NTP
National Toxicology Program

NTP Projects Utilizing the NIEHS Clinical Research Unit

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NTP Board of Scientific Counselors Meeting
June 15 – 16, 2016

Outline

- Background
- NTP projects
  - BPA oral and dermal pharmacokinetic studies (OHAT)
  - Cashier study (OHAT)
  - NIEHS EPA Pilot Study of Exposure to Chemicals in Consumer Products (OHAT)
  - Black cohosh (BSB)
- Process and responsibilities

Clinical Research Unit

Background

- Traditionally NTP has not had large human research portfolio
- CRU established in 2009
  - Designed for small efforts
  - New IRB submissions as well as opportunities for sub-studies under existing protocols, i.e., Environmental Polymorphism Registry
- NTP does not intend to conduct human toxicity studies following intentional dosing

Clinical Research Unit

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Clinical Research Unit

BPA Pharmacokinetic Oral and Dermal Studies

Overview

- Objective: To reduce uncertainties about the metabolism and excretion of BPA in humans following oral and dermal administration
  - Measurement of unconjugated (“free”) BPA and conjugates in serum and urine for several days after administration of d-BPA
  - Use more sensitive analytical chemistry methods than used in the past
- New IRB submission

BPA Oral Pharmacokinetic Study

- 14 subjects
  - 6 men, 8 women
  - 100 µg/kg bw d-BPA

Environment International 83 (2015) 107–115

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Full length article

Pharmacokinetics of bisphenol A in humans following a single oral administration

Kristina A. Thayer [a], Daniel R. Doerge [b], Dawn Hunt [c], Shepherd H. Schurman [c], Nathan C. Twaddle [b], Mona I. Churchwell [b], Stavros Garantziotis [c], Grace E. Kissling [d], Michael R. Easterling [e], John R. Bucher [a], Linda S. Birnbaum [f,*]

- Blood and urine collected over 3 days (most in first 8 hours)
  - Chemistry: Dan Doerge, NCTR/FDA; LOD 0.001–0.002 ng/ml
  - Individual subject data (anonymized) in supplemental files
- Findings consistent with data from animal and other human studies
  - Unconjugated BPA in blood following oral administration is <1% of total
  - Most subjects excreted >90% as conjugated metabolites within 24 h

BPA Dermal Pharmacokinetic Study

- Amendment to oral protocol
  - Same administered dose level
  - Trying to recruit same subjects who participated in oral
- Status: Pilot phase completed with carboxymethyl cellulose (suspension) and ethanol vehicles
  - Chemistry: Manish Arora, Mt. Sinai; LOD 0.00022 ng/ml for d-BPA
- Pilot data will be used to determine dermal sampling times
  - Current sampling based on oral protocol, expect to modify sampling times during first day and extend duration to >3 days

Cashier Study

Objective

- Assess whether handling receipt paper results in measurable absorption of BPA or the BPA alternatives bisphenol S (BPS) and 4-hydroxyphenyl 4-isoprooxyphenylsulfone (BPSIP)
- Amendment to existing IRB-approved protocol = fast approval

Cashier Study: Methods & Results

- Assesses presence of BPA and BPA alternatives in cash receipt paper → BPA, BPS, BPSIP
  - Chemistry: Dan Doerge, NCTR/FDA
- Measured urine and blood levels in non-cashiers (n=25) and cashiers (n=77) in samples collected before and after shift during 2011-2013
- Thermal receipt paper is a potential source of occupational exposure to BPA, BPS, and BPSIP

Bisphenol A, Bisphenol S, and 4-Hydroxyphenyl 4-Isoprooxyphenylsulfone (BPSIP) in Urine and Blood of Cashiers

Kristina A. Thayer,[1] Kyla W. Taylor,[1] Stavros Garantziotis,[2] Shepherd H. Schurman,[2] Grace E. Kissling,[3] Dawn Hunt,[2] Brenda Herbert,[2] Rebecca Church,[2] Rachael Jankowich,[2] Mona I. Churchwell,[4] Richard C. Scheri,[4] Linda S. Birnbaum,[5] and John R. Bucher[1]

[1]Division of the National Toxicology Program, [2]Clinical Research Unit, and [3]Biostatistics Branch, National Institute of Environmental Health Sciences, National Institutes of Health (NIH), Department of Health and Human Services (DHHS), Research Triangle Park, North Carolina, USA; [4]Division of Biochemical Toxicology, National Center for Toxicological Research, U.S. Food & Drug Administration, Jefferson, Arkansas, USA; [5]National Cancer Institute, NIH, DHHS, Research Triangle Park, North Carolina, USA

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Focusing on Chemicals in Consumer Products

- Presented as a concept to BSC during June 17-18, 2014 meeting
- Objective 1: Evaluate whether *historical use* of products as captured by a questionnaire can be used to accurately predict categories of *current use* as captured by daily diary, and of *biological levels* as measured from analysis of biological specimens.
  - Sister study Personal Care Product and Residential History and Environmental Exposures questionnaires + Food packaging and processed food questionnaire
- Objective 2: Inform and evaluate the questionnaire methods and EPA's exposure models designed to predict exposures to chemicals in the environment.

NIEHS EPA Pilot Study of Exposure

- High participant burden study, start with pilot (n=9)
  - Complete questionnaire and daily diary
  - Wear 5 small devices that measure air pollution, chemicals, and location
  - Record products used in diary and photograph them on an iPad and take iPad videos of requested products
  - Collect daily urine samples and 2 visits to CRU to give blood samples
  - Home visits by EPA field technicians
- Close to enrolling….

NIEHS-EPA Pilot Study of Exposure to Chemicals in Consumer Products

Open for Recruitment

Table of Contents

- Study Background
- Who can participate?
- What will you do?
- Study Location
- Enrollment and Contact Information
- Principal Investigator

Study Background

In a lifetime, a person may encounter tens of thousands of chemicals used as ingredients in the products they buy. It's not easy to measure these chemicals because the companies that sell the products aren't required to divulge the exact chemical ingredients.

Researchers want to compare how existing methods, such as surveys and models, measure exposure to chemicals in personal care and household products. This study intends to test and improve the ways that studies gather data about how chemicals in products come in contact with consumers.

Additional Information

Study Pocket Card (587KB)

Key Collaborators

- **Academic**
  - **Manish Arora,** Mt. Sinai
  - **Syam Andra,** Mt. Sinai
  - **Daniel Suarez,** Mt. Sinai
- **EPA**
  - **Timothy Buckley**
  - **Peter Egeghy**
  - **Kim Gaetz**
  - **Nicole Hagan**
  - **Daniel Stout**
- **FDA**
  - **Dan Doerge**
  - **Mona Churchwell**
  - **Richard Scheri**
  - **Nathan Twaddle**
- **NIEHS/NTP**
  - **Linda Birnbaum**
  - **John Bucher**
  - **Stavros Garantziotis**
  - **Grace Kissling**
  - **Kyla Taylor**
  - **Shepherd Schurman**
  - **Annette Rice**
  - **Suramya Waidyanatha**
- **Contract Support**
  - **Mike Easterling**
  - **Rebecca Church**
  - **Brenda Herbert**
  - **Dawn Hunt**
  - **Rachael Jankowich**

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Biomarker Studies with Black Cohosh in Women

DEPARTMENT OF HEALTH & HUMAN SERVICES • USA

What is Black Cohosh?

Herbal product used by women worldwide

- Black cohosh, or black cohosh extract, is the “active” component in a number of botanical products marketed to women of all ages for relief from a variety of menstrual and menopausal symptoms.
- Long history of use worldwide – marketed for sale at least as early as the 1880s in the US

Ad for black cohosh product in 1882

- 2014 sales figures: 4th most popular herbal product in the U.S., annual sales of $42 million (SPINS LLC and IRI, Chicago IL)

Black Cohosh Use

Herbal extract used by women worldwide

Black cohosh extracts

Black cohosh root

Black cohosh herbal mixtures

Black cohosh for premenopausal women

- Mechanism of action unclear, but contains no estrogen and no constituents that bind to estrogen receptors
- Use pattern may be periodic, short-term, or chronic

Nominated to NTP for testing

Black cohosh extract

- Nominated to NTP by the NCI and the NIEHS in 2000 based on wide spread use and lack of toxicity data

Actaea racemosa

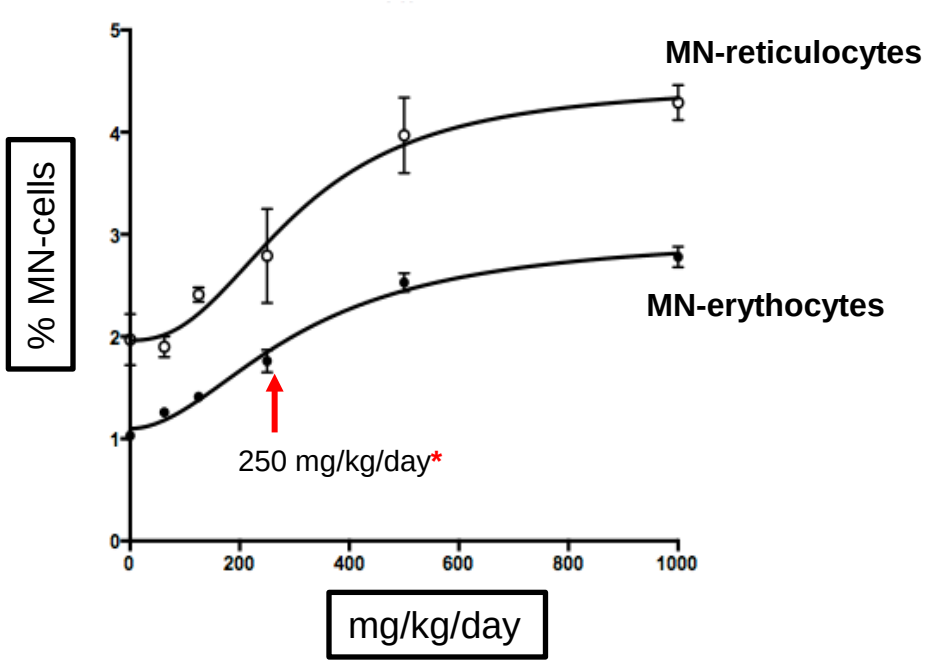
Perennial plant, native to eastern North America

NTP 13-week toxicity studies in female animals

Significant, dose-related increases in micronucleated RBCs

- Female Wistar Han rats, 15-1000 mg/kg for 13 weeks
- Female B6C3F1 mice, 30-1000 mg/kg for 13 weeks

MN in female mice at 13 weeks



*40x the recommended human dose of 40 mg/day

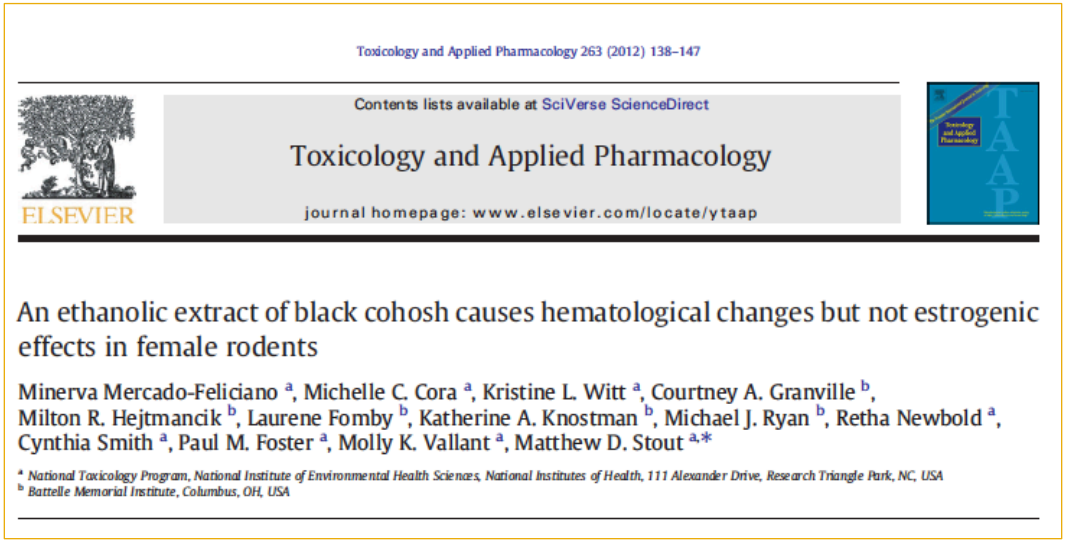
NTP 13-week toxicity studies in female animals

Additional findings in the 13-week studies

- Female Wistar Han rats, 15-1000 mg/kg for 13 weeks
- Female B6C3F1 mice, 30-1000 mg/kg for 13 weeks

Dose-dependent, non-regenerative macrocytic anemia in rats and mice

No estrogenic activity



Toxicology and Applied Pharmacology 263 (2012) 138–147

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Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

An ethanolic extract of black cohosh causes hematological changes but not estrogenic effects in female rodents

Minerva Mercado-Feliciano [a], Michelle C. Cora [a], Kristine L. Witt [a], Courtney A. Granville [b], Milton R. Hejtmancik [b], Laurene Fomby [b], Katherine A. Knostman [b], Michael J. Ryan [b], Retha Newbold [a], Cynthia Smith [a], Paul M. Foster [a], Molly K. Vallant [a], Matthew D. Stout [a,*]

[a] National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health, 111 Alexander Drive, Research Triangle Park, NC, USA
[b] Battelle Memorial Institute, Columbus, OH, USA

Rationale for a clinical study

NTP animal studies raised concerns

- Highly significant increases in micronucleated red blood cells (biomarkers of chromosomal damage) in female mice and rats
- Increased frequencies of MN are associated with increased risk for cancer
- Non-regenerative macrocytic anemia is consistent with folate and/or B12 deficiency
- Decreased folate levels are directly associated with increased erythrocyte MN frequencies (*MacGregor et al.*, 1997)
  - Folate deficiency in women of child-bearing age has significant implications for adverse reproductive outcomes (neural tube defects)

Cross-sectional study design

BCE-exposed and matched control populations; single clinic visit

Recruitment through the CRU

↓

Healthy women age 18+ taking a single-herb Black Cohosh product for at least 3 months

+

Matched controls: Black Cohosh naïve

Cross-sectional study design

Considerations

- Advantages
  - Simplified IRB review for this observational approach
  - Faster acquisition of data (only one sampling)
  - Recruit controls from current cohorts under study at the CRU
  - No attrition (repeat clinic visits not necessary)
  - No restrictions on menopausal status or age
- Disadvantages
  - Variety of black cohosh products rather than a single product
  - More difficult to interpret the data
  - Power of the study may be less than a prospective study

Endpoints evaluated

- **Blood samples**
  - Micronucleated reticulocyte frequencies
  - Folate, B12, homocysteine levels
  - Complete blood counts
  - Reticulocyte %, reticulocyte absolute, reticulocyte hemoglobin, immature reticulocyte fraction
  - Immature platelet fraction
  - Metabolomic profile

- **Black cohosh samples**
  - Chemical analysis

Division of responsibilities

CRU:

- Protocol preparation, questionnaires, consent forms, and IRB approval
- Advertising
- Telephone pre-screening and appointment scheduling
- Obtain consent and securely manage PII
- Blood sampling and specimen preparation/coding
- Specimen shipment to analytical laboratories

Study flyer

NTP:

- Study power determinations, assisting with designing the protocol, questionnaires, screening forms
- Data compilation and analysis

Current status

- **Enrollment totals (June 1, 2016)**
  - 6 Pilot study subjects
  - 18 Black cohosh users (2 were premenopausal)
  - 19 Matched (age, race) controls, black cohosh naïve
- **Recruiting problems**
  - Mixed herbal products
- **Next steps**
  - Continued advertising
  - Questionnaire information from the Environmental Polymorphism Registry regarding herbal product use
    - Potential source of additional study participants
  - Data compilation and analysis completed by late fall, 2016

Key Collaborators

- **NIEHS Clinical Research Unit**
  - Stavros Garantziotis
  - Shepherd Schurman
  - Annette Rice
- **CRU Contract Support**
  - Rebecca Church
  - Nicole Edwards
  - Lisa Barber
  - Cynthia Smith
  - Brittany Mosley
- **National Toxicology Program**
  - Linda Birnbaum
  - Chad Blystone
  - John Bucher
  - Michelle Cora
  - Paul Foster
  - Grace Kissing
  - Alex Merrick
  - Minerva Mercado-Feliciano*
  - Retha Newbold
  - Richard Paules
  - Stephanie Smith-Roe
  - Matt Stout
  - Suramya Waidyanatha

*Now at FDA