

# Synthetic Turf/Recycled Tire Crumb Rubber Research

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NTP Board of Scientific Counselors  
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# Variety of Uses for Recycled Tires





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# Why is There Public Health Concern?

- Reports in the media of health concerns among athletes, particularly soccer players
- Visible contact with rubber particles



- Potential for widespread and long term exposure
  - 12,000 fields in U.S., 1200 added annually, including youth sports fields
- Certain chemicals in tires are toxic in humans
  - E.g. polycyclic aromatic hydrocarbons (PAHs), heavy metals (lead, cadmium, arsenic), phthalates



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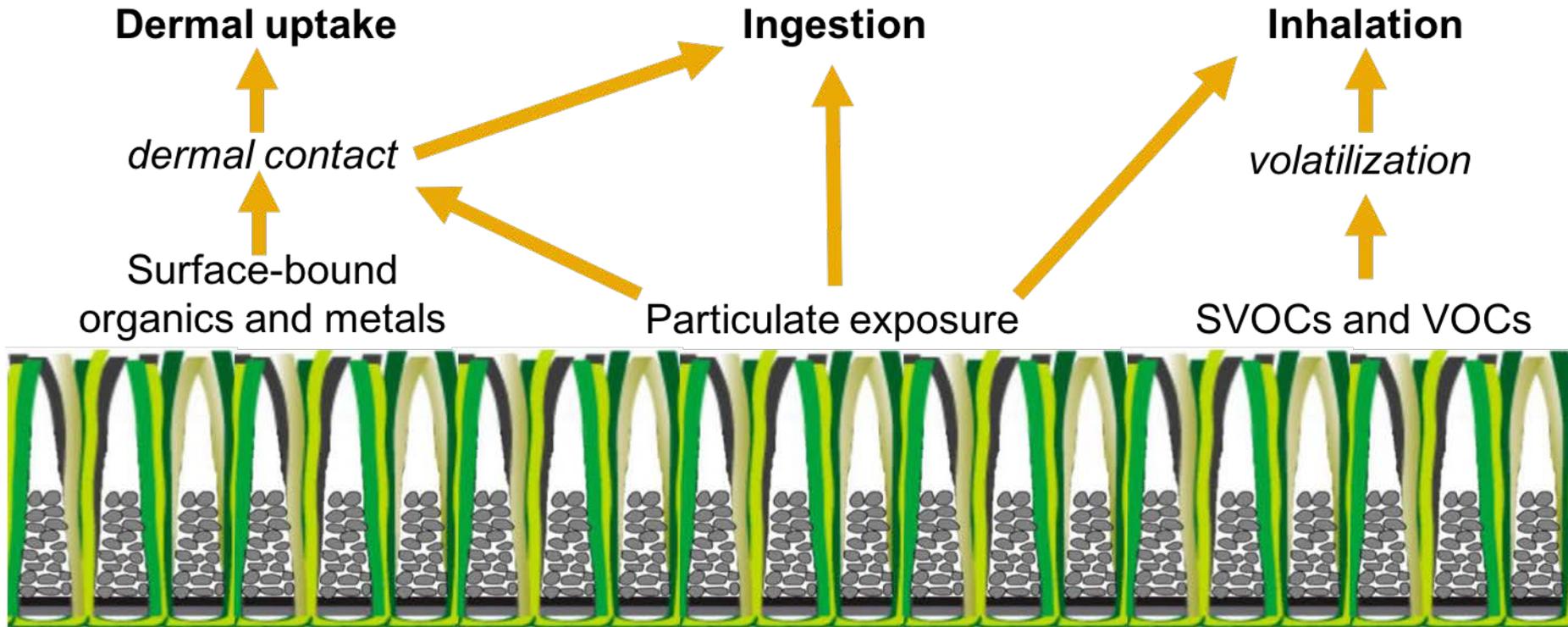
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# Is There Exposure To These Chemicals?

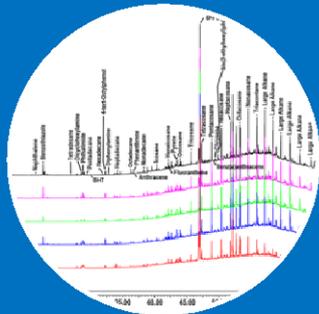
**RESEARCH FOCUS:** What conditions in an experimental setting have the potential to result in systemic exposure to crumb rubber constituents?





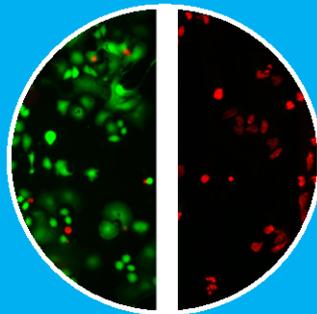
# NTP Research Approach

**RESEARCH FOCUS:** What conditions in an experimental setting have the potential to result in systemic exposure to crumb rubber constituents?



**Chemical  
Characterization**

Suramya  
Waidyanatha  
NIEHS



***In Vitro*  
Characterization**

William Gwinn  
NIEHS



**Feasibility to  
Conduct *In Vivo*  
Studies**

Jamie Richey  
Battelle



**14-Day *In Vivo*  
Studies**

Georgia Roberts  
NIEHS

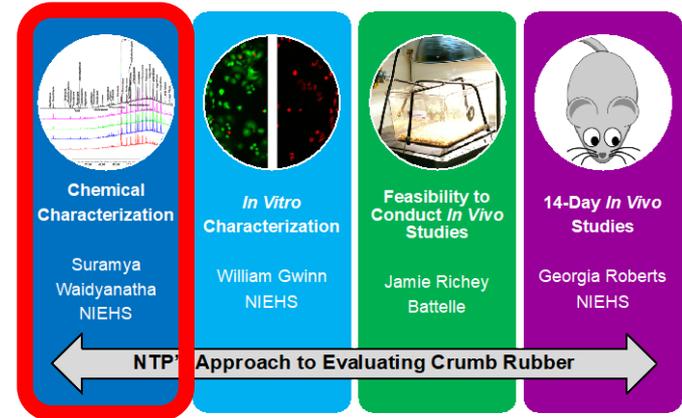
**NTP's Approach to Evaluating Crumb Rubber**



# Chemical Characterization

## • Goals:

- Characterize material used for *in vitro* and *in vivo* studies
  - Inform chemical analysis of biological samples collected from the *in vitro* and *in vivo* studies
- Allow comparison to other crumb rubber samples collected by California EPA and the Federal Research Action Plan
  - NTP studies used "fresh" recycled tire crumb rubber (NOT field samples)
    - Compare to samples from indoor and outdoor fields
    - Compare to samples from new and weathered fields



**Lead: Suramyia Waidyanatha, NIEHS**



- **Methods:**

- **Characterization**

- **Composition**

- Thermogravimetric analysis, optical and scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS)

- **Volatile and semivolatile organic compounds (VOCs, SVOCs), solvent extractables and metals**

- Gas chromatography-mass spectrometry, liquid chromatography-mass spectrometry, inductively coupled plasma-mass spectrometry (ICP-MS) or inductively coupled plasma-optical emission spectrometry (ICP-OES)

- **Bioaccessibility studies**

- **Extraction/leaching from simulated body fluids to mimic oral, dermal and inhalation exposures**

- Saliva, gastric fluid, intestinal fluid, sweat and lung fluid

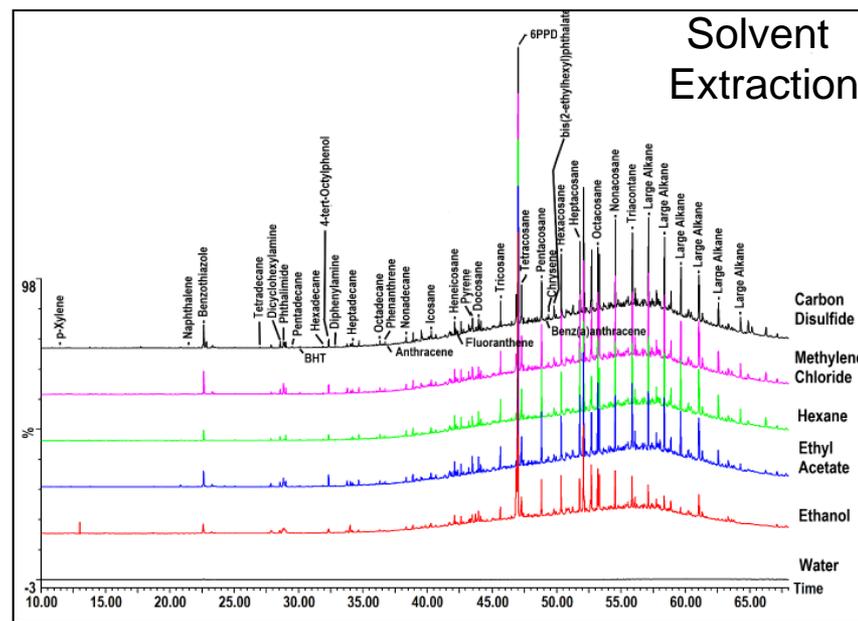
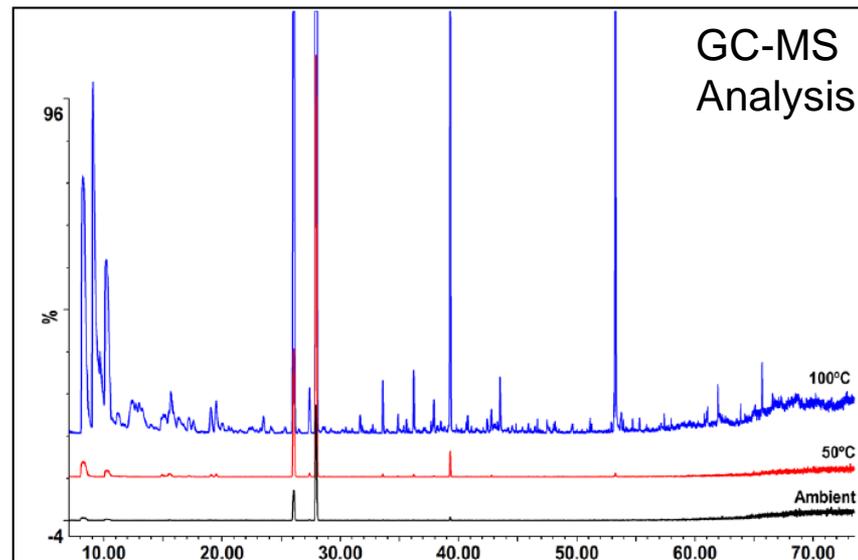




# Chemical Characterization

## • Results:

- Volatile and semivolatile organic compounds comprise ~ 0.0007% by weight of the bulk material
  - Methyl isobutyl ketone (3.4 ppm)
  - Aniline (1.1 ppm)
  - Benzothiazole (0.7 ppm)
- Solvent Extractables
  - Over 300 peaks, mostly non-polar
  - Very little extracted in water
- Metals account for 3% by weight of the bulk material
  - Zinc (1.68%)
  - Silicon (0.932%)
- Thermogravimetric
  - 67% polymers and carbon black





# In Vitro Evaluations

- **Goals:**

- Evaluate an experimental model for characterizing the toxicity of crumb rubber or bioaccessible constituents
- Evaluate the biological effects of bioaccessible crumb rubber constituents using human cell lines
  - Various cell lines were selected to reflect potential cellular targets of toxicity from dermal, inhalation, and oral routes of exposure

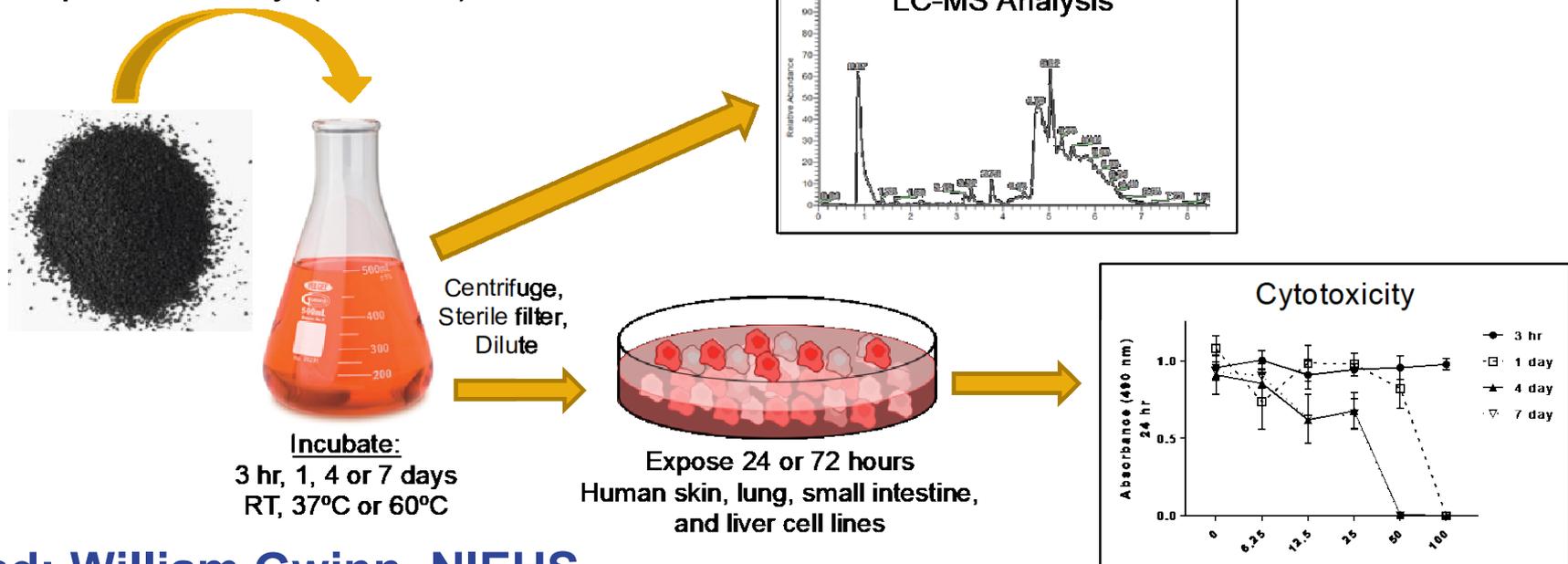


**Lead: William Gwinn, NIEHS**



## • Methods:

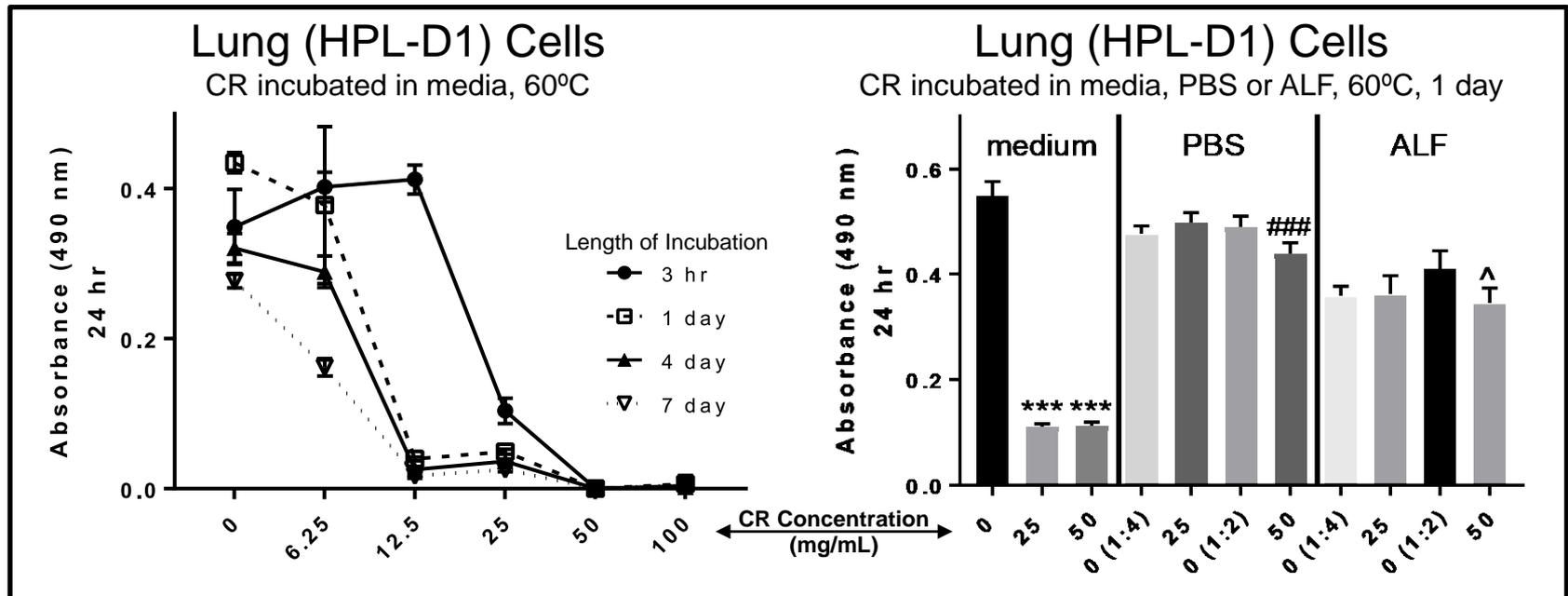
- Incubate crumb rubber in cell-type specific culture media at 100 mg/mL for varying times and temperatures to produce **Crumb Rubber Conditioned Media (CRCM)**
- CRCM was centrifuged, sterile filtered, and serially diluted with incubation time- and temperature-matched control media
- Cells were exposed for 24 or 72 hours to CRCM and cytotoxicity was evaluated
- CRCM was analyzed using an untargeted liquid chromatography mass spectrometry (LC-MS) method





## • Results: Cytotoxicity

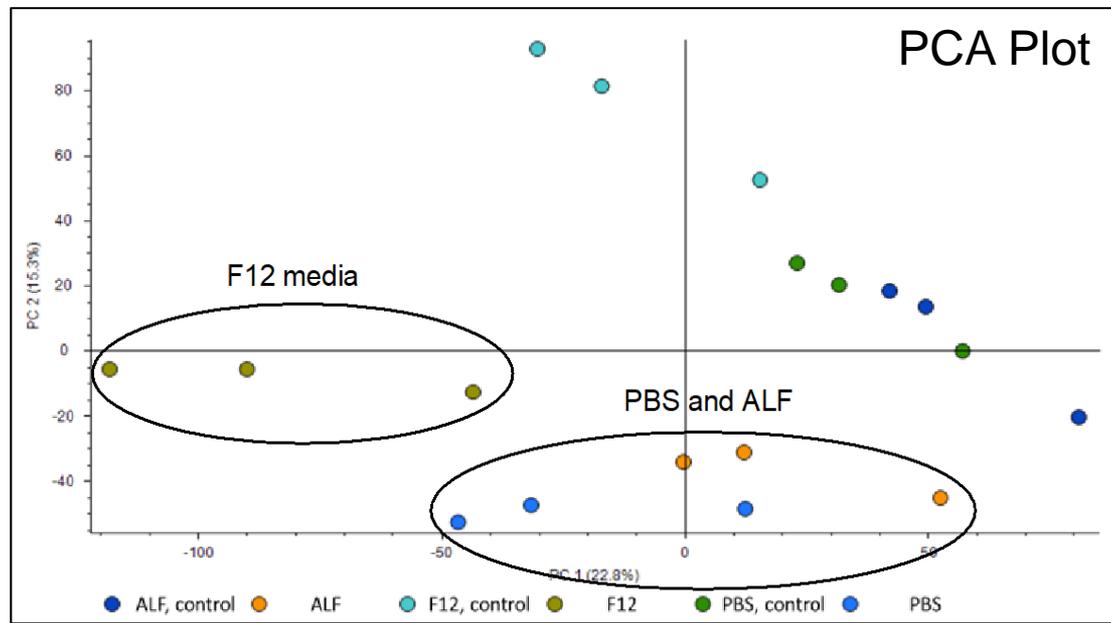
- Cytotoxicity was observed with human lung, skin and small intestine human cell lines, which was concentration-dependent, as well as incubation time- and temperature-dependent
- Cytotoxicity was **not** observed when crumb rubber was incubated in phosphate buffered saline (PBS) or artificial lung fluid (ALF) and diluted in culture media for cell exposures (only lung cells tested)
- Cytotoxicity was **not** observed in human liver cells





## • Results: LC-MS Analysis

- Used LC-MS to analyze control media, PBS and ALF, and crumb rubber conditioned media, PBS and ALF
- Principle component analysis showed separation between cytotoxic CRCM (F12 media) and non-cytotoxic crumb rubber conditioned-PBS and ALF

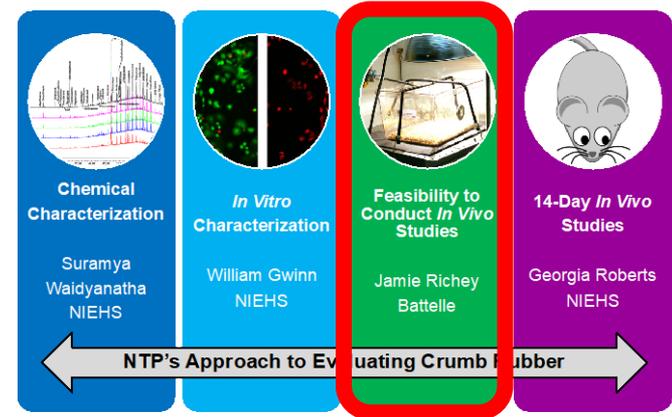




# Feasibility to Conduct *In Vivo* Studies

- **Goals:**

- Determine what routes of exposure are feasible to conduct *in vivo* studies in rodents
- Considerations included: practicality, homogeneity, particle sizes, doses, vehicles

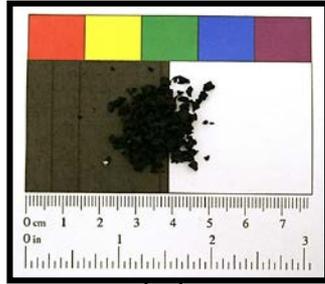


**Lead: Jamie Richey, Battelle**



# Feasibility to Conduct *In Vivo* Studies

## • Methods



Material was size fractionated



Dermal



Feed



Bedding



Gavage

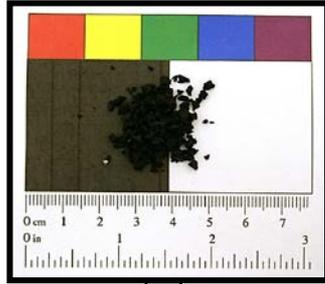
400 Mesh Only





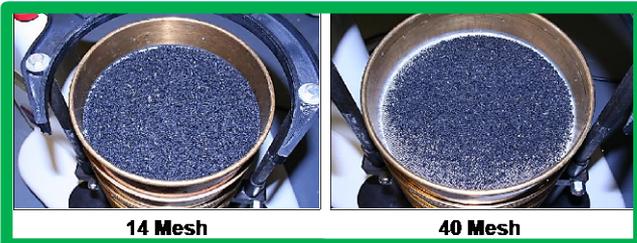
# Feasibility to Conduct *In Vivo* Studies

## • Results



Sieve Mesh	Size (µm)	% Total by Weight
14-80	170 to > 1410	> 99
400	37 - 170	0.34

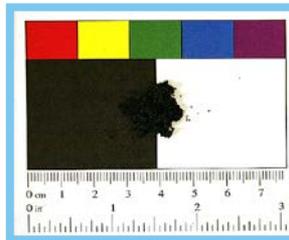
Material was size fractionated



14-80  
Mesh  
Combined



400 Mesh



- Bedding was visibly homogenous when mixed at 50%:50% weight
- Feed was visibly homogenous when prepared at 50,000 ppm

- 400 mesh formulation at 200 mg/mL was stable and acceptable for gavage studies

- Non-occluded dermal testing deemed not feasible using particles in suspension

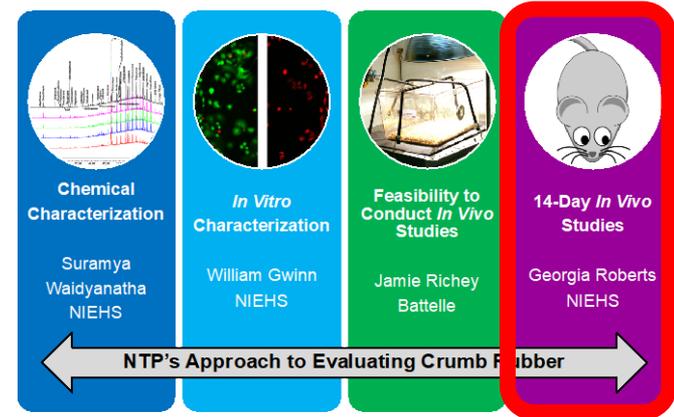


# 14-Day *In Vivo* Studies

- **Goals:**

- What routes of exposure are most likely to result in systemic exposure?

- What constituents of crumb rubber are bioaccessible and/or bioavailable?
- Use biological sampling and traditional measures of toxicity to determine if there is evidence of systemic exposure to crumb rubber constituents



**Lead: Georgia Roberts, NIEHS**



# 14-Day *In Vivo* Studies

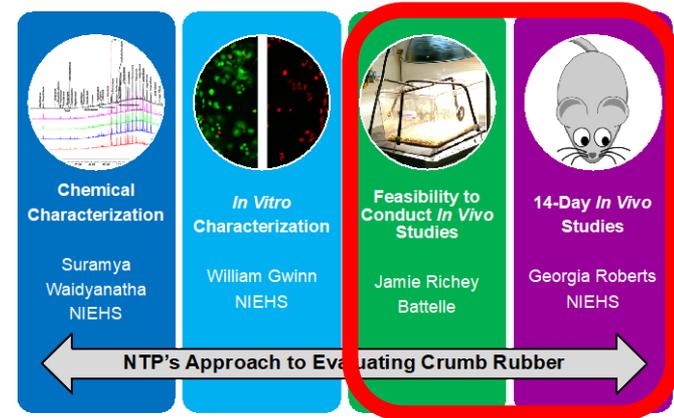
- **Methods** (based on feasibility)

- **Animal Model Selection:** (female B6C3F1/N mice)

- Conserve test material in gavage studies compared to rats
- Conserve test material in bedding and feed studies compared to male mice due to group housing

- **Routes/Doses:**

- Feed: 50,000 ppm, limit dose
- Bedding: 50%/50% by weight
  - Extend life of bedding, conserve material
- Oral gavage: 1250 mg/kg
  - Selected based on test material availability



- **Endpoints:**

- Characterize exposure (n=5)
  - Urine
  - Plasma
- Toxicity endpoints (n=10)
  - Histopathology
  - Hematology
  - Bone marrow cytology

**Lead: Georgia Roberts, NIEHS**



# 14-Day *In Vivo* Studies

## • Results:

- No impact on survival, body weight, histopathology, hematology or bone marrow cytology
- Evaluating crumb rubber consumption in the feed exposure study:
  - Attempts were made to quantify the amount of crumb rubber consumed by separation of feed and crumb rubber post-exposure



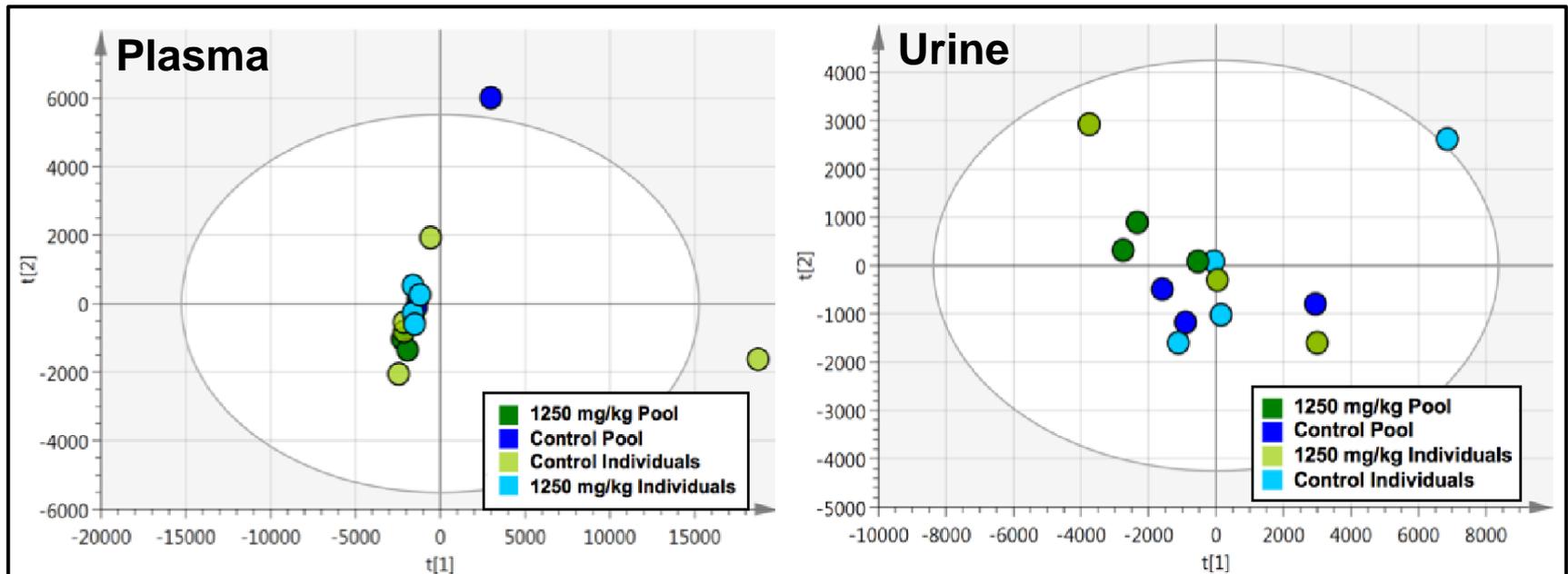
- Due to bedding and fecal contamination this was not possible
- Qualitatively, there was evidence (based on observation of feces) that animals consumed crumb rubber early in the study (days 0-4) and then began to avoid consumption later in the study



# 14-Day *In Vivo* Studies

- **Results: LC-MS Analysis**

- Plasma and urine were collected from 5 animals per group, and samples were analyzed using an LC-MS metabolomics approach
- Principle component analysis was unable to distinguish treated versus control samples for any route of exposure or matrix
  - **Example PCA from Oral Gavage Study:**





## • **Results:** LC-MS Analysis

- Plasma and urine were collected from 5 animals per group, and samples were analyzed using an LC-MS metabolomics approach
- Principle component analysis was unable to distinguish treated versus control samples for any route of exposure or matrix
- Evaluation of fold-change/p-value and feature identification
  - Untargeted ID criteria: exact mass, isotope ratio, fragmentation and retention time

	<b>Oral Gavage</b>		<b>Feed</b>		<b>Mixed-Bedding</b>	
Ion Mode	Plasma	Urine	Plasma	Urine	Plasma	Urine
<b>Positive</b>	25	0	5	0	0	0
<b>Negative</b>	13	1	2	0	4	1

Number of tentatively identified compounds with a **p-value<0.05** and a **fold change>5** in treated versus controls



## • Research Approach

- Used a streamlined, targeted approach to address a set of specific questions related to potential exposure
  - Nomination: December 2015
  - BSC Updates: June 2016, 2017, 2018
  - Publication of data: end of Summer 2018

## • Chemical Characterization

- Many of the compounds (VOCs, SVOCs, metals etc.) detected were expected based on what was known about crumb rubber
- We used a similar analysis pipeline to those in other on going efforts that will help us understand how these studies fit into the world of crumb rubber
- Generated data to inform these questions:
  - Are weathered fields very different than the material we used?
  - Is the material we used more similar to outdoor or indoor fields?



- ***In Vitro* Studies**

- Observed cytotoxicity in multiple cell lines using conditioned media
  - Dependent on time, temperature, concentration
- Experiments with more biologically relevant fluids resulted in little to no toxicity
- Using LC-MS/PCA, we were able to see a difference between the cytotoxic CRMC and the non-toxic PBS/ALF incubated with crumb rubber

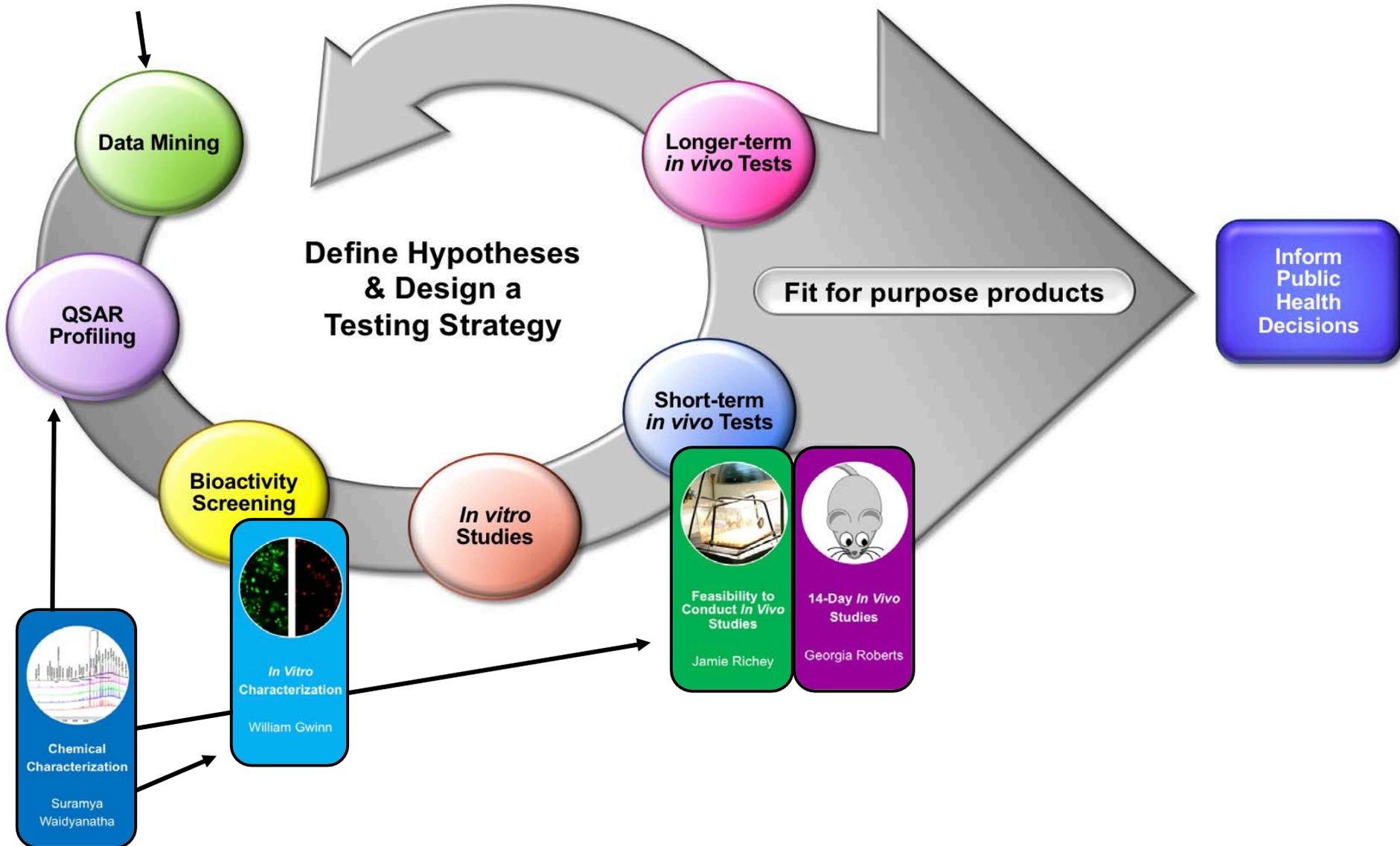
- ***In Vivo* Studies**

- No signs of traditional toxicity
- Some evidence of systemic exposure; based on our chemical characterization of the bulk, constituents would be present at extremely low levels (<100 µg/kg benzothiazole assuming 100% gastrointestinal extraction, absorption, distribution)
- Gavage and bedding studies were successful, however feed studies are not practical due to avoidance of the test material



# How Does Crumb Rubber Fit in the Pipeline?

We started here

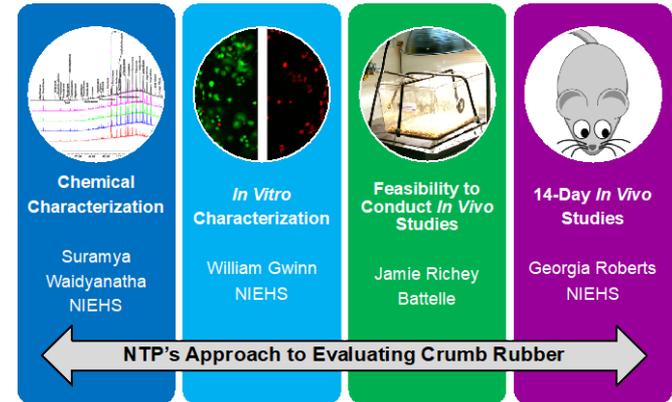




# Additional Information Available

- Results from these studies were presented at 2018 SOT Annual Meeting and the posters are available on the NTP website:

<https://ntp.niehs.nih.gov/go/turf>



- Chemical Characterization, Abstract 2415
  - *In Vitro* Evaluations, Abstract 2416
  - Feasibility to Conduct *In Vivo* Studies, Abstract 2417
  - 14-Day *In Vivo* Studies in Female Mice, Abstract 2414
- Project Outputs:
    - NTP Research Reports on each focus area published in summer 2018

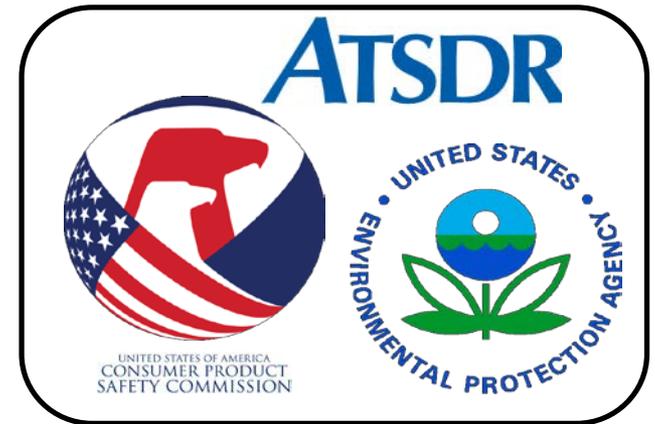


# Other Federal and International Efforts

- Other federal, state and international efforts focus primarily on exposure monitoring and field sampling
- NTP provides a unique capability to add information about crumb rubber in biological systems
- NTP participates in frequent communications with these groups to provide status updates and share information



## Federal Research Action Plan





# Acknowledgements

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Nigel Walker

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Dawn Fallacara

Jamie Richey

Brian Burbach (PI, Chemistry)

Tim Cristy

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Audrey Turley (PM)

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Questions