
EDTA	1.5 mg/mL	Y= 0.953X + 0.03; r = 0.999

LIMITATIONS

If urine samples are cloudy or turbid, it is recommended that they be centrifuged prior transfer to sample cups.

INTERFERENCES

1. The following substances were tested for interference with this methodology:

TABLE 4 INTERFERENCES

SUBSTANCE	SOURCE	LEVEL TESTED	OBSERVED EFFECT[a]
Bilirubin	Porcine	15.0 mg/dL	NSI[b]
		22.5 mg/dL	-0.5 mg/dL
Lipemia	Human	+4 (visual)	NSI
Hemoglobin	Human	500 mg/dL	NSI
Acetoacetate	Acetoacetic acid lithium salt	20 mg/dL	NSI
Pyruvate	Pyruvic acid	10 mg/dL	NSI
Methyl dopa	Methyl dopa HCl	5 mg/dL	NSI
Gentisic Acid	2,5-dihydroxybenzoic acid	50 mg/dL	NSI
Cephalothin	7-[2-thienylacetamido]-cephalosporanic acid sodium salt	100 mg/dL	NSI
Cefotaxime	Sodium Salt	50 mg/dL	NSI
Cefoxitin	Sodium Salt	12.5 mg/dL	NSI
		25.0 mg/dL	+0.7 mg/dL
Cephalosporin	Zinc salt	10 mg/dL	NSI

Refer to References (11,12,13) for other interferences caused by drugs, disease and preanalytical variables.

PERFORMANCE CHARACTERISTICS

There is no upper limit for Clinically Reportable Range. Values higher than the Analytical Measurement Range are diluted to obtain a result.

MCLNO ANALYTICAL MEASUREMENT RANGE:

TABLE 5 ANALYTICAL MEASUREMENT RANGE

SAMPLE TYPE	CONVENTIONAL UNITS	
Serum or plasma	0.3 – 25.0 mg/dL	
Urine	10.0 – 400.0 mg/dL	

Samples with concentrations exceeding the high end of the analytical range should be diluted with saline and reanalyzed.

EQUIVALENCY

Equivalency was assessed by Deming regression analysis of patient samples to accepted clinical methods.

Serum or Plasma (in the range of 0.3 to 25.0 mg/dL):

Y (UniCel DxC Systems) = 0.962X + 0.03

N = 105

Serum or Plasma (in the range of 0.3 to 25.0 mg/dL):

MEAN (UniCel DxC Systems)	= 3.8
MEAN (SYNCHRON CX Systems)	= 3.9
CORRELATION COEFFICIENT (r)	= 1.000

Urine (in the range of 17.1 to 391.5 mg/dL):

Y (UniCel DxC Systems)	= 1.002X + 3.67
N	= 75
MEAN (UniCel DxC Systems)	= 141.4
MEAN (SYNCHRON CX Systems)	= 137.4
CORRELATION COEFFICIENT (r)	= 0.999

Serum (in the range of 0.34 to 22.45 mg/dL):

Y (UniCel DxC Systems)	= 1.02X -0.08
N	= 39
MEAN (UniCel DxC Systems)	= 4.40
MEAN (Isotope Dilution Mass Spectroscopy reference procedure (14))	= 4.41
CORRELATION COEFFICIENT (r)	= 0.9997

Refer to References (15) for guidelines on performing equivalency testing.

PRECISION

A properly operating SYNCHRON® System(s) should exhibit precision values less than or equal to the following:

TABLE 6 PRECISION VALUES

TYPE OF PRECISION	SAMPLE TYPE	SD		CHANGEOVER VALUE[c]		% CV
		mg/dL	µmol/L	mg/dL	µmol/L	
Within-run	Serum/Plasma	0.2	18	10.0	600	2.0
	Urine	2.0	177	100	5,900	2.0
Total	Serum/Plasma	0.3	27	10.0	600	3.0
	Urine	3.0	266	100	5,900	3.0

Comparative performance data for the UniCel DxC System(s) evaluated using the NCCLS Proposed Guideline EP5-A appears in the table below.[16] Each laboratory should characterize their own instrument performance for comparison purposes.

TABLE 7 NCCLS EP5-A PRECISION ESTIMATE METHOD

TYPE OF IMPRECISION	SAMPLE TYPE	No. Systems	No. Data Points[d]	Test Mean Value (mg/dL)	EP5-A Calculated Point Estimates	
					SD	% CV
Within-run	Serum	Level 1	80	0.6	0.05	9.4
	Serum	Level 2	80	7.2	0.06	0.9
	Urine	Level 1	80	90.1	1.21	1.4
	Urine	Level 2	80	244.0	3.67	1.5
Total	Serum	Level 1	80	0.6	0.05	9.5
	Serum	Level 2	80	7.2	0.12	1.7
	Urine	Level 1	80	90.1	1.70	1.9
	Urine	Level 2	80	244.0	4.22	1.7

Refer to References (16) for guidelines on performing precision testing.

NOTICE

These degrees of precision and equivalency were obtained in typical testing procedures on UniCel DxC System(s) and are not intended to represent the performance specifications for this reagent.

ADDITIONAL INFORMATION

For more detailed information on UniCel DxC System(s), refer to the appropriate system manual.

SHIPPING DAMAGE

If damaged product is received, notify your Beckman Coulter Clinical Support Center.

REFERENCES

1. Jaffe, M. Z., *Physiol. Chem.*, 10:391 (1886).

2. Vasiliades, J., *Clin. Chem. Acta*, 22:1664 (1976).

3. Heinegard, D., Tiderstrom, G., *Clin. Chem. Acta*, 43:305 310 (1973).

4. Tietz, N. W., "Specimen Collection and Processing; Sources of Biological Variation", *Textbook of Clinical Chemistry*, 2nd Edition, W. B. Saunders, Philadelphia, PA (1994).

5. National Committee for Clinical Laboratory Standards, *Procedures for the Handling and Processing of Blood Specimens*, Approved Guideline, NCCLS publication H18-A, Villanova, PA (1990).

6. National Committee for Clinical Laboratory Standards, *Routine Urinalysis and Collection, Transportation and Preservation of Urine Specimens*, Tentative Guideline, NCCLS publication GP16-T, Villanova, PA (1992).

7. Tietz, N. W., *Clinical Guide to Laboratory Tests*, 3rd Edition, W. B. Saunders, Philadelphia, PA (1995).

8. National Committee for Clinical Laboratory Standards, *How to Define, Determine, and Utilize Reference Intervals in the Clinical Laboratory*, Approved Guideline, NCCLS publication C28-A, Villanova, PA (1994).

9. Tietz, N. W., ed., *Fundamentals of Clinical Chemistry*, 3rd Edition, W. B. Saunders, Philadelphia, PA (1987).

10. Henry, J. B., *Clinical Diagnosis and Management by Laboratory Methods*, 18th Edition, W. B. Saunders Company, Philadelphia, PA (1991).

11. Young, D. S., *Effects of Drugs on Clinical Laboratory Tests*, 3rd Edition, AACC Press, Washington, D.C. (1990).

12. Friedman, R. B., Young, D. S., *Effects of Disease on Clinical Laboratory Tests*, 2nd Edition, AACC Press, Washington, D.C. (1989).

13. Young, D. S., *Effects of Preanalytical Variables on Clinical Laboratory Tests*, AACC Press, Washington, D.C. (1993).

14. Stöckl D, Reinauer H., *Clin Chem* 39:993-1000 (1993).

15. National Committee for Clinical Laboratory Standards, *Method Comparison and Bias Estimation Using Patient Samples*, Tentative Guideline, NCCLS publication EP9-T, Villanova, PA (1993).

16. National Committee for Clinical Laboratory Standards, *Precision Performance of Clinical Chemistry Devices*, 2nd Edition, Approved Guideline, Vol. 19, No. 2, NCCLS publication EP5-A, Villanova, PA (1999).

EC REP Beckman Coulter Ireland Inc., Mervue Business Park, Mervue, Galway, Ireland (353 91 774068)

Beckman Coulter, Inc., 4300 N. Harbor Blvd., Fullerton, CA 92835

ENDNOTES

a Plus (+) or minus (-) signs in this column signify positive or negative interference.

b NSI = No Significant Interference (within ±0.4 mg/dL or 4%).

c When the mean of the test precision data is less than or equal to the changeover value, compare the test SD to the SD guideline given above to determine the acceptability of the precision testing. When the mean of the test precision data is greater than the changeover value, compare the test % CV to the guideline given above to determine acceptability. Changeover value = (SD guideline/CV guideline) x 100.

d The point estimate is based on the pooled data from one system, run for twenty days, two runs per day, two observations per run on an instrument operated and maintained according to the manufacturer`s instructions.

Research Operating Procedure PHTHMET-U01

Phthalate Metabolites in Urine by High Resolution-Accurate Mass Spectrometry for CHATS

Prepared by: Cherng-Zee Chuang., PhD., DABCC Date: 3/7/2013

Reviewed by:_____ Date: _____

Approved by: F. Avery Ragan, Jr., Ph.D., DABCC Date: 3/7/2013

Clinical Pharmacology-Toxicology Laboratory
Department of Pathology
LSU Interim Hospital

Table of Contents

Scope and Application: 4
Summary of Method: 4
Apparatus and Materials: 4
Personnel Qualifications: 5
Procedure: 6
Quality Control 8
Method Performance....9
References:....10

List of Revisions

Revision Number	Changes	Date
0	Original from RTI	

Scope and Application:

This procedure is for the detection and quantitation of phthalate metabolites in the urine of patients. Phthalates are ubiquitous chemicals in the environment and it is desirable to determine their scope and prevalence in humans. This will allow the determination of their role, if any, in increasing the risk for cancer and reproductive disorders.

Summary of Method:

A modification of CDC Method No. 6306.03 for urine phthalate metabolites (2010) was used. This method measures individual total phthalate metabolites with the addition of labeled internal standards after enzyme hydrolysis. Compound separation and identification were achieved with an ultra high performance liquid chromatography-mass spectrometer system (UHPLC-MS). High resolution and accurate mass (HR/AM) feature of mass spectrometer is used to select the target compounds specifically. Sample processing is simplified and a quick method is achieved.

Apparatus and Materials:

Instruments: Dionex 3000 Ultimate UHPLC system and Q-Exactive spectrometer (Thermo Scientific).
Column: Acquity UPLC HSS T3 2.1x150mm, 1.8um (Waters).

Standards and labeled standards are from Toronto Research Chemicals (Toronto, Canada) and Cambridge Isotopes Lab. (Andover, MA) (See Table1). HPLC water, acetonitrile, and ammonium acetate are LC-MS grade and are from Thermo Scientific. Glucuronidase is from Roche Co. and morphine-3-glucuronide-d3 is from Cerilliant Co.

Table 1. List of target compounds and internal standards

Abbreviation	Standards and Internal standards	Parent Compound
MMP	Monomethyl phthalate	Dimethyl-P (DMP)
MMP-IS	MMP-$^{13}C_4$	
MEP	Monoethyl phthalate	Diethyl-P (DEP)
MEP-IS	MEP-$^{13}C_4$	
MBP	Mono-n-butyl phthalate	Dibutyl-P (DBP)
MBP-IS	MBP-$^{13}C_4$	
MCPP	Mono(3-catboxypropyl) phthalate	Dibutyl-P (DBP)
MCPP-IS	MCPP-$^{13}C_4$	
MCHP	Monocyclohexyl phthalate	Dicyclohexyl-P (DCP)
MCHP-IS	MCHP-$^{13}C_4$	
MBzP	Monobenzyl phthalate	Bezyl butyl-P (BBP)
MBzP-IS	MBzP-$^{13}C_4$	
MEHP	Mono(2-ethylhexyl) phthalate	Bis(2-ethylhexyl)-P (DEHP)
MEHP-IS	MEHP-$^{13}C_4$	
MEOHP	Mono(2-ethyl-5-oxohexyl) phthalate	Bis(2-ethylhexyl)-P (DEHP)
MEOHP-IS	MEOHP-$^{13}C_4$	
MEHHP	Mono(2-ethyl-5-hydroxyhexyl) phthalate	Bis(2-ethylhexyl)-P (DEHP)
MCMHP	Mono-(2-carboxymethyl)-hexyl-phthalate	Bis(2-ethylhexyl)-P (DEHP)
MOP	Monooctyl phthalate	Di-n-octyl-P (DNOP)
MOP-IS	MOP-$^{13}C_4$	
MNP	Mono-isononyl phthalate	Di-isononyl-P (DINP)
MNP-IS	MNP-$^{13}C_4$	

Personnel Qualifications:

Personnel are trained in the safe and proper use of the required equipment and are licensed as Clinical Laboratory Scientists or Specialists by the Louisiana Board of Medical Examiners as required by Louisiana Statute to perform clinical testing on human patients. The personnel are appropriately trained and familiar with the requirements for maintaining our Accreditation by the College of American Pathologists. The laboratory

also maintains the appropriate CLIA licenses and has a DEA license to handle all scheduled controlled substances.

Procedure:

A. Preparation of stock standard/internal standard solutions

Stock solution: Use as it is from Cambridge Isotope Lab, 100ug/mL
Prepare MCHP stock solution from solids:
Weigh ~30mg and dissolved in 10mL acetonitrile (~3000 ug/mL).
Dilute to 100ug/mL with acetonitrile.
Store at -70C.

B. Preparation of mixed standard solution (MStd, 1000/2000 ng/mL)

1. Add 100uL of each standard (except 200uL for MEP) to a 10mL volumetric flask. Add water to the mark and mix well.
2. Aliquot 1mL in silanized vials. Store under -70C.

C. Preparation of mixed internal solution (MIS)

1. Add 500uL acetonitrile and 50uL of 8 internal standard (except MMP and MCPP, add 100uL) to a 10mL volumetric flask. Add water to the mark and mix well.
2. Aliquot 1.5mL in silanized vials. Store under -70C.

D. Preparation of mobile phase A (MP-A)

a. Stock solution (1500 mM, 100x)

1. Weigh 11.56g and dissolve in 50 mL MS-LC grade water.
2. Transfer to 100mL volumetric flask and add water to mark.
3. Store in 4C.

b. Working solution (15mM, pH 6.8)

1. Pour 11 mL to glass centrifuge tube and centrifuge at 3000 rpm for 10m.
2. Aspirate top 10 mL and add 1L water to make MP-A.
3. Pour out a few mL MP-A to a small beaker and check pH. Adjust the pH to 6.8 if necessary.

Note: Pour out a few mL to check pH each time when adjusting pH. Do not put pH meter to the bulk solution or pour back the solution contaminated with pH meter.

E. Preparation of ammonium acetate buffer (1M AAB) for glucuronidase

1. Dissolve 7.71g AA in ~20 mL HPLC water.
2. Transfer to 100mL volumetric flask and add water to mark.
3. Adjust pH to 6.5 with glacial acetic acid. Mix well and store 4C until use.

F. Preparation of glucuronidase solution (GCDase)

Prepare fresh working enzyme solution (Roche, cat# 03 707 598 001) for each batch: AAB:GCDase =1:1

G. Morphine-3-glucuronide-d3 (MG-d3) as hydrolysis control (HC)

Add 200uL MG-d3 (Cerilliant, Cat# M-017) to 10mL volumetric flask and add water to mark (2ug/mL). Aliquot in ~1mL and store under -70C.

H. Preparation of calibrators

1. Dilute MStd solution (1000ng/mL) to make calibrators 800, 400, 200, 100, and 50ng/mL with 10% acetonitrile.
2. Dilute calibrator 50ng/mL to make calibrators 25, 10, 5, 2.5, 1, 0.5, and 0.25ng/mL with 10% acetonitrile.
3. Prepare in silanized vials and store -70C until use.

Note: The concentrations for MEP are 2-fold of specified.

I. Procedure for Analysis

In 1.5mL microcentrifuge vials, Add followings:

				Hydrolysis controls	
		uL	uL	uL	uL
		RB (4)	**Sample**	**HC+E**	**HC-E**
Water		50		50	75
Working Enzyme		25	25	25	0
IS10		50	50		
Sample (Std, Controls, Unk)			50		
MG-d3				50	50
Mix well					
37Cx90m					
MP-A dilution, Mix well		375	375	375	375
centrifuge13000x10m					
Transfer to insert					
Inject vol	20uL				

Notes: Leave frozen samples at RT in the dark for 30m to thaw. Mix well by inversion and take aliquot for analysis.

A. HPLC parameters

Column: Acquity UPLC HSS T3 2.1x150mm, 1.8um, 40C

MP-A: 15mM ammonium acetate in water
MP-B: Acetonitrile

Gradient:	**Time, min**	**Flow, mL/min**	**%B**
	0	0.25	5
	9	0.25	100
	9.1	0.5	5
	11.6	0.5	5
	12	0.25	5

B. MS parameters

Detection in negative mode:

Sheath gas	30
Aux gas	20
Spray Voltage	3.0 KV
Capillary temp	300
Heater temp	300
Resolution	70,000

Quality Control

Hydrolysis control

The integrity of enzyme and completeness of enzyme hydrolysis were monitored by the complete hydrolysis of MG-d3 in HL+E sample. The absence of MG-d3 and presence of MG and glucuronic acid were monitored at m/z of 463.1801, 287.1481 and 193.0354 respectively.

Quality control Material

Three control samples (C1, C2 and C3) were prepared by spiking each of target compounds at 5, 50, and 200 ng/mL (except MEP was doubled) respectively to drug-free urine from a healthy subject. Precision, recovery and low level of detection (LOD) were shown in Table 2.

Method Performance

Table 2. Precision, Recovery and Level of Detection

	MCPP	MMP	MEP	MCMHP	MBP	MEHHP	MEOHP	MBzP	MCHP	MEHP	MOP	MNP
C1, ng/mL												
Mean	17.76	17.06	30.15	19.84	33.23	31.60	17.89	24.04	5.05	12.13	6.24	6.65
CV, %	24.5	14.2	8.3	11.4	5.1	5.2	2.7	2.0	5.3	7.2	4.7	10.1
Rec, %	1.7	90.7	103.0	100.7	94.4	85.6	82.3	80.9	88.4	97.1	111.3	121.8
C2, ng/mL												
Mean	68.9	67.1	105.8	72.6	79.1	80.0	63.4	71.2	49.9	55.2	51.9	51.9
CV, %	4.5	1.7	3.6	5.3	1.4	2.9	4.1	1.2	1.9	3.6	1.0	1.1
Rec, %	102.5	109.2	85.9	115.7	101.1	105.4	99.3	102.4	98.6	95.9	102.4	102.8
C3, ng/mL												
Mean	257.2	218.3	415.5	247.4	238.0	245.5	224.6	232.4	212.1	212.1	208.7	202.8
CV	7.3	3.1	0.2	4.9	2.1	0.6	1.9	1.2	1.1	1.9	2.2	1.0
Rec, %	119.7	102.9	98.9	116.3	104.7	109.1	105.4	106.2	105.7	102.4	104.0	101.1
LOD, ng/mL	1.31	0.88	0.50	0.26	0.26	0.27	0.32	0.26	0.26	0.52	0.30	0.36

References:

Blount, B.C.; Milgram, K.E.; Silva, M.J.; Malek, N.A.; Reidy, J.A.;Needham, L.L;Brock, J.W. Anal. Chem. **2000**,72, 4127-4134.

CDC Laboratory Procedure manual, Phthalate metabolites in Urine by HPLC/ESI-MS/MS, Method No. 6306.03, Revised July3, 2010.

Research Operating Procedure MERACIDVOC-U01 Synopsis

Mercapturic Acid Metabolites in Urine by High Resolution-Accurate Mass Spectrometry for CHATS

Prepared by: Cherng-Zee Chuang., PhD., DABCC Date: 3/7/2013

Reviewed by:_____ Date: _____

Approved by: F. Avery Ragan, Jr., Ph.D., DABCC Date: _3/7/2013

Clinical Pharmacology-Toxicology Laboratory
Department of Pathology
LSU Interim Hospital

Table of Contents

Scope and Application: 4
Summary of Method: 4
Apparatus and Materials: 4
Personnel Qualifications: 6
Procedure: 6
References: 9

List of Revisions

Revision Number	Changes	Date
0	Original from LSU	

Scope and Application:

This assay is used to identify and quantitate mercapturic acid metabolites of volatile organic compounds in urine. This assay is used to determine if the individual providing the sample has been exposed to volatile organic compounds by various routes of exposure.

Summary of Method:

A modification of the method of Alwis et al. (2012) was used. This method uses urine with the addition of labeled internal standards. Compound separation and identification were achieved with an ultra high performance liquid chromatography-mass spectrometer system (UHPLC-MS). High resolution and accurate mass (HR/AM) feature of mass spectrometer is used to select the target compounds specifically. Sample processing is simplified and a quick method is achieved.

Apparatus and Materials:

Instruments: Dionex 3000 Ultimate UHPLC system and Q-Exactive spectrometer (Thermo Scientific).
Column: Acquity UPLC HSS T3 2.1x150mm, 1.8um (Waters).
Standards and labeled standards are from Toronto Research Chemicals (Toronto, Canada) and Cambridge Isotopes Lab. (Andover, MA) (See Table1). Muconic acid, mandelic acid, and methylhupuric acids are from Sigma. HPLC water, acetonitrile, and ammonium acetate are of LC-MS grade and are from Thermo Scientific.

Table 1. List of target compounds and internal standards

Abbreviation	Standards and Internal Standards	Parent Compound
CEMA	N-Ac-S-(2-carboxylethyl)-L-cys	Acrolein
CEMA-d3	N-Ac-S-(2-carboxylethyl)-L-cys, d3	
GAMA	N-Ac-S-(2-hydroxy-3-propionamide)-L-cys	Acrylamide
GAMA-d3	N-Ac-S-(2-hydroxy-3-propionamide)-L-cys, d3	
CYMA	N-Ac-S-(2-cyanoethyl)-L-cys	Acrylonitrile
CYMA-d3	N-Ac-S-(2-cyanoethyl)-L-cys, d3	
PMA	N-Ac-S-(phenyl)-L-cys	Benzene
PMA-d5	N-Ac-S-(phenyl)-L-cys, d5	
MU	t-t-muconic acid	Benzene

Table 1. List of target compounds and internal standards, continued

Abbreviation	Standards and Internal Standards	Parent Compound
MU-d4	t-t-muconic acid, d5	
DHBMA	N-Ac-S-(3,4-dihydroxybutyl)-L-cys	1,3-Butadiene
DHBMA-d7	N-Ac-S-(3,4-dihydroxybutyl)-L-cys, d7	
MHBMA1+2	N-Ac-S-(1-hydroxymethyl-2-propen-1-yl)-L-cys	1,3-Butadiene
	+N-Ac-S-(2-hydroxy-3-buten-1-yl)-L-cys	
MHBMA1+2-d6	N-Ac-S-(1-hydroxymethyl-2-propenyl)-L-cys	
	+N-Ac-S-(2-hydroxymethyl-3-propenyl)-L-cys, d6	
HPMMA	N-Ac-S-(3-hydroxypropyl-1-methyl)-L-cys	Crotonaldehyde
HPMMA-d3	N-Ac-S-(3-hydroxypropyl-1-methyl)-L-cys, d3	
PHEMA	N-Ac-S-(1-pheny-2-hydroxyethyl)-L-cys	Styrene
	+N-Ac-S-(2-phenyl-2-hydroxyethyl)-L-cys	
MA	Mandelic acid (Phenylglycolic acid)	Styrene
PGA	Phenylglyoxylic acid	Styrene
BMA	N-Ac-S-(benzyl)-L-cys	Toluene
BMA-d3	N-Ac-S-(benzyl)-L-cys, d3	
1,2DCVMA	N-Ac-S-(1,2-dichlorovinyl)-L-cys	Trichloroethylene
1,2DCVMA-d3-C13	N-Ac-S-(1,2-dichloroethenyl)-L-cys, d3-C13	
2,2DCVMA	N-Ac-S-(2,2-dichlorovinyl)-L-cys	Trichloroethylene
HEMA	N-Ac-S-(2-hydroxylethyl)-L-cys	Vinyl chloride
		Acrylonitrile
		Ethylene oxide
HEMA-d4	N-Ac-S-(2-hydroxylethyl)-L-cys, d4	
24DPMA	N-Ac-S-(2,4-dimethylphenyl)-L-cys	Xylene
24DPMA-d3	N-Ac-S-(2,4-dimethylbenzene)-L-cys,d3	
25DPMA	N-Ac-S-(2,5-dimethylphenyl)-L-cys	Xylene
25DPMA-d3	N-Ac-S-(2,5-dimethylphenyl)-L-cys, d3	
34DPMA	N-Ac-S-(3,4-dimethylphenyl)-L-cys	Xylene
34DPMA-d3	N-Ac-S-(3,4-dimethylphenyl)-L-cys, d3	
2-MHA	2-methylhupuric acid	Xylene
3-MHA	3-methylhupuric acid	Xylene
4-MHA	4-methylhupuric acid	Xylene

Personnel Qualifications:

Personnel are trained in the safe and proper use of the required equipment and are licensed as Clinical Laboratory Scientists or Specialists by the Louisiana Board of Medical Examiners as required by Louisiana Statute to perform clinical testing on human patients. The personnel are appropriately trained and familiar with the requirements for maintaining our Accreditation by the College of American Pathologists. The laboratory also maintains the appropriate CLIA licenses and has a DEA license to handle all scheduled controlled substances.

Procedure:

A. Preparation of stock standard/internal standard solutions

1. Peel off the label completely.
2. Wipe off glue residues from glass completely with methanol.
3. Wipe the outside clean and dry completely.
4. Hold the vial by cap and do not touch the glass part of vial before weighing.
5. Tap the vial gently on hard surface several times.
6. Take off the cap.
7. Weigh the vial (BW) using forcep without touching the vial by hand.
8. Add ~1mL specific solvent to dissolve solids.
9. Transfer the dissolved solution to 10 mL volumetric flask.
10. Wash vial with ~1mL solvent and transfer wash to flask for additional four times.
11. Add solvent to 10-mL mark. Mix well and aliquot in storage vials.
12. Store in box and keep at -70C.
13. Wash the empty vial with ~1mL methanol, aspirate it as completely as possible, and discard it.
14. Dry the vial with nitrogen and wipe outside surface completely clean.
15. Wait for 5min and weigh again (AW).
16. Weigh the empty vial again every 30 min until you get a precise weight (within 0.05 mg).
17. Calculate the net weight (BW-AW) and correct salt amount (if any) to get actual stock concentration.

B. Preparation of mixed standard solution (MStd)

1. Aspirate the volume (in uL) needed to dilute to 6000ng/mL in a 10mL volumetric flask for each standard.
2. Add mobile phase A to the mark and aliquot ~0.5mL in silanized vials. Store under -70C.

Note: Dilute to 2000ng/mL for each of DPMA isomers, and to 3000ng/mL for 3MHA and 4MHA isomers.

C. Preparation of mixed internal solution (MIS)

1. Aspirate the volume (in uL) needed to dilute to 3000ng/mL in a 10mL volumetric flask for each internal standard.
2. Add mobile phase A to the mark and aliquot ~1.5mL in silanized vials. Store under -70C.

Note: Dilute to 1000ng/mL for each of DPMA isomers.

D. Preparation of mobile phase A (MP-A)

a. Stock solution (1500 mM, 100x)

1. Weigh 11.56g and dissolve in 50 mL MS-LC grade water.
2. Transfer to 100mL volumetric flask and add water to mark.
3. Store in 4C.

b. Working solution (15mM, pH 6.8)

1. Pour 11 mL to glass centrifuge tube and centrifuge at 3000 rpm for 10m.
2. Aspirate top 10 mL to a 1L-volumeteric flask and add water to the mark.
3. Adjust the pH to 6.8 if necessary.

Note: Pour out a few mL MP-A to a small beaker for checking pH. Do not put pH meter to the bulk solution or pour back the solution contaminated with pH electrode.

E. Preparation of calibrators

1. Dilute MStd solution (6000ng/mL) to make calibrators 3000, 1500, 900, and 600ng/mL with MP-A.
2. Dilute calibrator 600ng/mL to make calibrators 300, 150, 90, and 60ng/mL with MP-A.
3. Dilute calibrator 60ng/mL to make calibrators 30, 15, and 3ng/mL with MP-A.
4. Dilute calibrator 3ng/mL to make calibrators 1.5 and 0.3 ng/mL with MP-A.

F. Procedure for Analysis

Add followings in 1.5mL centrifuge vial:
MP-A: 400uL
ISM: 50uL
Calibrators, controls, or urine unknowns: 50uL
Mix well and centrifuge at 13000rpm for 10min.
Transfer 200uL to autosampler vial insert and inject 10-20uL.

Notes: Leave frozen samples at RT in the dark for 30m to thaw. Mix well by inversion and take aliquot for analysis.

G. HPLC parameters

Column: Acquity UPLC HSS T3 2.1x150mm, 1.8um, 40C

MP-A: 15mM ammonium acetate in water
MP-B: Acetonitrile

Gradient:	**Time, min**	**Flow, mL/min**	**%B**
	0	0.25	0
	3	0.3	10
	5	0.3	30
	6.5	0.3	40
	7	0.3	15
	7.5	0.3	10
	8	0.3	0
	11	0.25	0

H. MS parameters

Detection in negative mode:

Sheath gas	30
Aux gas	20
Spray Voltage	3.0 KV
Capillary temp	300C
Heater temp	300C
Resolution	140,000

Method Performance

Low and high control samples (LC and HC) were prepared by spiking each of the target compounds at 60 and 600ng/mL respectively to drug-free urine from a healthy subject. Precision, recovery and low level of detection (LOD) were shown in Table 2.

Table 2. Precision, Recovery and Level of Detection

		LC				HC			
	Conc, ng/mL	CV, %	Recovery		Conc, ng/mL	CV, %	Recovery		LOD, ng/mL
CEMA	170.48	27.3	118.2		825.2	6.8	121.1		4.45
GAMA	55.00	5.6	83.5		537.7	1.9	89.1		5.24
HEMA	57.12	8.4	90.1		591.8	1.1	98.6		1.80
MA	403.93	2.9	82.1		898.3	1.0	91.1		1.28
DHBMA	523.85	7.9	114.8		1145.2	2.7	115.1		3.53
CYMA	72.01	6.6	108.3		675.0	0.9	111.5		1.04
MHBMA	106.18	5.8	102.9		737.7	2.5	116.3		1.89
HPMMA	373.94	2.9	94.7		881.9	0.9	94.1		1.83
PGA	427.21	10.6	87.4		970.4	1.5	100.0		2.69
2MHA	31.95	5.9	35.1		275.0	3.7	44.5		1.17
34MHA	100.30	8.0	83.2		571.8	3.3	87.1		0.65
PHEMA	47.40	15.1	78.9		589.2	4.1	99.3		4.36
12DCVMA	51.31	5.4	87.3		614.0	2.2	103.4		1.38
PMA	54.06	4.9	89.9		587.4	1.7	98.3		1.80
BMA	54.47	3.5	88.3		570.8	2.0	95.3		0.60
22DCVMA	58.75	6.3	98.2		677.6	3.3	113.8		2.51
DPMA	52.48	5.2	86.7		581.6	2.200	97.4		1.43

References:

Alwis, K.U.; Blount, B.C.; Britt, A.S.; Patel, D.; Ashley, D.L. Anal. Chim. Acta. 750: 152-60, 2012.

Procedure Manual

Clinical Pharmacology-Toxicology Laboratory

Department of Pathology
LSU Interim Hospital

Analyte: **Nicotine-Cotinine**

Matrix: **Urine**

Method: **Nicotine Cotinine in Urine by Turboflow LC-Electrospray Tandem Mass Spectrometry**

Number: **NICCOT-U01 Synopsis**

Approved: **F. Avery Ragan, Jr., Ph.D., DABCC**

Scope and Application:

The nicotine-cotinine assay is used quantitate nicotine and cotinine in urine. The assay is applicable to the determination of parent nicotine and its primary metabolite cotinine in urine. This assay is used to determine if the individual providing the sample has been exposed to cigarette smoke either by passive inhalation or active smoking.

Summary of Method:

This method uses urine with the addition of labeled internal standards. Separation and concentration of the hydrolyzed metabolites is performed by turboflow liquid chromatography on a Thermo Scientific Aria TLX2 multiplex LC system which allows for a vigorous cleaning cycle of the column to reduce the possibility of carryover and also allows diversion of waste while directing sample to the tandem mass spectrometry. The use of tandem mass spectrometry improves specificity and sensitivity in determination of nicotine and cotenine. Turboflow hplc eliminates many of the problems created by ion suppression in tandem mass spectrometry.

Apparatus and Materials:

Thermo Scientific Aria TLX2 multiplex LC system which allows for a vigorous cleaning cycle of the column to reduce the possibility of carryover and also allows diversion of waste while directing sample to the mass spectrometer. The mass spectrometers will be either a Thermo Quantum Access or Quantum Ultra tandem mass spectrometer depending on the requirements of the assay.
Standards and labeled standards are from Cerilliant, Round Rock, Texas

Personnel Qualifications:

Personnel are trained in the safe and proper use of the required equipment and are licensed as Clinical Laboratory Scientists or Specialists by the Louisiana Board of Medical Examiners as required by Louisiana Statute to perform clinical testing on human patients. The personnel are appropriately trained and familiar with the requirements for maintaining our Accreditation by the College of American Pathologists. The laboratory also maintains the appropriate CLIA licenses and has a DEA license to handle all scheduled controlled substances.

Procedure:

The procedure followed will be modifications of the listed references will include the fact that we will be using turboflow chromatography. We will make modifications of the procedure based on our equipment, as noted in apparatus and material, and our workflow. All modifications will meet the sensitivity and specificity requirements of this project. The turboflow procedure will meet statistical parity with the referenced methods.

All samples will be batched to provide an optimum mix of controls and patient samples and to fit in timeline requirements of the project and produce reliable analytical results.

The procedure will meet all of the College of American Pathologists requirements for a fully documented clinical laboratory procedure used to produce reportable results for patient care.

Samples from time of receipt will be stored at minus –70 degrees until analysis to reduce the chance of sample degradation and contamination.

References:

Gabr, R.Q.; Elsherbiny, M.E.; Somayaji, V.; Pollak, P.T.; Brocks, D.R. Biomed. Chromatogr. 2011 Online library DOI 10.002/bmc.1581.

Laboratory Procedure Manual, Emergency Response and Air Toxicants Branch, NCEH, Method 03-OD December 5, 2003.

.

Research Operating Procedure EAR-CHATS-22

Determination of Phthalate Metabolites in Urine

for

Children's Health after the Storms (CHATS)

Prepared by: Daniel K. Briggs Date: 4/26/2012

Reviewed by: Cynthia Ann Salmons Date: 2/8/2013

Reviewed by: James W. Rogers Date: 2/26/2013

Approved by: Brian K. Wagoner Date: 2/27/2013

RTI International
Exposure Analysis Research
3040 Cornwallis Road
Research Triangle Park, NC 27709

List of Revisions

Revision Number	Changes	Date
0	Original from RTI	

Table of Contents

1.0 Scope & Application 4
2.0 Summary of Method 4
3.0 Definitions 5
4.0 Cautions 5
5.0 Interferences 5
6.0 Apparatus & Materials 6
7.0 Personnel Qualifications 6
8.0 Procedures 7
9.0 Method Performance including MDLs 12
10.0 Records Management 12
11.0 References 12

1.0 Scope & Application

The analytical procedures described in this protocol are intended for the determination of selected phthalate metabolites from urine samples that will be collected as part of the Children's Health after the Storms (CHATS) Study. This protocol addresses:

- Extraction of urine samples by enzymatic deconjugation followed by automated solid phase extraction (auto-SPE)
- Analysis of phthalate metabolite sample extracts by high performance liquid chromatography-electrospray ionization-tandem mass spectrometry (HPLC-ESI-MS/MS)
- Laboratory quality control (QC) procedures
- Data processing and documentation

Target analytes include:

- Monomethylphthalate (MMP)
- Monoethylphthalate (MEP)
- Monobutylphthalate (MBP)
- Mono(3-carboxypropyl)phthalate (MCPP)
- Monocyclohexylphthalate (MCHP)
- Mono(2-ethylhexyl)phthalate (MEHP)
- Mono-n-octylphthalate (MOP)
- Monobenzylphthalate (MBzP)
- Monoisononylphthalate (MIP)
- Mono(2-ethyl-5-oxohexyl)phthalate (MEOHP)
- Mono(2-ethyl-5-hydroxyhexyl)phthalate (MEHHP)
- Mono-[(2-Carboxymethyl)Hexyl]phthalate (MCMHP)

2.0 Summary of Method

The method is based on two procedures from the Centers for Disease Control and Prevention (CDC). The sample preparation method is taken from the procedure developed by Dr. Dana Barr (NHANES 2001-2002). The instrumental method is taken from the procedure developed by Dr. Antonia Calafat (NHANES 2007-2008). Urine samples are processed using enzymatic deconjugation of the glucuronides followed by automated solid phase extraction (auto-SPE) and concentration of the resulting eluate. The phthalate metabolites are then chromatographically resolved by reversed phase high performance liquid chromatography-electrospray ionization-tandem mass spectrometry (HPLC-ESI-MS/MS) and quantified by isotope dilution. QC samples include reagent blanks, reagent controls, matrix blanks, matrix spikes, and calibration checks. The HPLC is calibrated using a minimum of a six-point standard curve. Chromatograms are processed using Analyst 1.4.2 data system and data are output as Microsoft Excel Spreadsheets

(*.xls). If data are required to be entered into the CHATS database, the laboratory supervisor will be responsible for organizing the data in the format required by CHATS ROP #21 and uploading it to the ESN using FileZilla.

3.0 Definitions

None.

4.0 Cautions

The analyst is responsible for maintaining awareness of OSHA regulations regarding the safe handling of chemicals used in this method. The toxicity and carcinogenicity of chemicals used in this method have not been precisely defined; therefore, each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Appropriate care should be exercised in handling extracts, reagents, and solvents. All solvents, pure standard materials and stock standard solutions of target compounds should be handled exclusively in a chemical fume hood. Personal protective equipment (gloves, lab coat and eye protection) appropriate for handling hazardous materials should be worn. Exercise caution in the handling of biological samples. Observe universal precautions: wear safety glasses, protective gloves, and lab coat during all steps of this method because of both infectious and chemical related hazards. The Hepatitis B vaccination series is strongly recommended for all testing personnel. Laboratory personnel handling human fluids and tissues are required to take Bloodborne Pathogens training.

5.0 Interferences

5.1 The extent of interferences may vary considerably from sample to sample. Interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences.

5.2 Since phthalates are used as plasticizers, avoid using plastic labware and other sources of plastic to the extent possible.

5.3 Carryover contamination may occur when a sample containing low concentrations of compounds is analyzed immediately after a sample containing relatively high concentrations of similar compounds. Syringes and injectors must be thoroughly cleaned between each injection or replaced, as needed, to avoid this problem.

6.0 Apparatus & Materials

6.1 Individual native standards (phthalate monoester metabolites) and internal standards (13C4-labeled phthalate monoesters) are purchased from Cambridge Isotope Laboratories (Andover, MA)
6.2 Acetonitrile, LC-MS grade, Honeywell (B&J) LC015-2.5, 99.99%
6.3 Deionized water, HPLC grade, Hydro Picosystem UV Plus
6.4 Sodium phosphate, monobasic monohydrate, Ultrapure Bioreagent, J.T. Baker 4011-01
6.5 Phosphoric acid, ACS reagent grade, J.T. Baker 0260-02
6.6 Ammonium hydroxide, ACS reagent grade, Mallinckrodt 6665-14
6.7 Ammonium acetate, ACS reagent grade, BDH0204-500G (VWR, Suwanee, GA)
6.8 Glacial acetic acid, certified ACS Plus, Fisher A38^{C}-212, 100.0%
6.9 pH meter, Mettler Toledo SevenMulti
6.10 Top loading precision balance (3 decimal places), Mettler Toledo PR1203
6.11 β-glucuronidase from *E. coli*, Roche Diagnostics GmbH REF 03 707 598 001, 5-mL solution
6.12 Male human urine, unfiltered, Bioreclamation HMURINE-M
6.13 Micropipettes, 100- and 1000-µL volumes (Eppendorf Reference) and 200-µL volume (VWR)
6.14 Water bath, circulating thermostated, VWR Model 1265PC (VWR, Suwanee, GA)
6.15 Solid phase extraction (SPE) cartridges, 3 mL/60 mg, ABS Elut-Nexus, Agilent 12103101
6.16 Solid phase extraction (SPE) cartridges, 6 mL/200 mg, ABS Elut-Nexus, Agilent 12103102
6.17 Automated liquid handler (auto-SPE unit), Gilson GX-271
6.18 Ethyl acetate, HPLC/pesticide grade, Honeywell (B&J) 100-4, 99.9+%
6.19 Concentration workstation, TurboVap LV evaporator, Zymark (Caliper Life Sciences)
6.20 Disposable centrifuge tubes, 15-mL, Type 1 glass, screw-thread, Teflon-lined caps, Kimble Chase 73785-15
6.21 Disposable culture tubes, 12x75 mm, borosilicate glass, Kimble Chase 73500-1275
6.22 Disposable culture tubes, 13x100 mm, borosilicate glass, VWR 47729-572
6.23 Disposable culture tubes, 16x100 mm, borosilicate glass, VWR 47729-576
6.24 AB Sciex API-5000 Triple Quadrupole Mass Spectrometer with Waters Acquity UHPLC system.
6.25 Betasil Phenyl 3µm 2.1x150mm column with Betasil Phenyl 3µm 2.1x10mm guard column

7.0 Personnel Qualifications

Personnel should read the ROP carefully and have this documented by the laboratory supervisor in their training file. All staff performing this method will have demonstrated proficiency by recovering 70% - 130% of target analytes, spiked into urine at 1x – 5x the method lower limit of quantitation (LLOQ), for each of two duplicate samples.

8.0 Procedures

8.1 Standards:

8.1.1 Stock solutions are prepared by accurately transferring approximately 5 mg of material to a 10 mL Pyrex beaker (methanol rinsed). The phthalate monoester is then dissolved in acetonitrile and quantitatively transferred to a 50 mL volumetric flask. This stock solution is stored at –20°C in a Teflon-capped glass bottle (methanol rinsed) until use.

8.1.2 Internal standards (13C4-labeled phthalate monoesters) are prepared similarly to the native standards and stored sealed at –20°C until use, except for the working solution (4°C). The isotopic purity of each internal standard is confirmed empirically.

8.1.3 Eleven unique working standards with all eight analytes were prepared in water from the stock solutions of native and the 13C4-labeled internal standards to cover the linear range of the assay for each analyte (approx. 1–1000 ppb). The linear range for mEP was extended to 2500 ppb due to the relatively high levels of this analyte found in human urine.

8.2 A minimum sample volume of 3 mL is required for the assay. Specimens may be stored in a 5 mL plastic cryovials as long as the vials are tightly sealed to prevent desiccation of the sample. Urine samples received for analysis will be logged in and stored in a freezer at (< -20°C) until extraction. Specimens may be stored frozen at –20°C to –70°C for one year prior to analysis.

8.3 Samples will be analyzed in batches of up to 12 study samples, plus laboratory quality control samples. Analysis of each batch will be documented in a laboratory notebook.

8.4 Reagent Preparation

8.4.1 Basic Buffer for Solid-Phase Extraction

8.4.1.1 Measure 500 mL of acetonitrile using a 500-mL graduated cylinder and transfer to a 1-L amber glass bottle, using a glass funnel.

8.4.1.2 Measure 500 mL of reagent water using the same cylinder and add to bottle.

8.4.1.3 Measure 5.0 mL of concentrated ammonium hydroxide solution (30%) using a 5-mL graduated pipette and add to bottle.

8.4.1.4 Seal bottle with Teflon-lined cap and invert several times to mix thoroughly.

8.4.1.5 Store buffer at room temperature and ***discard after one week***.

8.4.2 Acidic Buffer for Solid Phase Extraction

8.4.2.1 Weigh 20.0 g of monosodium phosphate monohydrate (NaH_2PO_4) (ultrapure bioreagent) and transfer to a 1-L amber glass bottle, using a glass funnel.

8.4.2.2 Measure 1 L of reagent water using a 1-L graduated cylinder and add to bottle.

8.4.2.3 Measure 10.0 mL of phosphoric acid (H_3PO_4) (85%) using a 10-mL graduated pipette and add to bottle.

8.4.2.4 Seal bottle with Teflon-lined cap and invert several times to mix thoroughly.

8.4.2.5 Store buffer at room temperature and ***discard after one month***.

8.4.3 Glucuronidase/Ammonium Acetate Solution for Hydrolysis

8.4.3.1 Prepare 1 M ammonium acetate buffer (pH = 6.5) as follows: Weigh 7.708 g of ammonium acetate into a small beaker, dissolve in reagent water, and transfer to a 100-mL volumetric flask. Rinse beaker several times with reagent water, transferring to flask each time. Adjust flask to volume with reagent water, stopper, and invert several times to mix thoroughly. Use a pH meter to adjust pH to 6.5, adding concentrated glacial acetic acid dropwise (~5-6 drops). Transfer to a 125-mL amber glass bottle with Teflon-lined cap and store in refrigerator when not in use.

8.4.3.2 Accurately measure 4.00 mL of ammonium acetate buffer using a 5-mL disposable graduated pipette and transfer to a rinsed beaker. Note: This volume is enough to prep ~16 samples. Adjust accordingly for actual number of samples in batch (250 µL per sample).

8.4.3.3 Measure 80 µL of β-glucuronidase from *E. coli* using a 10-100 µL micropipette and add to beaker. Note: This volume is enough to prep ~16 samples. Adjust accordingly for actual number of samples in batch (5 µL per sample).

8.4.3.4 Swirl solution to mix. **Note: *Mix this solution fresh just prior to addition to samples.***

8.4.4 Mobile Phases for LC-MS

8.4.4.1 "Mobile Phase A" (Water + 0.1% acetic acid) is prepared by addition of 1.0 mL of glacial acetic acid to 1000 mL of water. A different volume may be prepared as long as the specified proportion of components is maintained.

8.4.4.2 "Mobile Phase B" (Acetonitrile + 0.1% acetic acid) is prepared by addition of 1.0 mL of glacial acetic acid to 1000 mL of acetonitrile. A different volume may be prepared as long as the specified proportion of components is maintained

8.5 Hydrolysis

8.5.1 Measure 1.0 mL of each unknown urine sample using a disposable 2-mL glass graduated pipette and transfer into properly labeled disposable 15-mL centrifuge tube with screw thread finish (Type 1 glass). Measure 1.0 mL reagent water for reagent blank and 1.0 mL each of QC High and QC Low.

8.5.2 Measure 75 µL of Internal Standard (labeled mixture) spiking solution using a 10-100 µL micropipette and transfer to each tube.

8.5.3 Measure 255 µL of glucuronidase/ammonium acetate solution using a 100-1000 µL micropipette and transfer to each tube.

8.5.4 Seal tubes with Teflon-lined caps, mix gently by vortexing, and incubate at 37°C for 90 minutes.

8.6 Auto-SPE Method #1 (Phthalate monoesters – 3 mL 60 mg)

8.6.1 Transfer conjugated urine samples to 12x75 mm glass culture tubes using glass Pasteur pipettes.

8.6.2 Dilute by adding 1.0 mL of basic buffer solution (measured using 100-1000 µL micropipette) to each and vortexing for 5 seconds. Note: These last 2 steps should be performed just prior to SPE.

8.6.3 Agilent Nexus solid phase extraction cartridge (3 mL/60 mg) is conditioned with 1.0 mL of acetonitrile at 1 mL/min flow rate.
8.6.4 Cartridge is then conditioned with 2.0 mL of basic buffer at 1 mL/min flow rate.
8.6.5 Sample (2.45 mL) is then loaded on cartridge and collected in 12x75 mm glass culture tube at 1 mL/min flow rate.
8.6.6 1.0 mL of basic buffer is then dispensed into original sample tube.
8.6.7 Buffer from tube is then loaded onto cartridge at 1 mL/min flow rate and collected in same tube as 8.6.3.
8.6.8 Cartridge is discarded. Note: This method is located under the bed layout: Phthalate monoesters – glass solvent containers. Run time for this method is ~17 minutes per sample.

8.7 Auto-SPE Method #2 (Phthalate monoesters – 6 mL 200 mg)
8.7.1 Combined eluants from method #1 are manually transferred to 13x100 mm glass culture tubes using glass Pasteur pipettes.
8.7.2 Acidify by manually adding 3.0 mL of acidic buffer (measured using disposable 5-mL graduated pipette) to each and mix by vortexing carefully. Note: Tubes will be very full; exercise caution to avoid spills.
8.7.3 Agilent Nexus solid phase extraction cartridge (6 mL/200 mg) is conditioned with 2.0 mL of acetonitrile at 1 mL/min flow rate.
8.7.4 Cartridge is then conditioned with 3.0 mL of acidic buffer at 1 mL/min flow rate.
8.7.5 Sample (3.45 mL + 3.0 mL = 6.45 mL total) is then loaded on cartridge at 1 mL/min flow rate, discarding as waste.
8.7.6 Cartridge is then washed with 3.0 mL acidic buffer at 1 mL/min flow rate, discarding as waste.
8.7.7 Cartridge is then washed with 9.0 mL reagent water (4.5 mL + 4.5 mL) at 1 mL/min flow rate, discarding as waste.
8.7.8 Cartridge is then dried by purging with nitrogen for 1 minute.
8.7.9 Cartridge is then eluted with 2.0 mL of acetonitrile at 1 mL/min flow rate, collecting in 16x100 mm glass culture tube.
8.7.10 Cartridge is then eluted with 2.0 mL of ethyl acetate at 1 mL/min flow rate, collecting in same tube as 8.7.9. Note: This method is located under the bed layout: Phthalate monoesters – glass solvent containers. Run time for this method is ~53 minutes per sample.

8.8 Turbovap Concentration
8.8.1 Vortex samples to mix thoroughly.
8.8.2 Transfer eluants to 12x75 mm glass culture tubes using glass Pasteur pipettes.
8.8.3 Evaporate to dryness under a stream of dry nitrogen (UHP grade) in a Turbovap evaporator at 55°C water bath. Note: Begin with nitrogen pressure at ~5 psi, increasing to ~15 psi as samples concentrate.
8.8.4 Add 200 µL of 6% acetonitrile/94% reagent water to resuspend residues (measured using a 20-200 µL micropipette). Note: 6% acetonitrile/94% reagent water is prepared by measuring 6 mL of acetonitrile using a graduated pipette, transferring to a 100-mL volumetric flask, and diluting to volume with reagent water. Flask is

stoppered and inverted several times to mix thoroughly. Solution is transferred to a 125-mL amber glass bottle with Teflon-lined cap and stored at room temperature.

8.8.5 Transfer to glass inserts in HPLC autosampler vials using glass Pasteur pipettes and seal with PTFE/silicone caps for LC-MS analysis.

8.8.6 Store samples in refrigerator at 4°C for up to 2 weeks prior to analysis.

8.9 Analysis:

8.9.1 HPLC conditions:

Column: Betasil Phenyl 3µm 2.1x150mm column with Betasil Phenyl 3µm 2.1x10mm guard column
Mobile Phase A: Water + 0.1% acetic acid
Mobile Phase B: Acetonitrile + 0.1% acetic acid
Detector: ESI-MS, MRM

General MS Parameters:

Parameter	Value
Scan Type	MRM
Polarity	Negative
Ion Source	Turbo Spray
Collision Gas (CAD)	5
Curtain Gas (CUR)	10
Gas 1	45
Gas 2	45
Ion Spray Voltage (IS)	-4500 V
Temperature (TEM)	450°C
Interface Heather (ihe)	On
Entrance Potential (EP)	-10 V

Compound Specific MS Parameters:

Compound	Q1 mass (amu)	Q3 mass (amu)	Dwell (ms)	DP (V)	CE (V)	CXP (V)	Internal Standard
MMP	179	77	37.5	-25	-26	-9	MMP-$^{13}C_4$
MEP	193	77	37.5	-15	-30	-11	MEP-$^{13}C_4$
MBP	221	77	37.5	-45	-26	-11	MBP-$^{13}C_4$
MCPP	251	103	37.5	-5	-12	-15	MCPP-$^{13}C_4$
MCHP	247	97	37.5	-95	-24	-15	MCHP-$^{13}C_4$
MBzP	255	183	37.5	-65	-16	-11	MBzP-$^{13}C_4$
MEHP	277	134	37.5	-45	-22	-11	MEHP-$^{13}C_4$
MEOHP	291	121	37.5	-70	-26	-7	MEOHP-$^{13}C_4$
MEHHP	293	121	37.5	-95	-26	-17	MEOHP-$^{13}C_4$
MCMHP	307	159	37.5	-40	-15	-9	MOP-$^{13}C_4$
MOP	277	125	37.5	-33	-24	-20	MOP-$^{13}C_4$
MIP	291	141	37.5	-55	-36	-30	MIP-$^{13}C_4$
MMP-$^{13}C_4$	183	79	37.5	-25	-32	-13	N/A
MEP-$^{13}C_4$	197	79	37.5	-20	-28	-11	N/A
MBP-$^{13}C_4$	225	79	37.5	-15	-28	-11	N/A
MCPP-$^{13}C_4$	255	103	37.5	-15	-14	-19	N/A
MCHP-$^{13}C_4$	251	97	37.5	-95	-24	-15	N/A
MBzP-$^{13}C_4$	259	186	37.5	-55	-16	-11	N/A
MEOHP-$^{13}C_4$	295	124	37.5	-95	-24	-21	N/A
MOP-$^{13}C_4$	281	127	37.5	-42	-25	-17	N/A
MIP-$^{13}C_4$	295	141	37.5	-55	-27	-30	N/A
MEHP-$^{13}C_4$	281	137	37.5	-80	-22	-7	N/A

Flow Rate: 0.350 mL/min
Sample Injection Volume: 10 µL
Gradient: 9.5 minute gradient from 30% B to 65% B, 0.6 minute gradient to 100% B, held at 100% B for 1.4 minutes, reversed to 30% B in 1.0 minute, held at 30% B for 6.0 minutes.
Total Run time: 18.5 minutes

8.10 Quality control

8.10.1 The following metrics are targets:

8.10.1.1 Solvent blanks and method blank - < 3 x MDL

8.10.1.2 Calibration checks: ± 15% of nominal value

8.10.1.3 Method control and fortified samples – spike recovery ± 30% of nominal value

8.10.1.4 Duplicate analyses (injections) should be performed for at least one sample in each batch. Study data quality objectives for precision are ± 15% for intralaboratory analyses.

8.10.2 Batches that meet all QC criteria described above are automatically accepted. Batch data that do not meet the criteria must be approved or rejected by the project manager or PI.

8.11 Data reporting

8.11.1 Data will be exported from the Analyst software to Excel format and delivered to project management electronically. No operator calculations will be performed.

9.0 Method Performance including MDLs

9.1 MDLs will be created by spiking urine at a concentration near the lowest level of the calibration curve. Seven aliquots will then be transferred to separate vials, extracting and analyzing each sample according to the method above in section 8.

9.2 Calculate the mean, standard deviation and RSD for all seven replicates.

9.3 The MDL will be calculated by multiplying the standard deviation by 3.143 to get the value in ng units for each analyte.

10.0 Records Management

10.1 Following review by the analyst, instrument analytical data will be exported to a Microsoft Excel file format and sent to the CHATS organics manager for review.

10.2 All raw instrument data is to be maintained on the instrument or saved to the CHATS share directory until completion of the project.

11.0 References

NHANES Method for Phthalate Monoesters in Urine (2001-2002), Dana Barr, Centers for Disease Control (CDC), National Center for Environmental Health, Division of Laboratory Sciences, Toxicology Branch

NHANES Method for Phthalate Metabolites in Urine (2007-2008), Method No. 6306.03, Revised July 3, 2010, Antonia Calafat, Centers for Disease Control (CDC), National Center for Environmental Health, Division of Laboratory Sciences, Organic Analytical Toxicology Branch, Personal Care Products Laboratory

Research Operating Procedure EAR-CHATS-23

Determination of VOC Metabolites in Urine

for

Children's Health after the Storms (CHATS)

Prepared by: Michael S. Gardner Date: 12/28/2012

Reviewed by: Cynthia Ann Salmons Date: 2/8/2013

Reviewed by: James W. Rogers Date: 2/26/2013

Approved by: Brian K. Wagoner Date: 2/27/2013

RTI International
Exposure Analysis Research
3040 Cornwallis Road
Research Triangle Park, NC 27709

List of Revisions

Revision Number	Changes	Date
0	Original from RTI	

Table of Contents

1.0 Scope & Application 4
2.0 Summary of Method 5
3.0 Definitions 5
4.0 Cautions 5
5.0 Interferences 5
6.0 Apparatus & Materials 6
7.0 Personnel Qualifications 6
8.0 Procedures 6
9.0 Method Performance including MDLs 10
10.0 Records Management 10
11.0 References 11

1.0 Scope & Application

The analytical procedures described in this protocol are intended for the determination of selected VOC metabolites from urine samples that will be collected as part of the Children's Health after the Storms (CHATS) Study. This protocol addresses:

- Preparation of urine samples by dilution
- Analysis of urine samples by ultra high performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UHPLC-ESI-MS/MS)
- Laboratory quality control (QC) procedures
- Data processing and documentation

List of Target Analytes:

Analyte name	Analyte acronym
N-Acetyl-S-(1,2-dichloroethenyl)-L-cysteine	12DCVMA
N-Acetyl-S-(2,2-dichloroethenyl)-L-cysteine	22DCVMA
N-Acetyl-S-(2,4-dimethylbenzene)-L-cysteine	24DPMA
N-Acetyl-S-(2,5-dimethylbenzene)-L-cysteine	25DPMA
N-Acetyl-S-(3,4-dimethylbenzene)-L-cysteine	34DPMA
2-Methylhippuric acid	2MHA
3-Methylhippuric acid	3MHA
4-Methylhippuric acid	4MHA
N-Acetyl-S-benzyl-L-cysteine	BMA
N-Acetyl-S-(2-carboxyethyl)-L-cysteine Bis(dicyclohexylamine) Salt	CEMA
N-Acetyl-S-(2-cyanoethyl)-L-cysteine	CYMA
N-Acetyl-S-(3,4-dihydroxybutyl)-L-cysteine (mixture of diastereomers)	DHBMA
N-Acetyl-S-(2-hydroxy-3-pripionamide)-L-cysteine Dicyclohexylammonium Salt	GAMA
Hippuric acid	HA
N-Acetyl-S-(2-hydroxyethyl)-L-cysteine Dicyclohexylammonium Salt	HEMA
N-Acetyl-S-(3-hydroxyropyl-1-methyl)-L-cysteine Dicyclohexylammonium Salt (mixture of diastereomers)	HPMMA
(R)-(-)-Mandelic Acid	MA
(R,S)-N-Acetyl-S-[1-(hydroxymethyl)-2-propen-1-yl]-L-cysteine	MHBMA1+2
(R,S)-N-Acetyl-S-(2-hydroxy-3-buten-1-yl)-L-cysteine	MHBMA1+2
trans, trans-Muconic acid	MU
Phenylglyoxylic acid	PGA
N-Acetyl-S-(2-hydroxy-1-phenylethyl)-L-cysteine	PHEMA
N-Acetyl-S-(2-hydroxy-2-phenylethyl)-L-cysteine	PHEMA
S-Phenylmercapturic Acid	PMA

2.0 Summary of Method

The method is adapted from a procedure from the Centers for Disease Control and Prevention (CDC), developed by K. Udeni Alwis et.al. Urine samples are diluted tenfold and filtered. The VOC metabolites are then chromatographically resolved by reversed phase ultrahigh performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UHPLC-ESI-MS/MS) and quantified by isotope dilution. QC samples include reagent blanks, reagent controls, matrix blanks, matrix spikes, and calibration checks. The HPLC is calibrated using a minimum of a six-point standard curve. Chromatograms are processed using Analyst 1.4.2 data system and data are output as Microsoft Excel Spreadsheets (*.xls). After QA review of the individual data files, data will be uploaded electronically into the study database from the output files using FileZilla.

3.0 Definitions

None

4.0 Cautions

The analyst is responsible for maintaining awareness of OSHA regulations regarding the safe handling of chemicals used in this method. The toxicity and carcinogenicity of chemicals used in this method have not been precisely defined; therefore, each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Appropriate care should be exercised in handling extracts, reagents, and solvents. All solvents, pure standard materials and stock standard solutions of target compounds should be handled exclusively in a chemical fume hood. Personal protective equipment (gloves, lab coat and eye protection) appropriate for handling hazardous materials should be worn. Exercise caution in the handling of biological samples. Observe universal precautions: wear safety glasses, protective gloves, and lab coat during all steps of this method because of both infectious and chemical related hazards. The Hepatitis B vaccination series is strongly recommended for all testing personnel. Laboratory personnel handling human fluids and tissues are required to take Bloodborne Pathogens training.

5.0 Interferences

5.1 The extent of interferences may vary considerably from sample to sample. Interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baselines in chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences by analysis of blanks.

5.2 Carryover contamination may occur when a sample containing low concentrations of compounds is analyzed immediately after a sample containing relatively high concentrations of similar compounds. The wash steps employed in the instrument method are designed to minimize carryover. Carryover must be measured by analyzing a blank sample immediately following a high standard.

6.0 Apparatus & Materials

6.1 Individual native standards (VOC metabolites) and internal standards (13C4-labeled phthalate monoesters) are purchased from Toronto Research Chemicals (North York, Ontario, Canada) or Sigma-Aldrich (St. Louis, MO)
6.2 Acetonitrile, High Purity, Honeywell (B&J) 015-4
6.3 Water, High Purity, Honeywell (B&J) 365-4
6.4 Methanol, High Purity, Honeywell (B&J) 365-4
6.5 Isopropanol, High Purity, Honeywell (B&J) 365-4
6.6 Ammonium acetate, ACS reagent grade, BDH0204-500G (VWR, Suwanee, GA)
6.7 Top loading precision balance (3 decimal places), Mettler Toledo PR1203
6.8 Male human urine, unfiltered, Bioreclamation HMURINE-M
6.9 Micropipettes, 100- and 1000-µL volumes (Eppendorf Reference)
6.10 Disposable centrifuge tubes, 2-mL, polypropylene
6.11 Centrifugal filters: Millipore Durapore PVDF 0.1µm
6.12 Disposable culture tubes, 13x100 mm, borosilicate glass, VWR 47729-572
6.13 Disposable culture tubes, 16x100 mm, borosilicate glass, VWR 47729-576
6.14 AB Sciex API-5000 Triple Quadrupole Mass Spectrometer with Waters Acquity UHPLC system.
6.15 Waters Acquity HSS T3 1.8µm 2.1x150mm column with Waters Acquity HSS T3 1.8µm 2.1x5mm guard column

7.0 Personnel Qualifications

Personnel should read the ROP carefully and have this documented by the laboratory supervisor in their training file. All staff performing this method will have demonstrated proficiency by recovering 70% - 130% of target analytes, spiked into urine at 1x – 5x the method lower limit of quantitation (LLOQ), for each of two duplicate samples.

8.0 Procedures

8.1 Standards
- 8.1.1 Stock solutions are prepared by solvating in an appropriate solvent, quantitatively transferring to a volumetric flask and bringing to volume, weighing the original container before and after (dried) to determine the weight by difference. This stock solution is stored at –20°C in a plastic vial.
- 8.1.2 Internal standards (13C4-labeled phthalate monoesters) are prepared similarly to the native standards and stored sealed at –20°C until use. The isotopic purity of each internal standard is confirmed empirically.
- 8.1.3 Eight unique working standards with all analytes were prepared in water from the stock solutions of native standards to cover the linear range of the assay for each analyte.

8.2 A minimum sample volume of 50µL is required for the assay. Specimens may be stored in plastic cryovials as long as the vials are tightly sealed to prevent desiccation of the sample. Urine samples received for analysis will be logged in and stored in a freezer at until extraction. Specimens may be stored frozen at approx. –20°C to –70°C for one year prior to analysis.

8.3 Samples will be analyzed in batches of up to 12 study samples, plus laboratory quality control samples. Analysis of each batch will be documented in a laboratory notebook.

8.4 Reagent Preparation

8.4.1 Solvents for LC-MS

8.4.1.1 "Mobile Phase A" (15 mM ammonium acetate in water) is prepared by weighing 1.156g of ammonium acetate and dissolving in 1000mL water. A different volume may be prepared as long as the specified proportion of components is maintained. Store at room temperature in amber glass and ***discard after one month***. Note: This is also used as the sample dilution solvent.

8.4.1.2 "Mobile Phase B" (Acetonitrile) is used directly without further preparation. Store at room temperature.

8.4.1.3 Weak Wash" (Water) is used directly without further preparation. Store at room temperature in amber glass.

8.4.1.4 "Strong Wash" (1:1:1:1 Methanol : Acetonitrile : Water : Isopropanol) is prepared by measuring separately 250mL of each of the four solvents and mixing together in a 1L glass bottle by agitation. Store at room temperature.

8.5 Sample Preparation Procedure

8.5.1 To labeled sample containers, add the appropriate amount of 15mM ammonium acetate diluent (500µL for blank and filter blank, 475µL for solution control, 425µL for all others). Standards, QCs and blanks are directly prepared in autosampler vials. Urine samples, QCs and a filter blank are initially prepared in 1.5mL polypropylene tubes prior to filtration as given below.

8.5.2 Add 25µL ISWS, except to the blank and filter blank (FBLK).

8.5.3 For the standards and QCs, add 50µL of the appropriate spiking solution.

8.5.4 For the urine samples, add 50µL of the appropriate sample.

8.5.5 Vortex all containers to mix.

8.5.6 For urine samples, QCs and filter blank, filter with 0.1 uM Millipore microcentrifuge filters at 16000 RCF for 5 min., then transfer to labeled autosampler vials.

8.6 Analysis:

8.6.1 HPLC conditions:

Column: Waters Acquity HSS T3 1.8µm 2.1x150mm column with Waters Acquity HSS T3 1.8µm 2.1x5mm guard column
Mobile Phase A: 15mM ammonium acetate in water
Mobile Phase B: Acetonitrile
Detector: ESI-MS, MRMGeneral MS Parameters:

Parameter	Value
Scan Type	MRM
Polarity	Negative
Ion Source	Turbo Spray
Collision Gas (CAD)	5
Curtain Gas (CUR)	10
Gas 1	50
Gas 2	50
Ion Spray Voltage (IS)	-4500 V
Temperature (TEM)	650°C
Interface Heather (ihe)	On
Entrance Potential (EP)	-10 V
Declustering Potential (DP)	Optimized for compound
Collision Energy (CE)	Optimized for compound
Collision Cell Exit Potential (CXP)	Optimized for compound
Dwell Time	15 ms per transition

Compound Specific MS Parameters:

Analyte(s)	MRM Transition (Q1/Q3, amu)	Internal Standard
12DCVMA	257.02/127.00	12DCVMA-13C-d3
22DCVMA	256.92/126.80	12DCVMA-13C-d3
24DPMA+25DPMA+34DPMA*	266.12/137.20	24DPMA-d3
2MHA	191.96/148.00	12DCVMA-13C-d3
3MHA + 4MHA*	191.96/148.00	12DCVMA-13C-d3
BMA	252.03/122.80	BMA-d3
CEMA	234.02/162.00	CEMA-d3
CYMA	215.04/85.90	CYMA-d3
DHBMA	250.10/121.00	DHBMA-d7
GAMA	249.20/119.90	GAMA-d3
HA	177.96/77.00	12DCVMA-13C-d3
HEMA	206.25/77.00	HEMA-d4
HPMMA	234.03/104.90	HPMMA-d3
MA	150.94/107.20	CYMA-d3
MHBMA1+2*	232.00/103.00	MHBMA1+2-d6
MU	140.91/97.00	MU-d4
PGA	148.97/77.00	PMA-d5
PHEMA	282.23/152.80	PMA-d5
PMA	238.07/109.00	PMA-d5
CEMA-d3	237.05/165.00	N/A
GAMA-d3	252.05/119.90	N/A
CYMA-d3	218.01/85.90	N/A
PMA-d5	243.04/114.10	N/A
MU-d4	145.02/101.10	N/A
DHBMA-d7	257.20/128.10	N/A
HPMMA-d3	236.99/104.90	N/A
12DCVMA-13C-d3	261.06/127.00	N/A
BMA-d3	255.28/122.80	N/A
HEMA-d4	209.94/80.80	N/A
24DPMA-d3	269.02/137.20	N/A
MHBMA1+2-d6*	238.07/109.00	N/A

*Mixture quantitated as single component.

Sample Injection Volume: 10 µL
Flow Rate, Gradient and Run Time (Table):

Time (min)	Flow Rate (mL/min)	% B
0.0	0.250	3.0
2.0	0.250	5.0
3.0	0.300	10.0
5.0	0.300	30.0
6.5	0.300	40.0
7.5	0.300	100
8.0	0.300	100
8.5	0.300	3.0
11.5	0.300	3.0

8.7 Quality control
- 8.7.1 The following metrics are targets:
  - 8.7.1.1 Solvent blanks and method blank - < 3 x MDL
  - 8.7.1.2 Calibration checks: ± 15% of nominal value
  - 8.7.1.3 Method control and fortified samples – spike recovery ± 30% of nominal value
- 8.7.2 Duplicate analyses (injections) should be performed for at least one sample in each batch. Study data quality objectives are ± 15% for intralaboratory precision. Batches that meet all QC criteria described above are automatically accepted. Batch data that do not meet the criteria must be approved or rejected by the project manager or PI.

8.8 Data reporting
- 8.8.1 Data will be exported from the Analyst software to Excel format and delivered to project management electronically. No operator calculations will be performed.

9.0 Method Performance including MDLs

9.1 MDLs will be created by spiking urine at a concentration near the lowest level of the calibration curve. Seven aliquots will then be transferred to separate vials, prepared and analyzing each sample according to the method above in section 8.
9.2 Calculate the mean, standard deviation and RSD for all seven replicates.
9.3 The MDL will be calculated by multiplying the standard deviation by 3.14 to get the value in ng units for each analyte.

10.0 Records Management

10.1 Following review by the analyst, instrument analytical data will be exported to a Microsoft Excel file format and sent to the CHATS organics manager for review.

10.2 All raw instrument data is to be maintained on the instrument or saved to the CHATS share directory until completion of the project.

11.0 References

"Simultaneous analysis of 28 urinary VOC metabolites using ultra high performance liquid chromatography coupled with electrospray tandem mass spectrometry (UPLC-ESI/MSMS)", K. Udeni Alwis, Benjamin C. Blount, April N. Sheppard, and David L. Ashley; Centers for Disease Control (CDC), National Center for Environmental Health