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Best practices for biomarker collection, analysis, and interpretation:

Perspectives from EPA's Chemical Safety for Sustainability (CSS) research program

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Human Exposure and Atmospheric Sciences Division

Presentation outline

- EPA labs, centers, and research programs
- “Biomarkers” research projects
  - Better uses of existing data
    - Computational case studies
  - Better collection of new data
    - Biomonitoring field studies
  - Take-home points

The EPA in Research Triangle Park, NC

ORD Research Laboratories:
NERL: Exposure Lab
NHEERL: Effects Lab
NRMRL: Engineering Lab

ORD Research Centers:
NHSRC: Homeland Security
NCEA: Environmental Assessment
NCCT: Computational Toxicology
NCER: Extramural Research

What's happening in ORD?

- ORD performs research to support regulatory decisions/actions

- Research programs:
  - ACE (Air, Climate, and Energy)
  - CSS (Chemical Safety for Sustainability)
  - SHC (Sustainable and Healthy Communities)
  - SSWR (Safe and Sustainable Water Resources)
  - Homeland Security Research
  - Human Health Risk Assessment

- Focus on integration, innovation, and sustainability

Biomarkers research in CSS

- Project 1: Defining best practices for interpreting existing biomarker data via computational case studies
  - Goal 1: review the uses of existing data
  - Goal 2: identify data gaps and challenges
  - Goal 3: propose new methods and best practices

- Project 2: Studies to identify, measure, and evaluate biomarkers of exposure and effect
  - Goal 1: identify new biomarkers
  - Goal 2: collect targeted data for model evaluation
  - Goal 3: use new data for model development/refinement

Project 1 team members

NERL

- Cecilia Tan
- Joachim Pleil
- Martin Phillips
- Seungho Lee
- Elin Ulrich
- Jon Sobus

NCEA

- Krista Christensen
- Rob Dewoskin

NHEERL

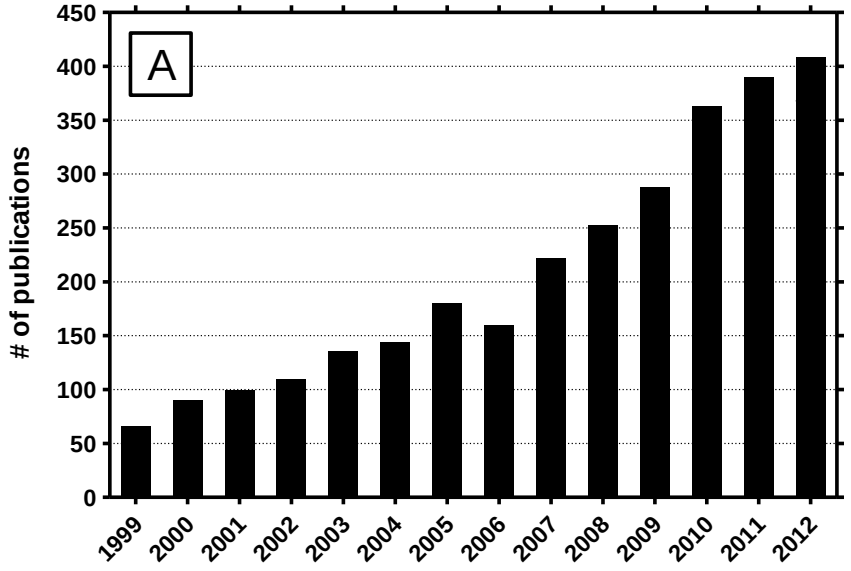
- Stephen Edwards
- Dina Schreinemachers
- Rory Conolly
- Shannon Bell
- BJ George
- Judy Schmid
- *Marc Williams*

NCCT

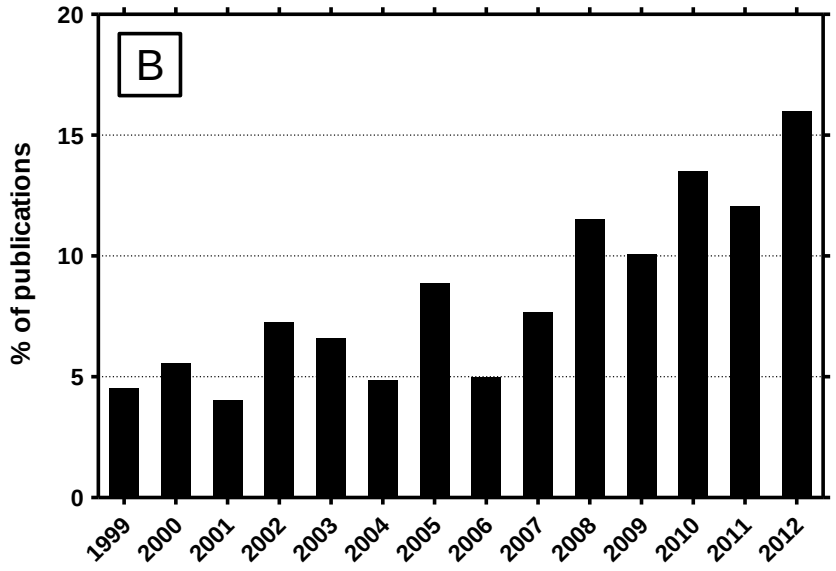
- Elaine Cohen-Hubal

What biomarker data are used?

Number of NHANES publications

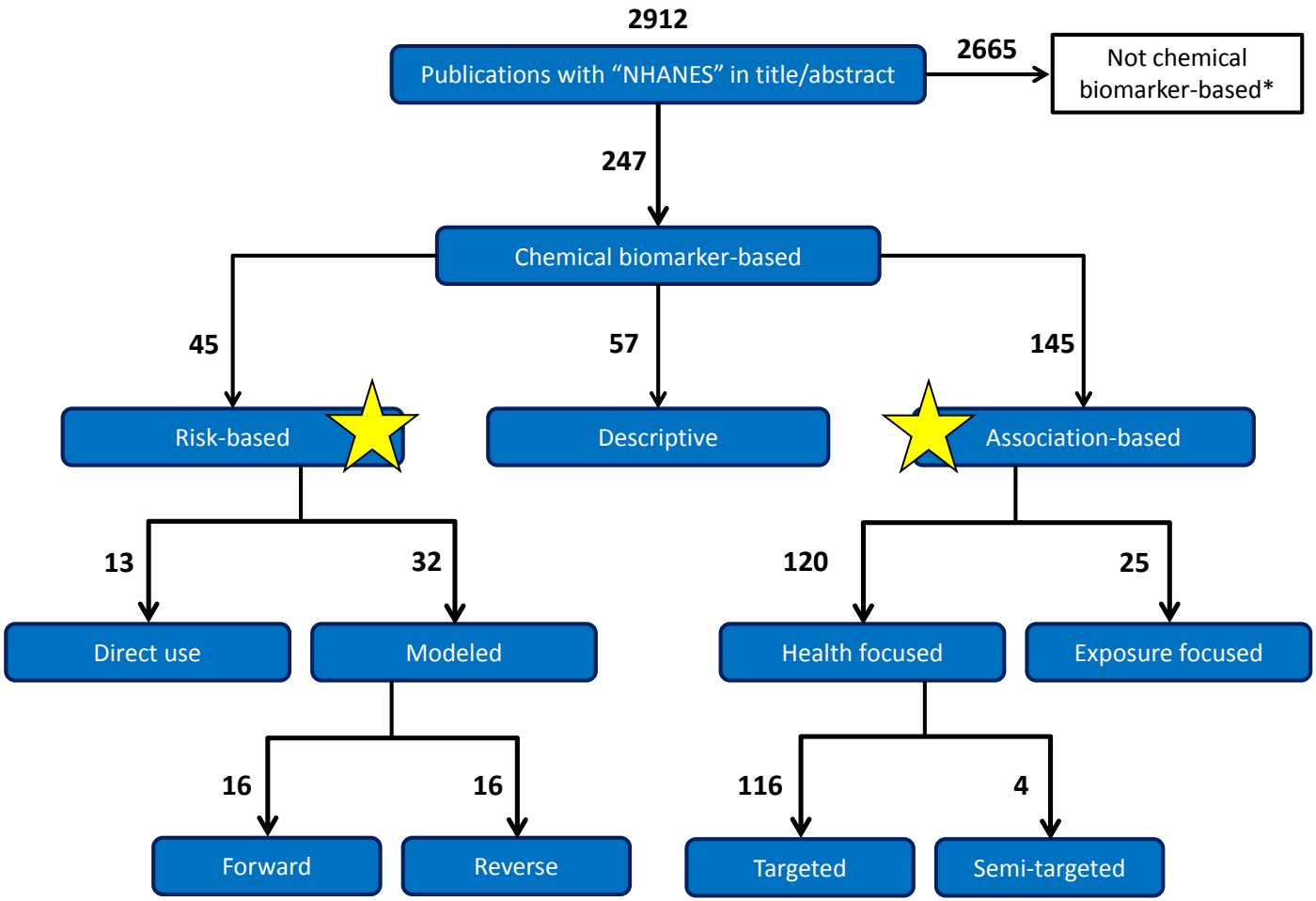


Percentage using biomarkers of environmental chemicals



Increasing use of NHANES biomarker data for environmental health research

How are the data being used?



Case study 1
(association-based)

"Changes in epidemiologic associations with different exposure metrics: A case study of phthalate exposure associations with body mass index and waist circumference"

K. Christensen, J. Sobus, M. Phillips, T. Blessinger, M. Lorber, and Y.M. Tan, submitted to *Environ. Int.*

Challenge: different exposure metrics produce different results in epidemiology studies

Research question: what are the best practices in selecting an exposure metric?

Approach:

1) evaluate NHANES associations using different exposure metrics
2) simulate random exposures and evaluate using different metrics
3) compare simulation results to NHANES results

Results from NHANES 2009-2010

Adjusted regression coefficients for effect of phthalate levels on ln(Body Mass Index). All models adjusted for age, sex, race/ethnicity, height, and PIR. Results presented for models treating phthalate exposures as ln-transformed variables.

	Outcome is ln(Body Mass index)				
Phthalate	nmole/min: β (SE),	nmole/mL: β (SE),	nmole/mL + crt: β (SE),	nmole/g crt: β (SE),	nmole/kg-day: β (SE),
DBP	**0.022 (0.005)****	**0.023 (0.004)*****	**0.014 (0.006)***	0.007 (0.006)	**0.040 (0.006)******
BBzP	**0.019 (0.005)****	**0.021 (0.004)*****	**0.011 (0.005)***	0.006 (0.006)	**0.033 (0.006)*****
DEHP[a]	**0.019 (0.005)****	**0.025 (0.004)*****	**0.017 (0.005)***	0.008 (0.006)	**0.033 (0.005)*****
DiNP	**0.020 (0.004)*****	**0.023 (0.004)******	**0.017 (0.004)****	**0.013 (0.004)***	**0.028 (0.004)******
DiBP	**0.022 (0.005)****	**0.025 (0.005)*****	**0.014 (0.006)***	0.003 (0.007)	**0.045 (0.007)******
DEP	**0.013 (0.004)****	**0.016 (0.003)****	**0.010 (0.004)***	0.005 (0.004)	**0.018 (0.004)****

[a]Represents the molar sum of 4 DEHP metabolites (MEHP, MEHHP, MEOHP, MECPP)

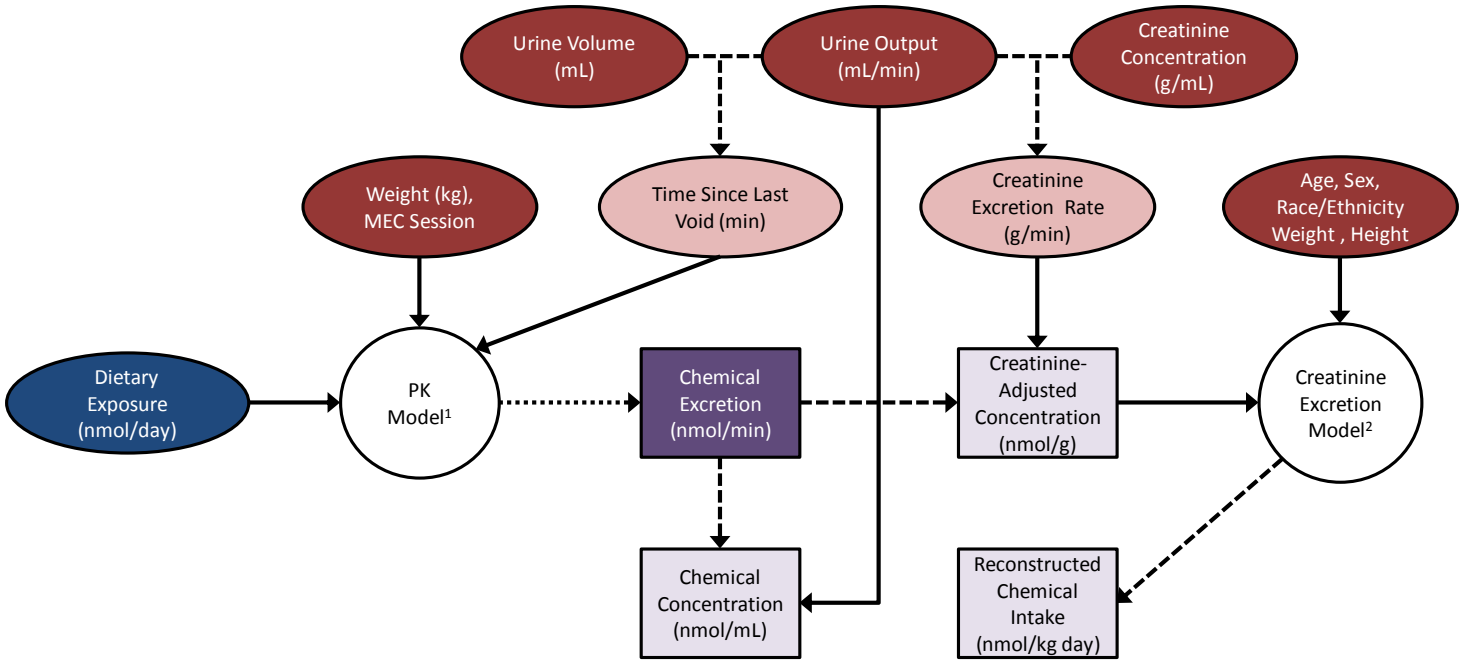
* $p < 0.05$

** $p < 0.001$ (1×10^{-3})

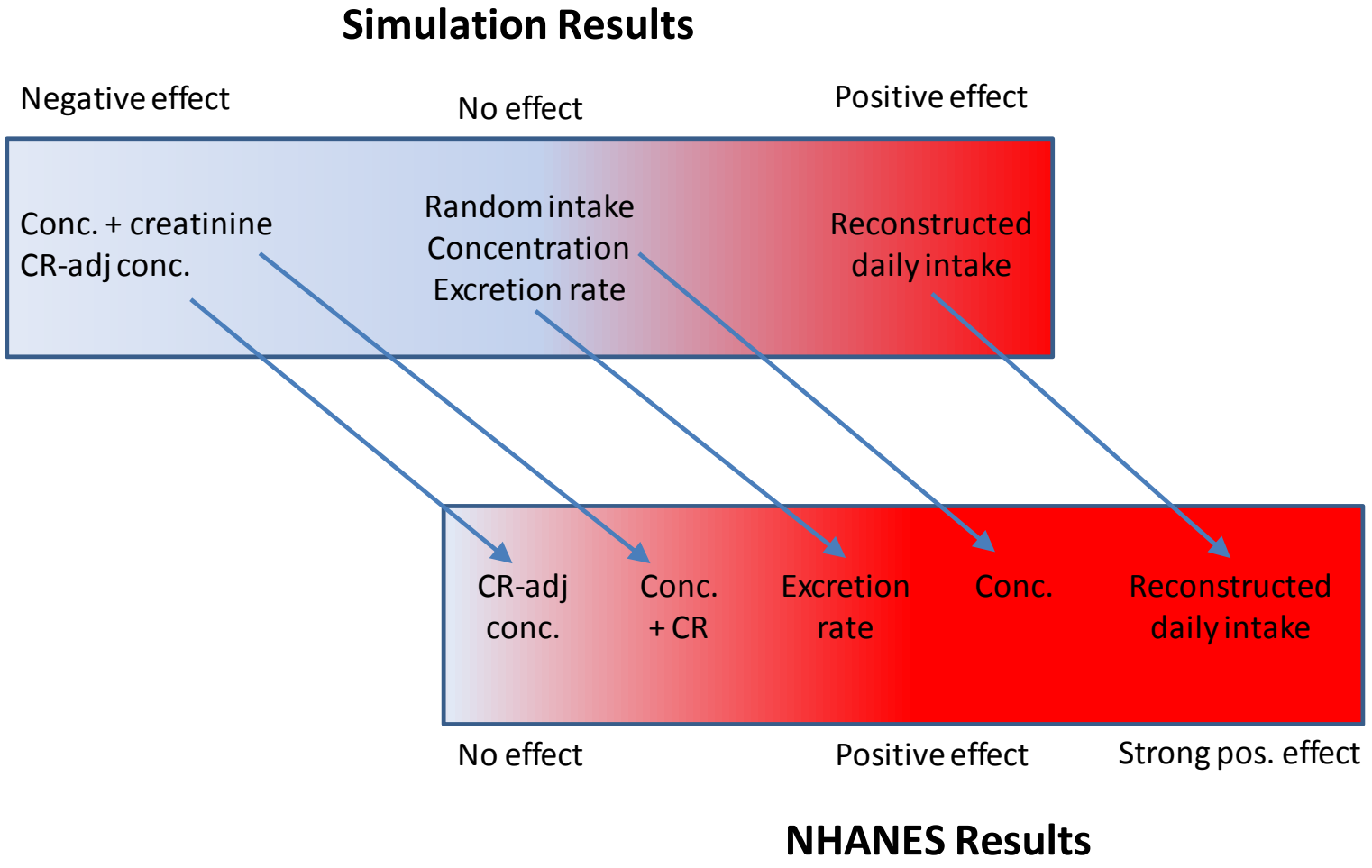
*** $p < 0.000001$ (1×10^{-6})

**** $p < 0.000000001$ (1×10^{-9})

Exposure simulation



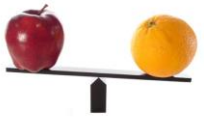
Results comparison



Case study 2
(risk-based)

“Estimating lifetime risk from spot biomarker data and intraclass correlation coefficients (ICC)”

J. Pleil and J. Sobus, *Journal of Toxicology and Environmental Health, Part A*, 76:747–766, 2013



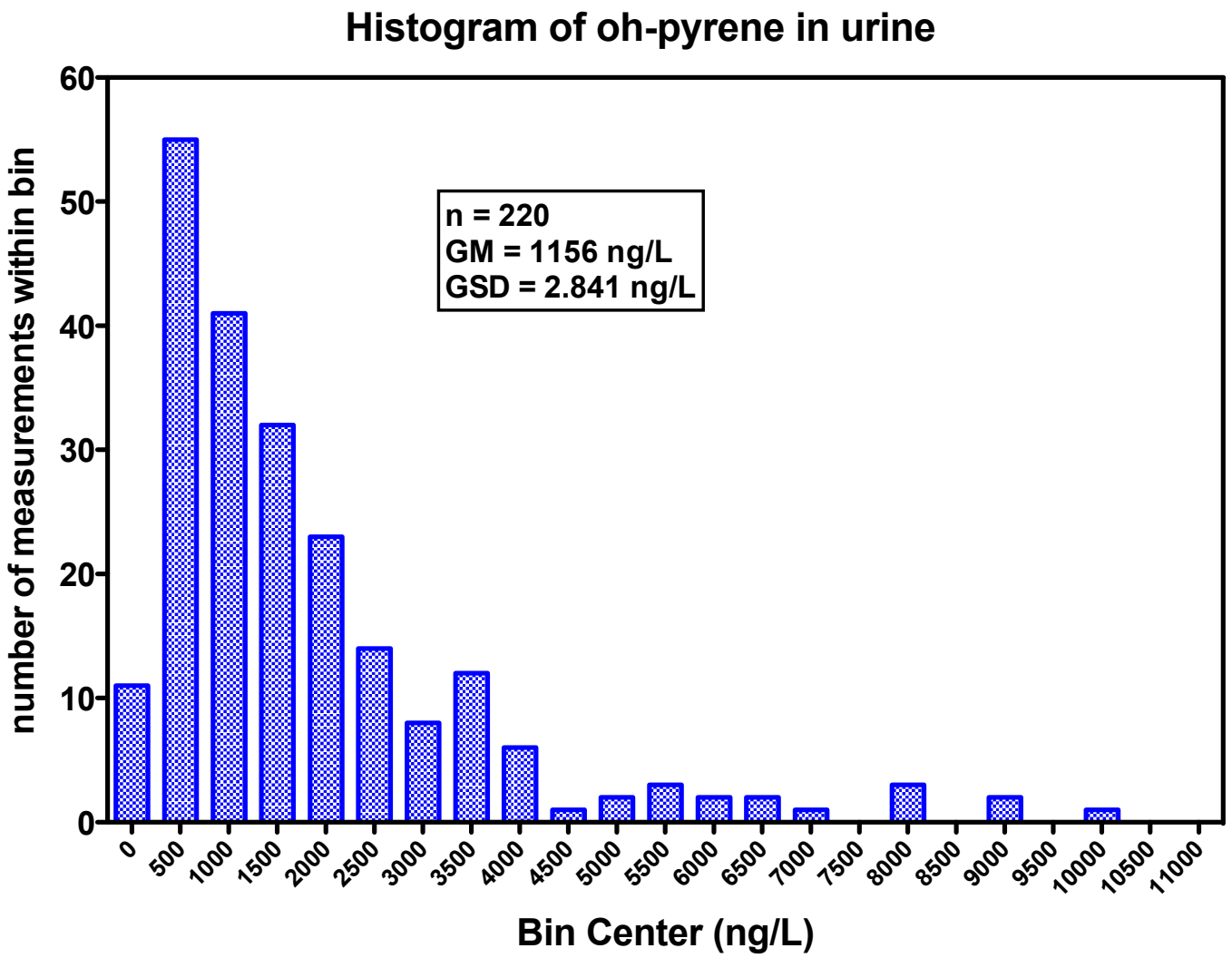
Challenge: “Spot” data are compared to ref. levels based on long-term exposure

Research question: What % of the pop. has long-term exposure above a ref. level?

Approach:

1) develop approach for converting dist. of spots to dist. of averages
2) calculate population exceedance above ref. level
3) develop tool for rapid calculations across chemicals

“Spot” measurements

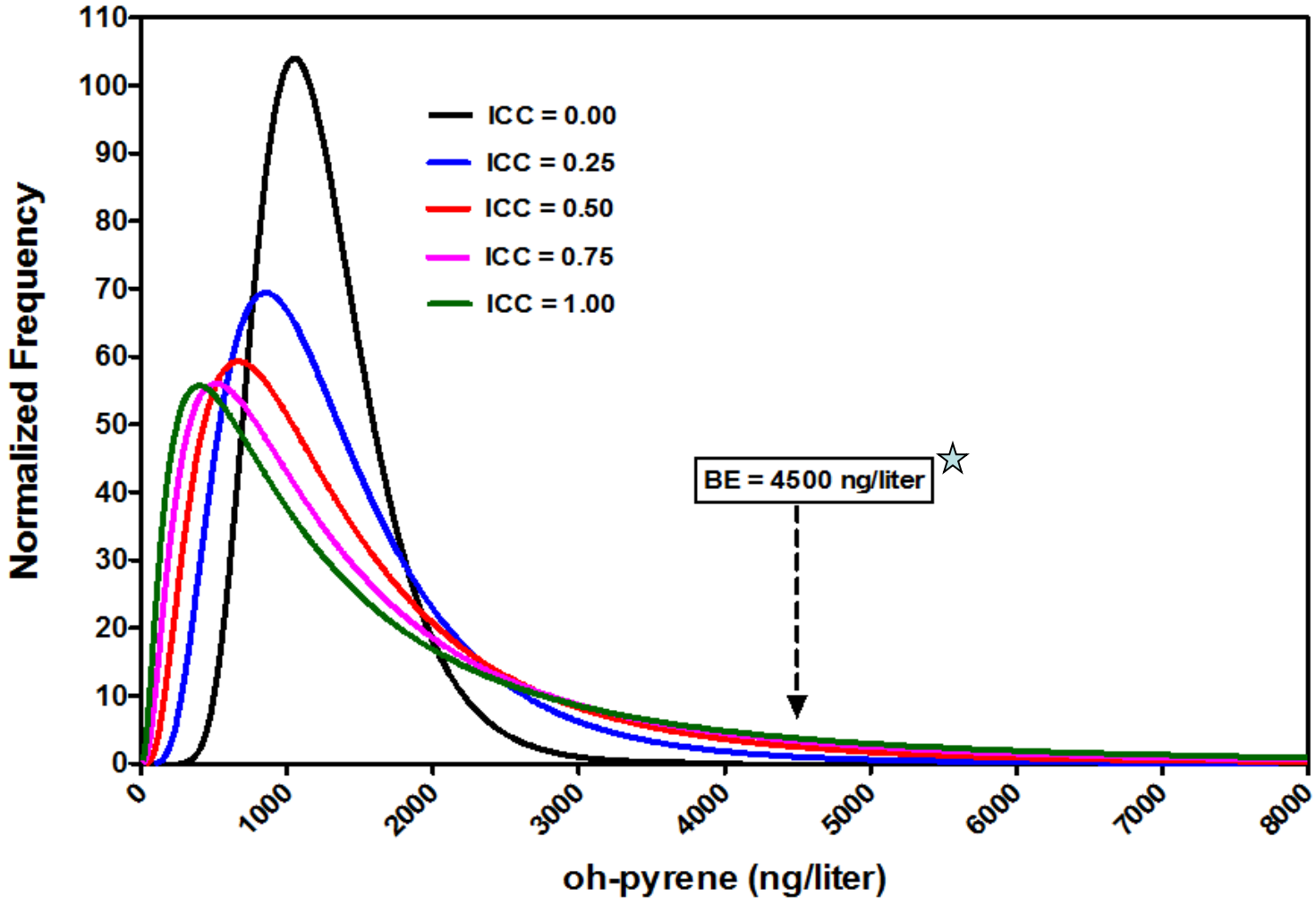


Pleil and Sobus, *J Toxicol Environ Health Part A*, 76:747–766, 2013

Using ICC to predict averages

- ICC = $\sigma_b^2 / (\sigma_b^2 + \sigma_w^2)$
- ICC has a possible range from 0 to 1
- If repeat measures are spread across the overall distribution:
  - σ_b^2 is ~ 0 (very small "between-subject" variance)
  - ICC is ~ 0
- If repeat measures are all approximately the same:
  - σ_w^2 is ~ 0 (very small "within-subject" variance)
  - ICC is ~ 1

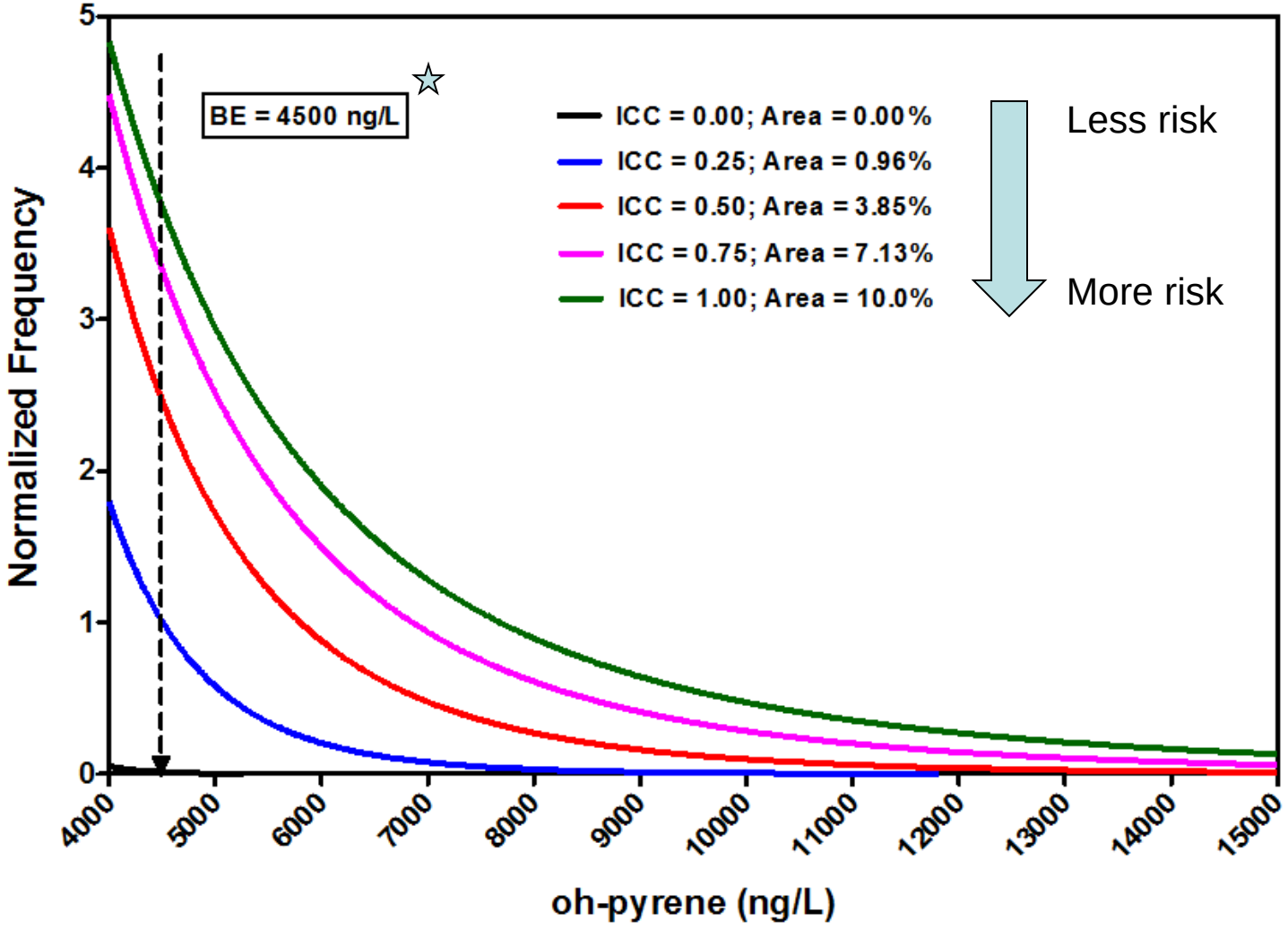
Predicted distributions of averages



Pleil and Sobus, *J Toxicol Environ Health Part A*, 76:747–766, 2013

☆ *Not a calculated "BE". Used here for illustrative purposes.*

Calculated exceedance



Pleil and Sobus, *J Toxicol Environ Health Part A*, 76:747–766, 2013

☆ *Not a calculated "BE". Used here for illustrative purposes.*

Other challenges and studies

- Association-based studies:
  - Challenge: limited standards for analysis and reporting
  - Study:

    "A Proposal for Assessing Study Quality: Biomonitoring, Environmental Epidemiology, and Short-Lived Chemicals (BEES-C) Instrument"

    J. LaKind, J. Sobus, M. Goodman, D. Barr, P. Fürst, R. Albertini, T. Arbuckle, G. Schoeters, Y.M. Tan, J. Teeguarden, R. Tornero-Velez, C. Weisel, submitted to *Environ. Int.*

  - Challenge: one chemical or outcome at a time
  - Study:

    "Building associations between markers of environmental stressors and adverse human health impacts using frequent itemset mining"

    S. Bell, S. Edwards**,** *Proceedings of the 2014 SIAM International Conference on Data Mining*

Other challenges and studies

- Risk-based studies:
  - Challenges: no evaluation at individual subject level
  - Study:

    “A New Method for Generating Distributions of Biomonitoring Equivalents to Support Exposure Assessment and Prioritization”

    M. Phillips, J. Sobus, B.J. George, K. Isaacs, R. Conolly, Y.M. Tan, submitted to *Regulatory Toxicology and Pharmacology*

The Exposure Reconstruction "Ex-R" Study

Major Objectives:

- To assess variability in urinary pyrethroid metabolite levels in non-occupationally exposed adults over a six-week period of time

- To estimate exposures and absorbed doses of selected pyrethroids for study participants by the ingestion route of exposure using an exposure reconstruction approach

Ex-R study contributors

- PI: Marsha Morgan

- Field team:
  - Lillian Alston
  - Erik Andersen
  - Jim Baugh
  - Fu-Lin Chen
  - Scott Clifton
  - Louis DeLaine
  - Jon Sobus
  - Richard Walker
  - Andrea Ware

- Analytical team:
  - Erik Andersen
  - Dana Barr
  - Carry Croghan
  - Candice Cunningham
  - Joe Evans
  - Paul Jones
  - John Kenneke
  - Denise MacMillan
  - Joachim Pleil
  - Jon Sobus
  - Jim Starr
  - Matthew Stiegel

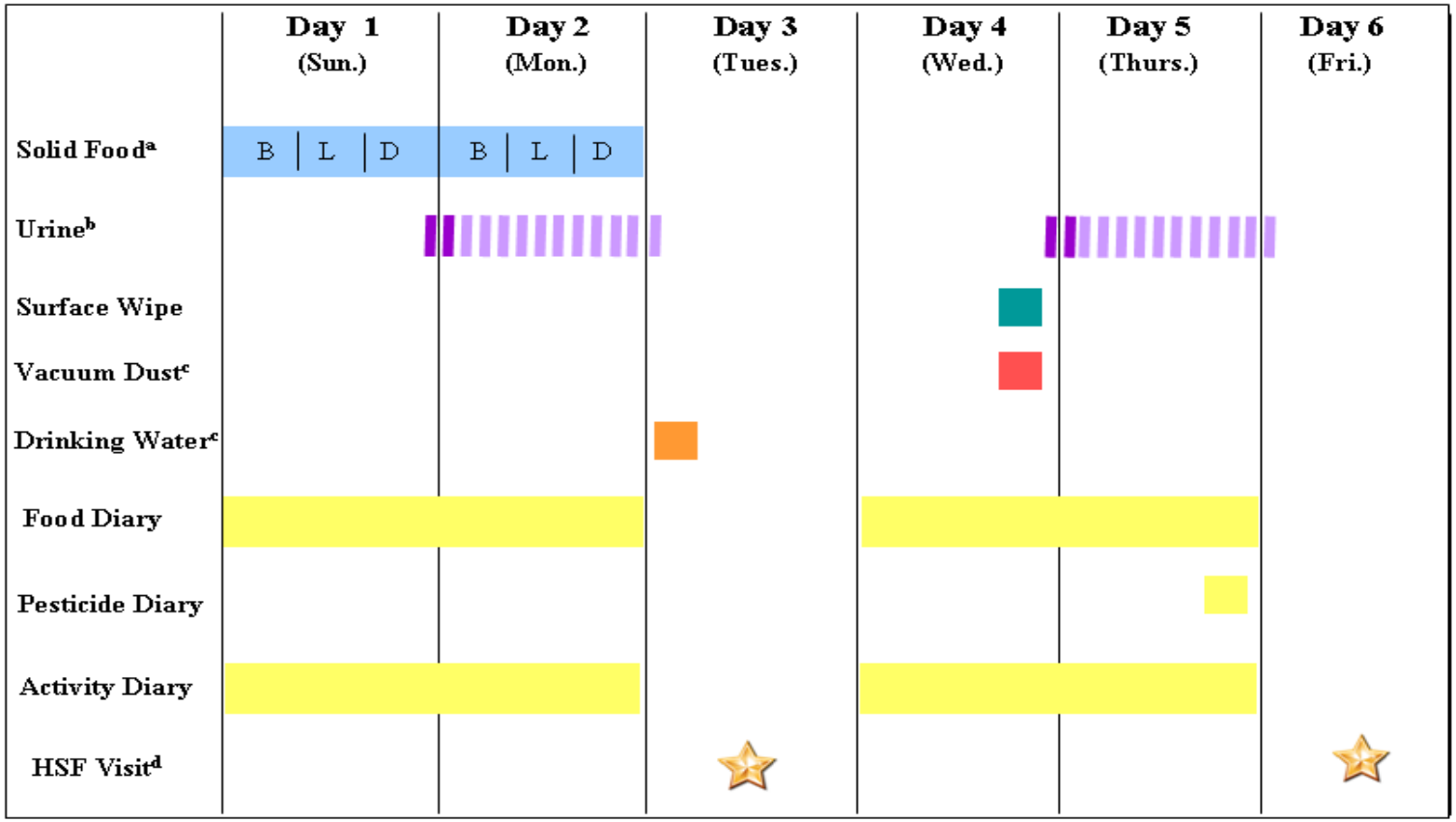
- Management team:
  - Roy Fortmann
  - Linda Sheldon
  - Kent Thomas
  - Donald Whitaker
  - Ronald Williams

- QA team:
  - Elizabeth Betz
  - Sania Tong-Argao

Study information

- Location: US EPA's Human Studies Facility in Chapel Hill, NC and participant's homes w/in a 40-mile radius of this facility.
- Study population: 50 adults (18 to 50 years old)
- Participation: 6-week monitoring period
- Diaries & questionnaires: food, activities, and pesticide-use
- Multimedia samples: solid food, drinking water, surface wipe, dust, and urine
- Sample analysis (primary):
  - Environmental: pyrethroids and metabolites
  - Urine: pyrethroid metabolites
- Field sampling duration: Nov 2009 – May 2011

Participant weekly schedule



[a]B, L, D equal breakfast, lunch, and dinner respectively.
[b]A bedtime void (dark purple), a FMV (dark purple), and a 24-h void (light purple) was collected on days 1-3 and days 4-6 each sampling week.
[c]Drinking water and vacuum dust samples were collected only on day 3 and day 4, respectively, of the last sampling week (week 6).
[d]Participants dropped off coolers containing study items on day 3 or day 6, respectively, of each sampling week at the Human Studies Facility (HSF).

Portable thermoelectric coolers

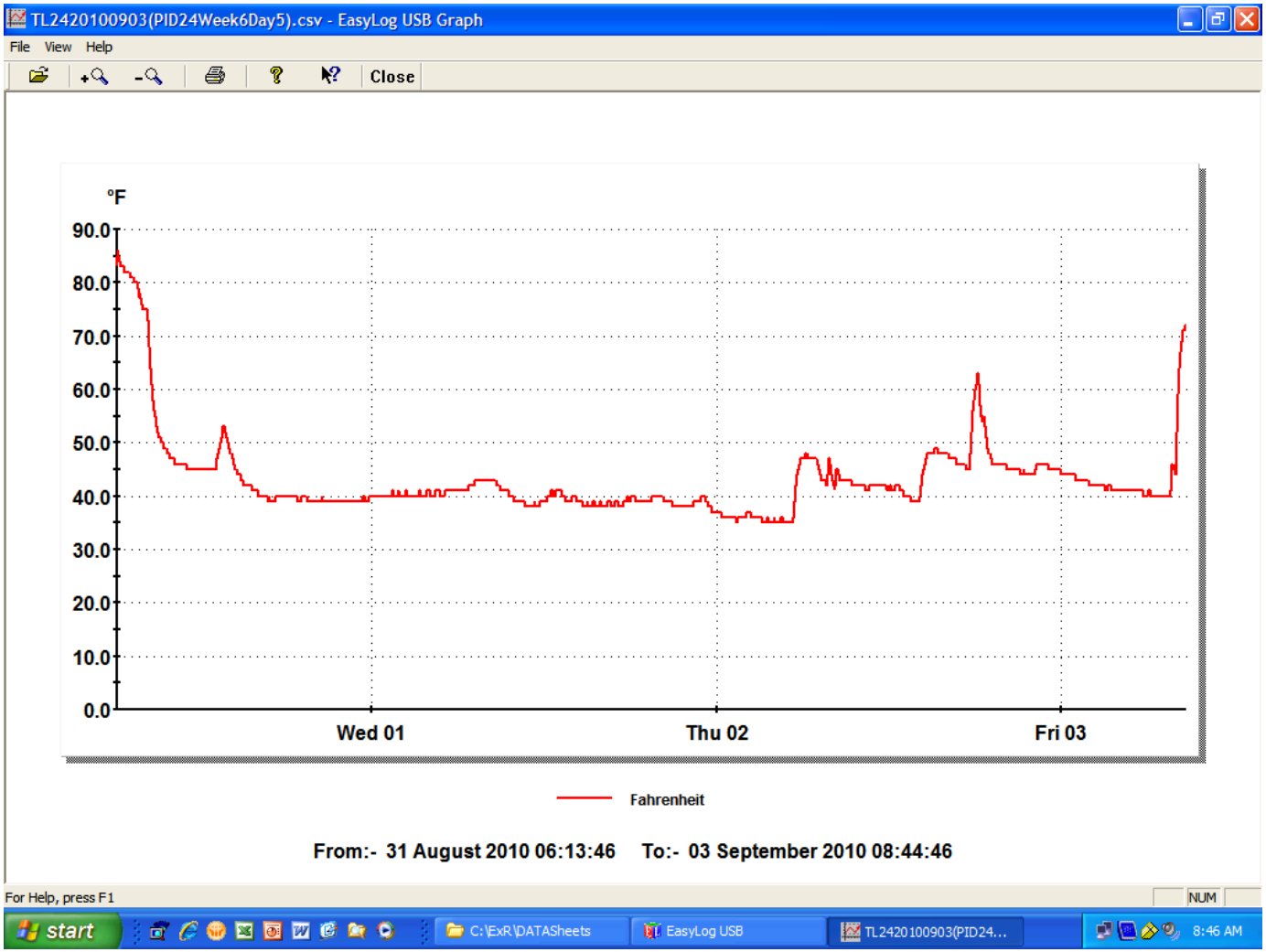
Items color coded and/or bar coded:

-Cooler label
-Diaries
-Instruction manuals
-Checklists
-Sampling containers

Other items:

-Pens
-Gloves
-Wall charger
-Adapter
-Velcro connection strap
-Temperature loggers

Temperature readings



Work at the EPA HSF

United States Environmental Protection Agency

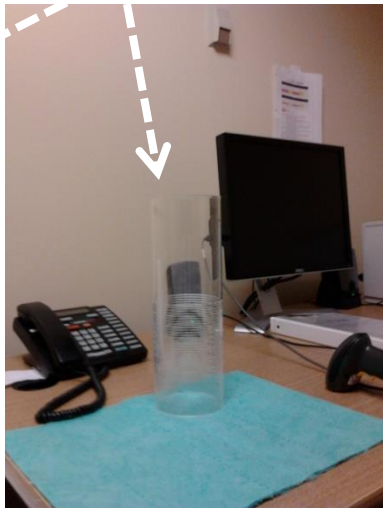
Assembly

Organization

2 kits/participant;
5 participants/day

Training

“Sobusizer”



Check-in

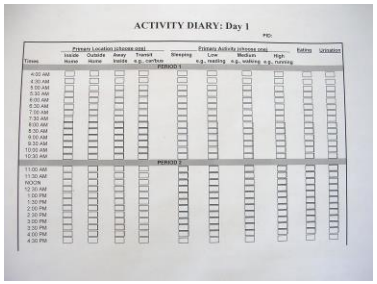
Daily instruction manuals

Instructions for Day 1 Sample Collection

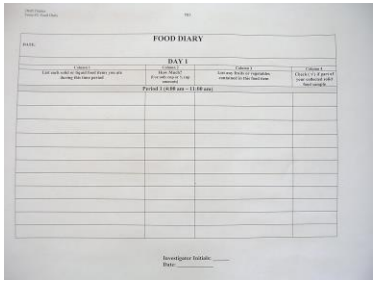
****Make sure your cooler is plugged in as much as possible.****

****Throughout sampling Day 1 (Sunday) please carry with you and fill out the Day 1 Activity Diary and the Day 1 Food Diary (both located in the outside pocket of the cooler in separate yellow folders).****

Activity Diary: Indicate your primary location and primary activity for **each 30 minute interval of the day**. Also indicate for each 30 minute interval when you ate something (meal or snack) or urinated.



Food Diary: Indicate the type and quantity of food that you ate between the hours of: **4:00 am – 11:00 am, 11:00 am – 5:00 pm, and 5:00 pm – 4:00 am.**



Page 1

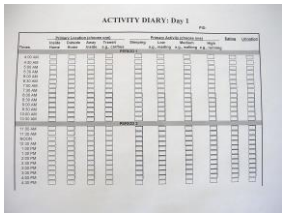
Instructions for Day 1 Sample Collection

Bedtime Urine Sample Collection

4) Unscrew the cap of the plastic jar and urinate directly into it, **providing your entire urine void.**

5) Immediately recap the plastic jar and screw closed tightly.

6) With a pen record the time of urination on the label of the plastic jar **and** in the Day 1 Activity Diary. (Example: 9:15 pm)



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Urine samples
(up to eleven 1L bottles per cooler)

Daily checklists

Instructions for Checklist for Day 1 Sample Collection

Day 1 sample collection is complete. Make sure that all urine and solid food samples have been sealed and placed into the cooler. Complete all sections of the activity and food diaries. ** Leave the cooler plugged in until you return to the clinic on Day 3 (Tuesday).

note: you will have 1 empty plastic jar labeled "Urine Sample 1 (FMV): Day 3 (Tuesday)" in this cooler. You will collect this sample in the morning on Day 3.

Day 1 Checklist (check boxes):

- ☐ Food Sample 1: 4:00 am – 11:00 am
- ☐ Food Sample 2: 11:00 am – 5:00 pm
- ☐ Food Sample 3: 5:00 pm – 4:00 am
- ☐ Bedtime Urine Sample
- ☐ Completed Food Diary
- ☐ Completed Activity Diary

Describe below any problems, if any, that occurred during Day 1:
(examples: Missing urine sample or food sample, cooler stopped running)

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Instructions for Checklist for Day 2 Sample Collection

Day 2 sample collection is complete. Make sure that all urine samples and solid food samples have been sealed and placed into the cooler. Complete all sections of the activity and food diaries. Leave the cooler plugged in until you return to the clinic on Day 3 (Tuesday).

Day 2 (Monday) Checklist:

- ☐ Urine sample 1 (FMV)
- ☐ Urine Sample 2
- ☐ Urine Sample 3
- ☐ Urine Sample 4
- ☐ Urine Sample 5
- ☐ Urine Sample 6
- ☐ Urine Sample 7
- ☐ Urine Sample 8
- ☐ Urine Sample 9
- ☐ Urine Sample 10
- ☐ Urine Sample 11
- ☐ Food Sample 1: 4:00 am – 11:00 am
- ☐ Food Sample 2: 11:00 am – 5:00 pm
- ☐ Food Sample 3: 5:00 pm – 4:00 am
- ☐ Completed Food Diary
- ☐ Completed Activity Diary

Describe below any problems, if any, that occurred during Day 2:
(examples: Missing urine sample or food sample, cooler stopped running)

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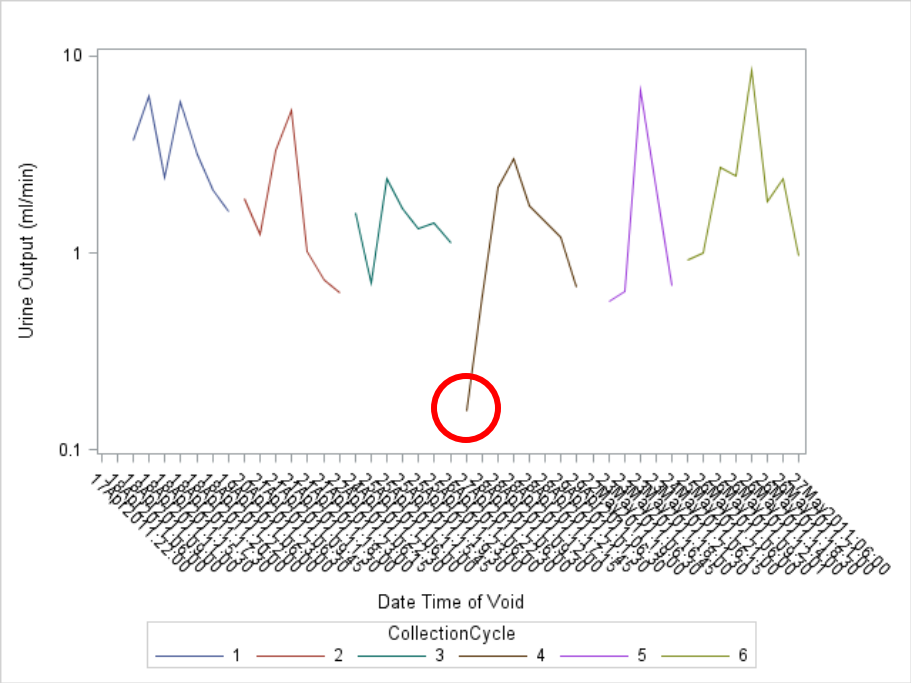
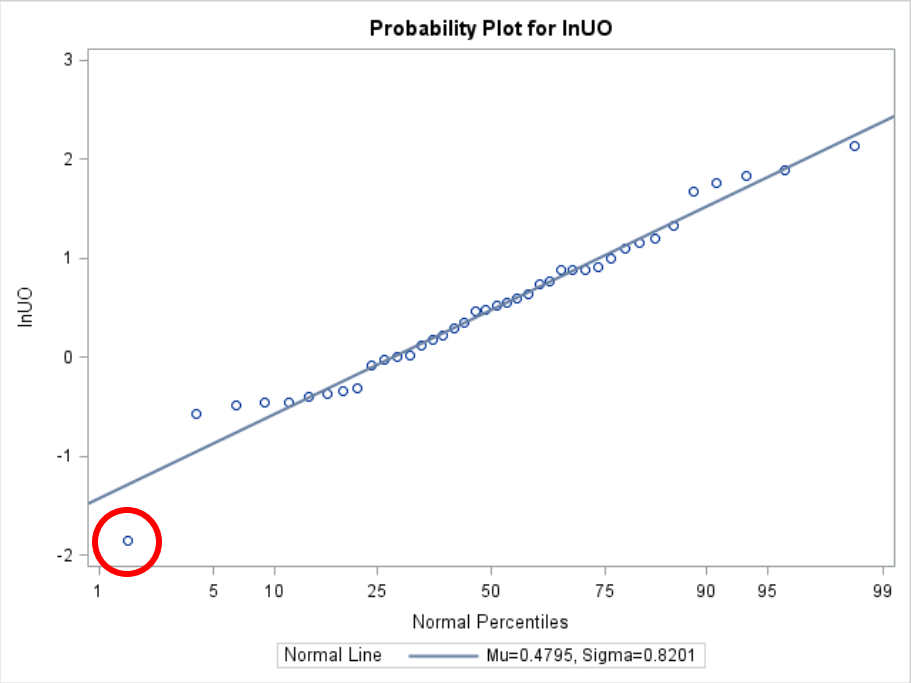
Completion statistics

Sampling metric	Number	Percent
Total urine sampling containers	3900	--
Total void events during collection periods	2577	--
Total samples collected	2489	97%
Acknowledged missing samples	88	3%
Acknowledged partial voids	4	0.2%
Suspected missing or partial voids ★	17	--

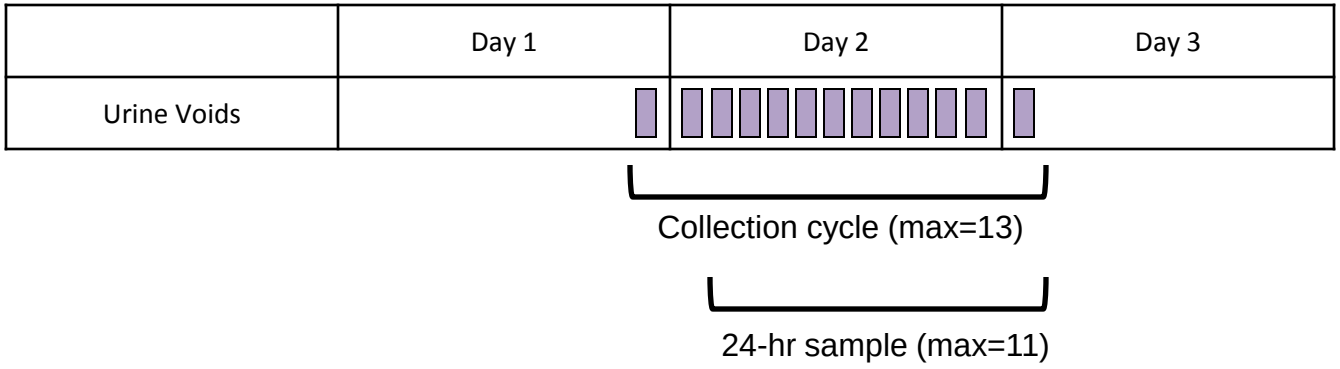
Visual inspection of urine output data





Void events and volumes

	1st	5th	25th	50th	75th	95th	99th
Void volume (mL)	24	56	150	250	390	650	860
Void events (# per cycle)	5	5	7	8.5	10	12	14
Void events (# per "24 hrs")	3	4	5	7	9	12	14



Keys to success

- Participant-based sampling (↑samples, ↓$, and ↑privacy)
- Individual training session / *ad hoc* refresher training
- Instruction manuals with color photos
- Contact email and phone numbers with instructions
- Positive reinforcement to encourage complete collection
- Daily checklists
- Recruiter with established database of volunteers
- Multiple QA checkpoints (field and lab)

<u>Technology-based:</u>

- Direct data uploads
- Barcodes on everything
- Temperature loggers (cooler and subject performance)

United States Environmental Protection Agency

Opportunities for improvements

- Smartphone/tablet applications:
  - electronic diaries with reminder alarms
  - consumer product barcode scans
  - sampling container barcode scans
  - real-time data uploads
  - real-time data validation
  - web apps

Take-home points

- Biomarkers research is advanced using innovative strategies to support:
  - Targeted field studies:
    - Sample collection, transport, storage, and analysis
    - Data collection, synthesis, and interpretation
  - Computational case studies:
    - Identifying associations between stressors and health
    - Evaluating biomarker levels against reference levels
    - Prioritizing chemicals by exposure