NTP
National Toxicology Program

# Where the Rubber Meets the Road

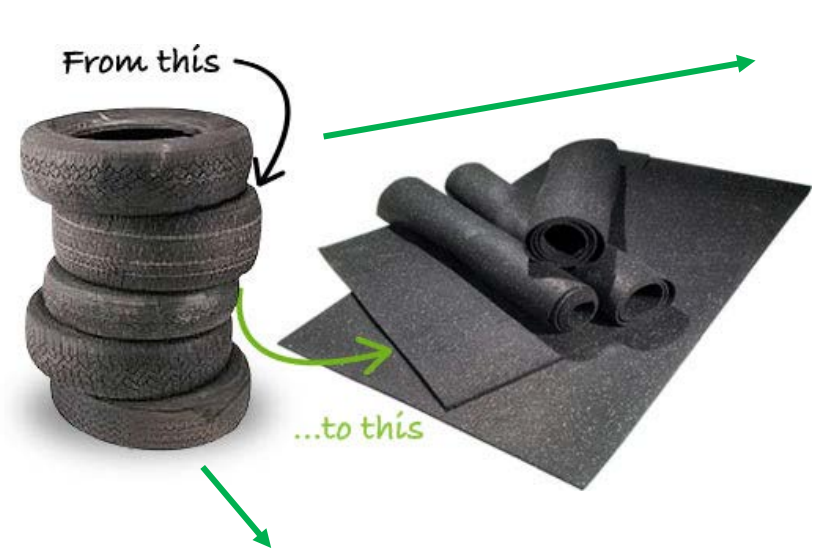
*Update on NTP Crumb Rubber Research*

Georgia K. Roberts, PhD
Program Operations Branch
National Institute of Environmental Health Sciences

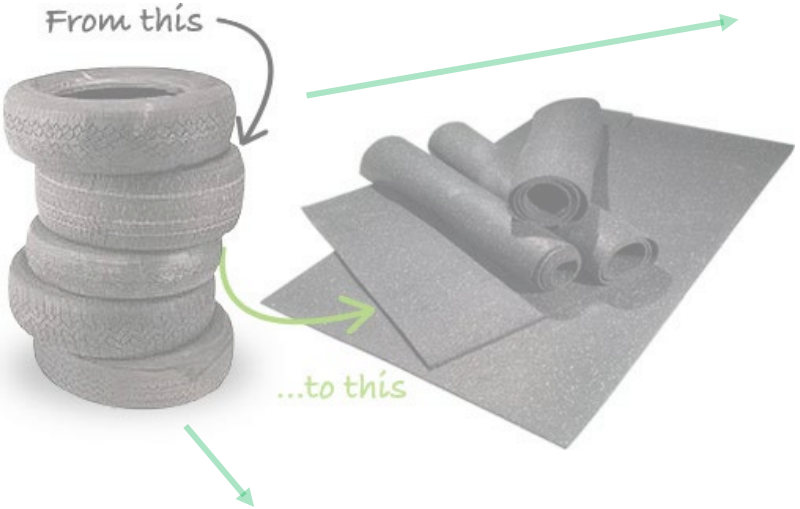
NTP Board of Scientific Counselors
June 29, 2017

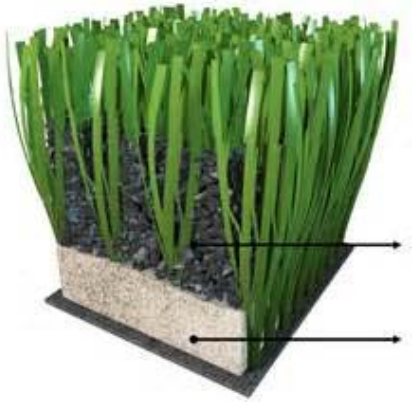
# What is Crumb Rubber?

# Variety of Uses for Recycled Tires



# Variety of Uses for Recycled Tires





# Crumb Rubber in the News

HEALTH | CANCER

**Did Artificial Turf Give More Than 200 Soccer Players Cancer?**

Here's what the research says about turf safety

BY ALISA HRUSTIC *February 1, 2017*

February 1, 2017

http://www.menshealth.com/health/artificial-turf-cancer-risk

- Amy Griffin, soccer coach at University of Washington
  - In 2009: 2 goalies diagnosed with "blood cancers"
  - By 2014: her list had 36 people (34 were goalies) diagnosed with "blood cancers"

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- Amy Griffin, soccer coach at University of Washington
  - In 2009: 2 goalies diagnosed with "blood cancers"
  - By 2014: her list had 36 people (34 were goalies) diagnosed with "blood cancers"
- Follow up analyses showed that the cancer rate among this group was lower than expected compared to a similar demographic in Washington state during the time period analyzed (2002-2015)

http://www.doh.wa.gov/CommunityandEnvironment/Schools/EnvironmentalHealth/SyntheticTurf

# Why is There Public Health Concern?

- Reports in the media of young soccer players, particularly goalies, developing ‘blood cancers’
- Frequently used for youth sports, including soccer
  - Life time exposure
  - Exposure during critical windows of development
- Visible exposure (players often take home rubber particles)
- Potential for widespread exposure
  - 11,000 fields in U.S., 1,200 added annually
- Certain chemicals in tires are toxic in humans

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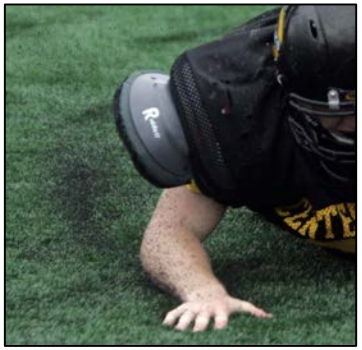
But are we exposed?

# Potential Routes of Exposure



**Dermal uptake**

**Ingestion**

**Inhalation**

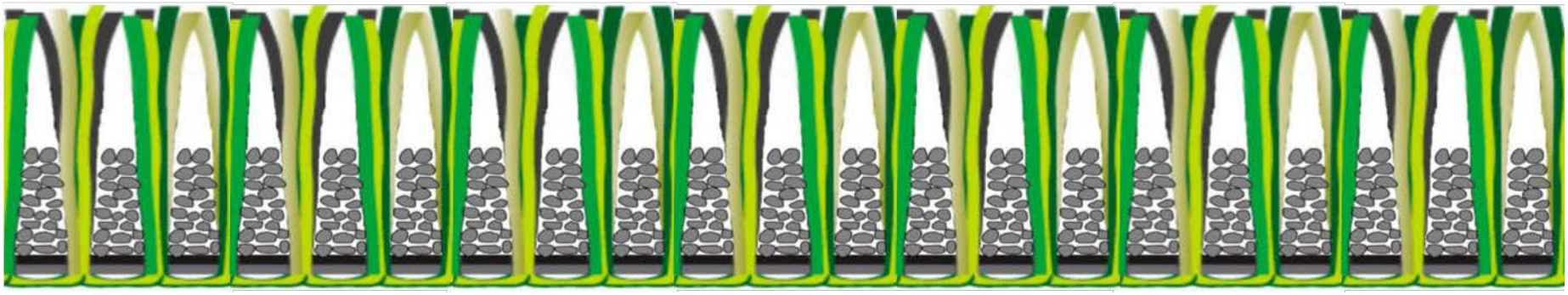
*dermal contact*

*volatilization*

Surface-bound organics and metals

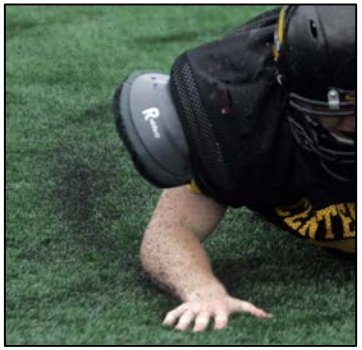
Particulate exposure

SVOCs and VOCs

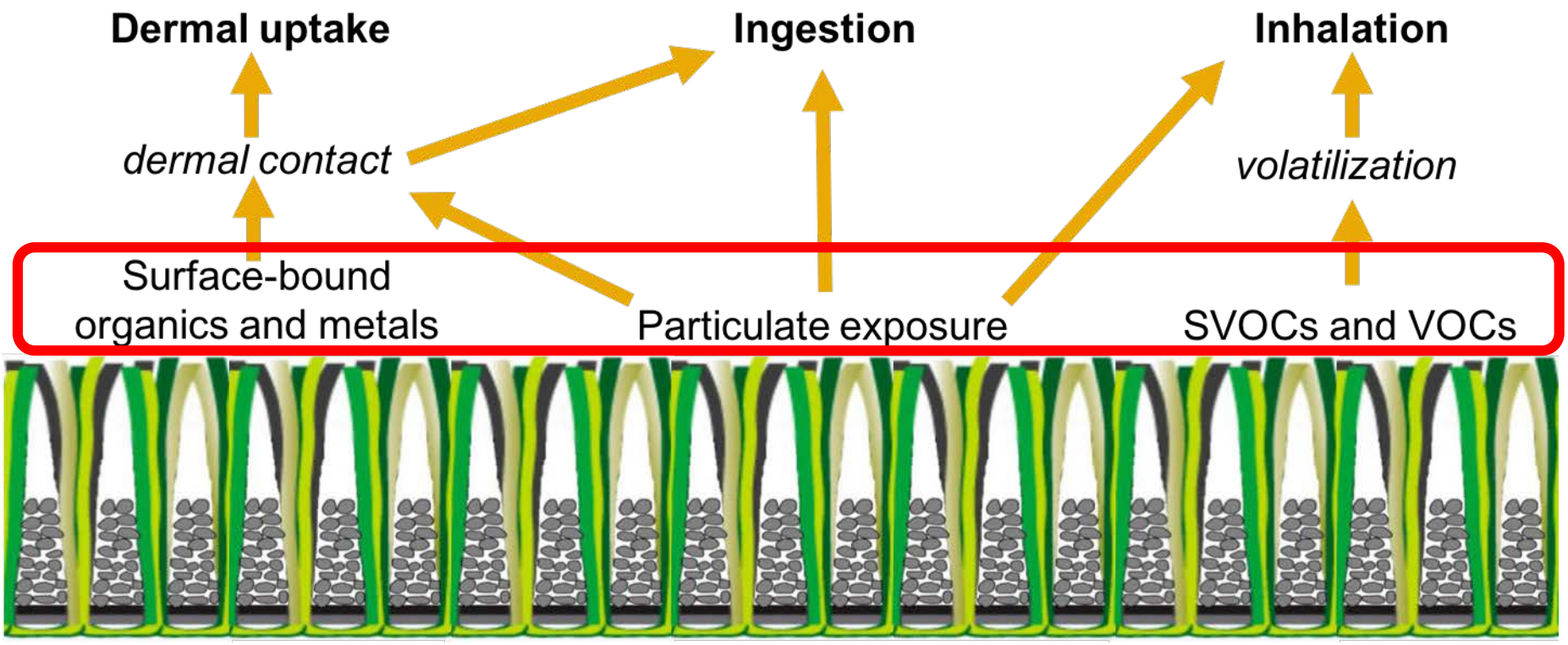


Adapted from Cheng, et al. (2014)

# Potential Routes of Exposure



Presence and abundance of constituents likely change over the life of a field

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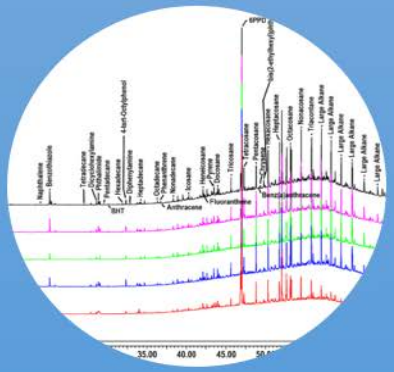
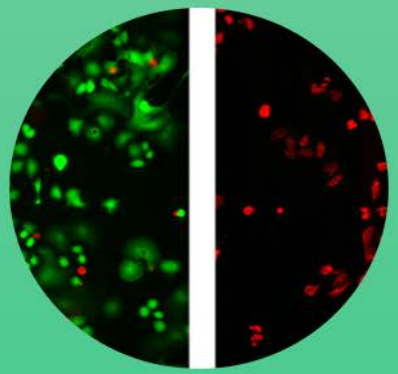
# NTP's Approach



**Chemical Characterization**

Suramya Waidyanatha



**In Vitro Characterization**

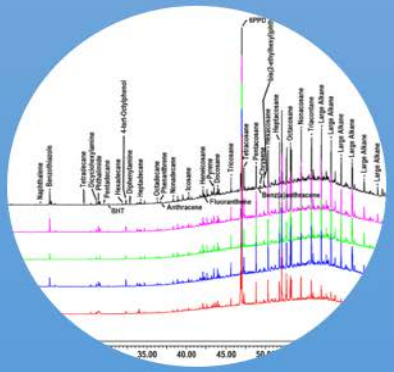
Will Gwinn

**In Vivo Feasibility**

Georgia Roberts

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

# NTP's Approach



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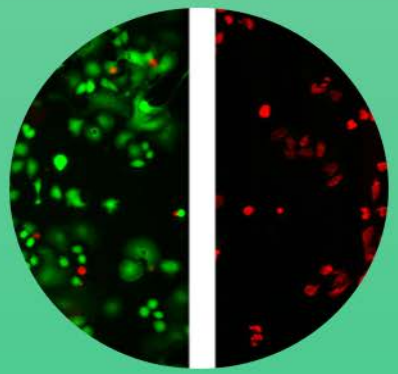

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**NTP's Approach to Evaluating Crumb Rubber**

**Interagency Communication**

# **NTP's Approach:** Interagency Communication

OEHHA
SCIENCE FOR A HEALTHY CALIFORNIA

ATSDR

UNITED STATES OF AMERICA
CONSUMER PRODUCT
SAFETY COMMISSION

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

NTP
National Toxicology Program
U.S. Department of Health and Human Services

**NTP Lead: Scott Masten**

# NTP's Approach: Interagency Communication

**Nomination/Request for Testing**

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**Federal Research Action Plan**

NTP
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# NTP's Approach: Interagency Communication

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ECHA
EUROPEAN CHEMICALS AGENCY

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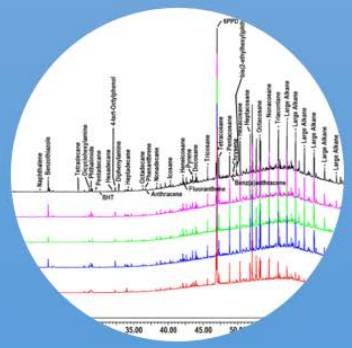
# NTP's Research Approach



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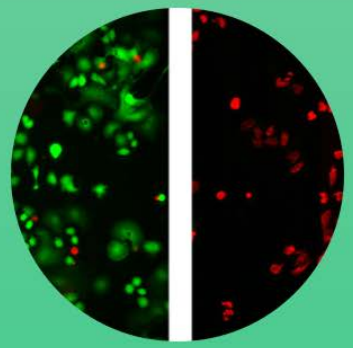

**In Vitro Characterization**

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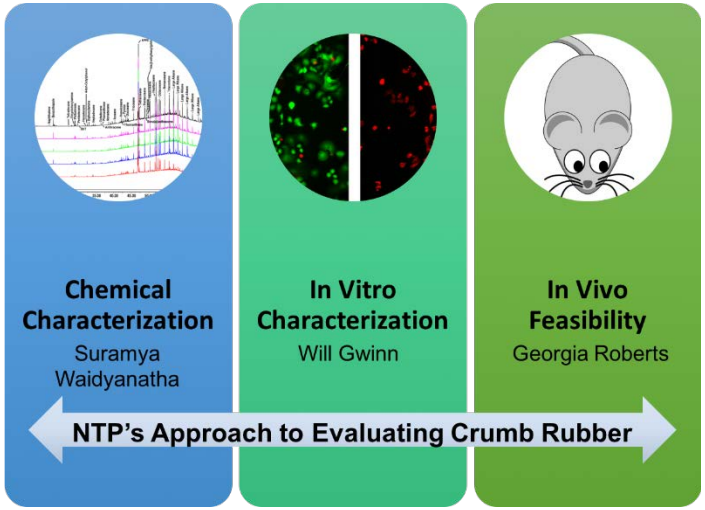
**NTP's Approach to Evaluating Crumb Rubber**

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

# NTP's Research Approach: Overview

- Key questions
  - What experimental models are useful for characterizing the toxicity of crumb rubber or bioaccessible constituents?
  - What routes of exposure are most likely to result in systemic exposure?
  - What constituents of crumb rubber are bioaccessible and/or bioavailable?
  - How does the bioaccessibility/bioavailability of constituents vary based on route of exposure?




https://ntp.niehs.nih.gov/results/areas/syntheticturf/research.html

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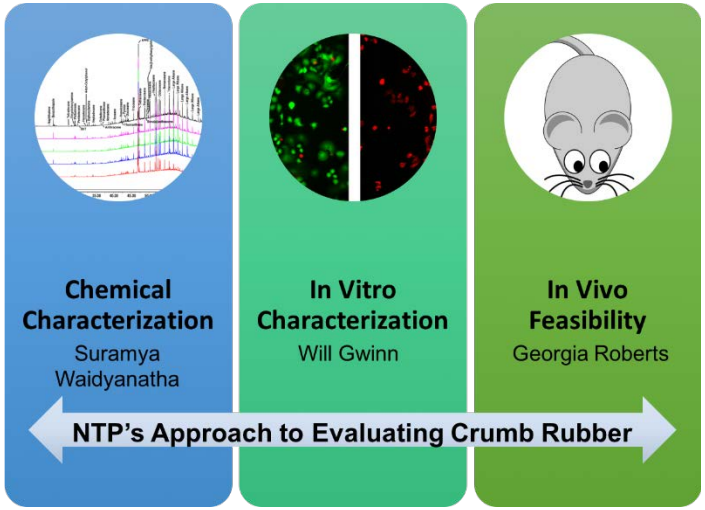
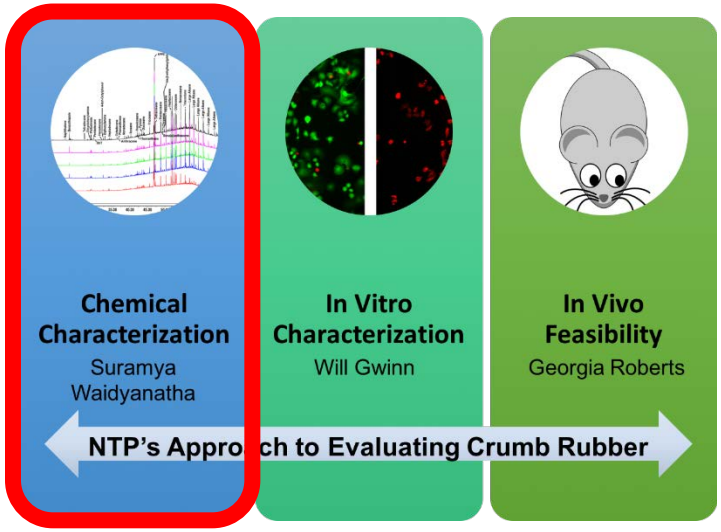



https://ntp.niehs.nih.gov/results/areas/syntheticturf/research.html

# NTP's Research Approach: Chemistry

- Chemical characterization of material used for in vitro and in vivo testing
  - Inform chemical analysis of biological samples collected from the in vitro and in vivo studies




  - Allow comparison of the tested material to samples collected from new and **weathered** synthetic turf fields as part of the OEHHA and FRAP studies

**NTP Lead: Suramya Waidyanatha**

# NTP's Research Approach: Chemistry

- Test material was received from OEHHA
  - Represents 2 CA manufacturers; from 1 manufacturer the materials received were from 2 manufacturing processes
- Characterization of combined material
  - 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

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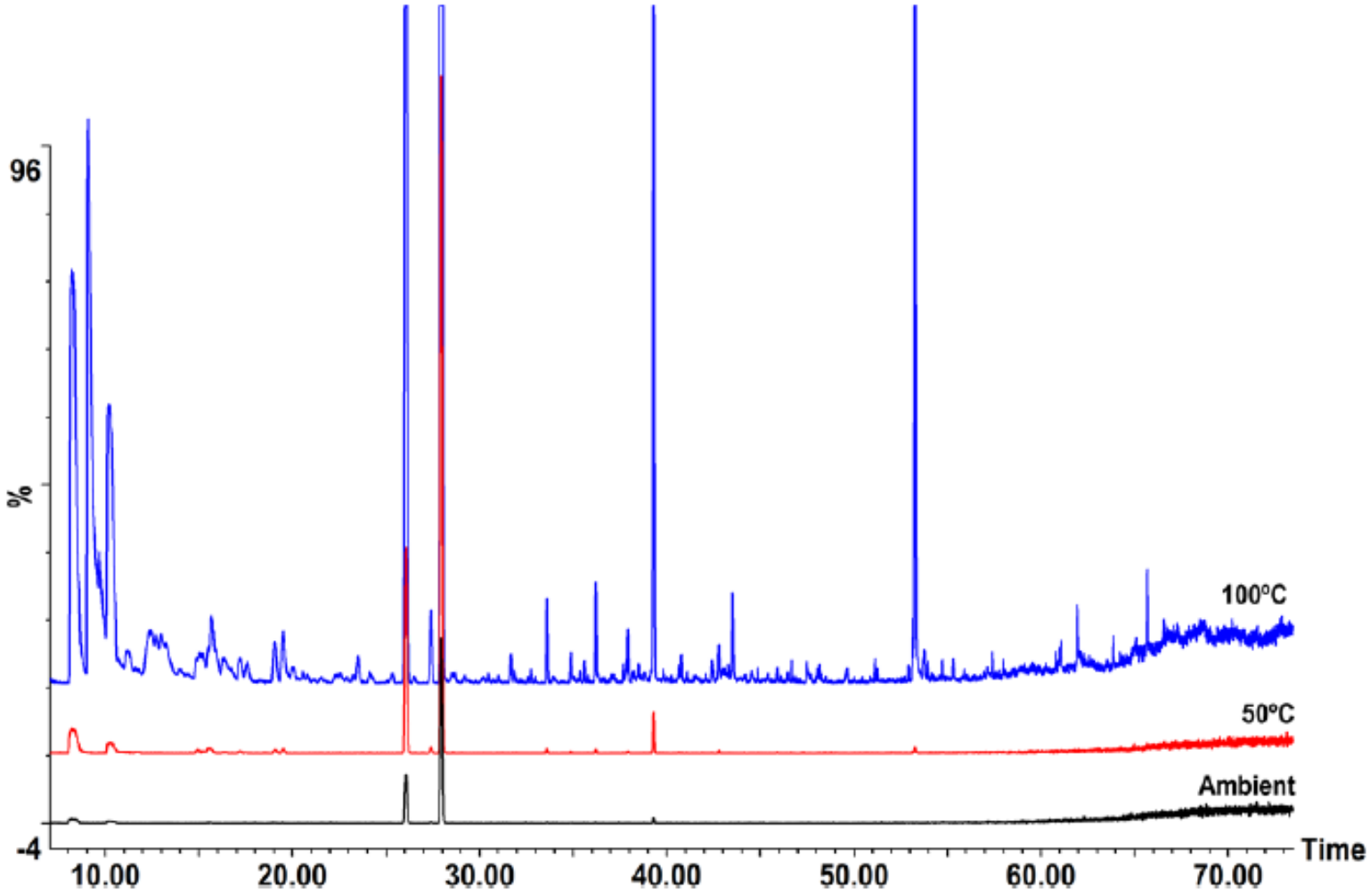
# NTP's Research Approach: Chemistry

## GC-MS Analysis; VOC Profiles at Different Incubation Temperatures




**NTP Lead: Suramya Waidyanatha**

# NTP's Research Approach: Chemistry

## VOCs and Estimated Concentrations

| Compound | Average (ppm) |
|---|---|
| Methyl Isobutyl Ketone | 3.35 |
| Aniline | 1.05 |
| Benzothiazole | 0.719 |
| Toluene | 0.428 |
| Cyclohexanone | 0.366 |
| 2,4,4- Trimethylpentene | 0.120 |
| Methyl Vinyl Ketone | 0.106 |
| Hexamethylcyclotrisiloxane | 0.100 |
| 5-Methyl-2-hexanone | 0.0870 |
| Trichloroethylene | 0.0665 |
| Butyraldehyde | 0.0303 |
| m/p-Xylene | 0.0167 |
| Hexanal | 0.0189 |
| Phenol | 0.0817 |
| 3-Methylhexane | 0.0103 |

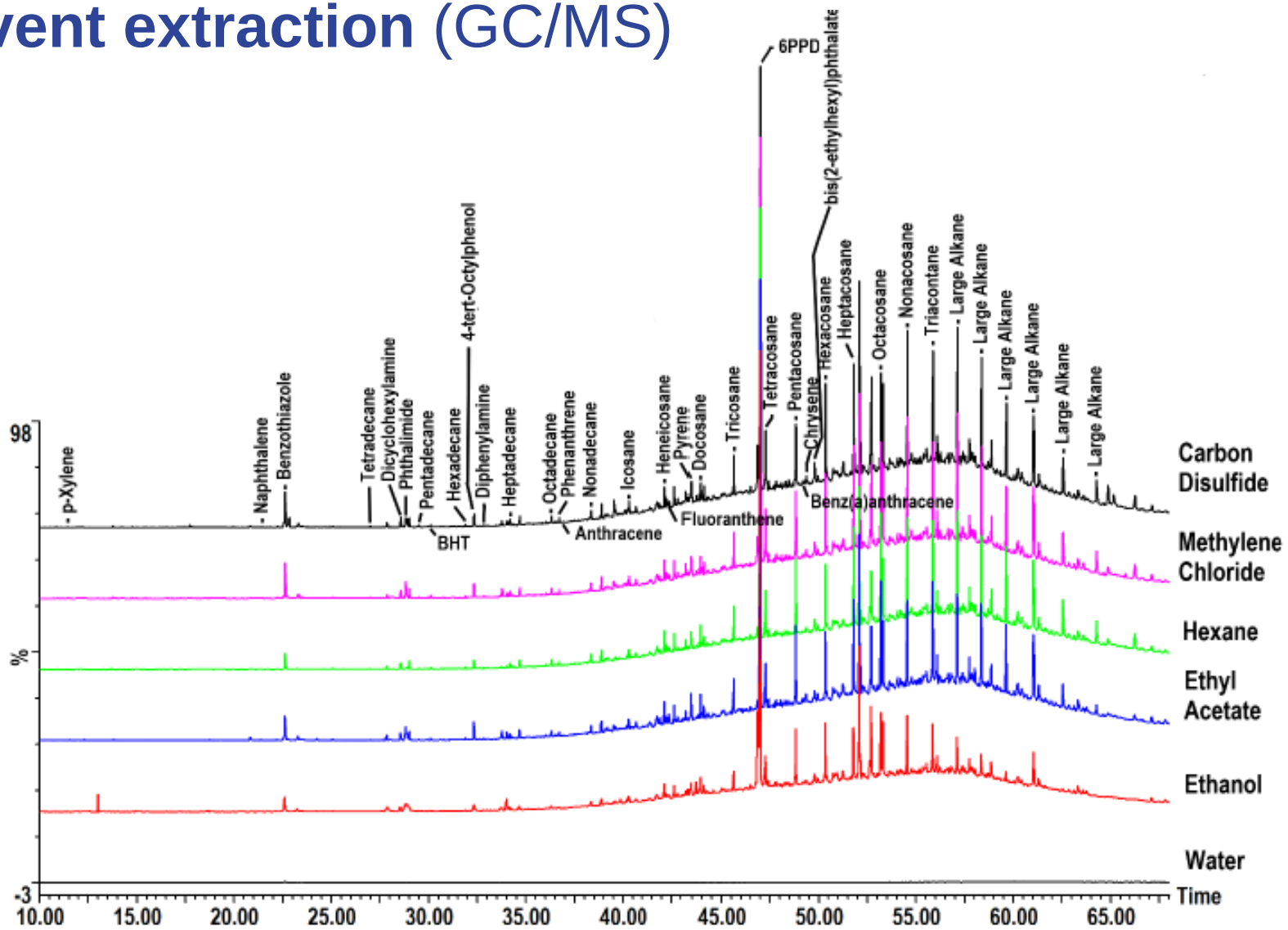
| Compound | Average (ppm) |
|---|---|
| 1-Methylnaphthalene | 0.00904 |
| Napthalene | 0.00644 |
| Styrene | 0.00632 |
| Furfural | 0.00539 |
| 1,2,4- Trimethylbenzene | 0.00520 |
| 2-Methylnaphthalene | 0.00511 |
| Ethanol | 0.00495 |
| Pentadecane | 0.00454 |
| Decane | 0.00377 |
| Tetradecane | 0.00371 |
| Butylated Hydroxytoluene | 0.00250 |
| Tridecane | 0.00182 |
| 3-Ethyltoluene | 0.00135 |
| t-Butylamine | 0.00132 |
| o-Xylene | 0.00123 |
| Ethylbenzene | 0.00108 |
| Acetone | 0.00499 |
| Heptane | 0.00228 |

**Total is ~ 0.0007%**

**NTP Lead: Suramya Waidyanatha**

# NTP's Research Approach: Chemistry

## Solvent extraction (GC/MS)

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# NTP's Research Approach: Chemistry

## Quantitation of Metals (ICP-MS or ICP-OES)

**Total quantified ~ 3 %**

| Compound | Average |
|---|---|
| Zinc | 1.68% |
| Silicon | 0.932% |
| Aluminum | 0.106% |
| Potassium | 456 ppm |
| Iron | 432 ppm |
| Magnesium | 344 ppm |
| Sodium | 302 ppm |
| Cobalt | 145 ppm |
| Copper | 44.6 ppm |
| Lead | 12.5 ppm |
| Nickel | 5.93 ppm |
| Manganese | 5.89 ppm |
| Barium | 5.22 ppm |
| Tin | 1.95 ppm |
| Arsenic | 0.805 ppm |
| Cadmium | 0.649 ppm |
| Chromium | BLOQ (<13 ppm) |

**NTP Lead: Suramya Waidyanatha**

# NTP's Research Approach: Chemistry

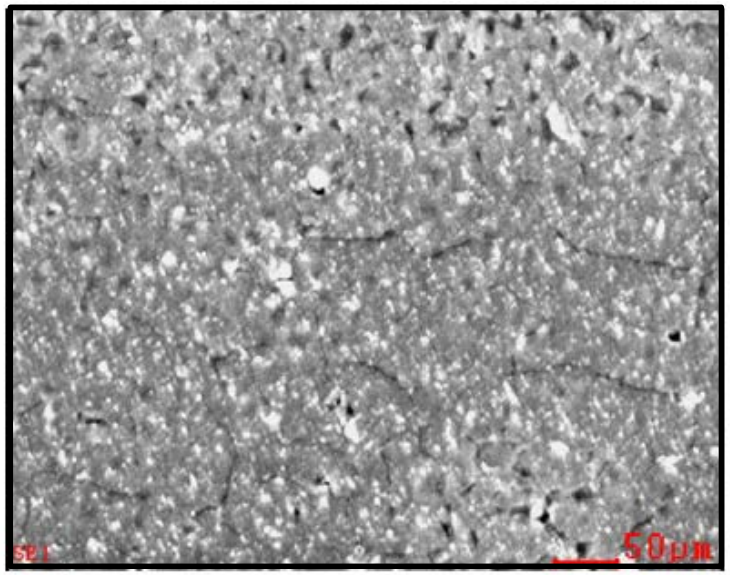
## Morphology and Elemental Composition (Optical Microscopy)

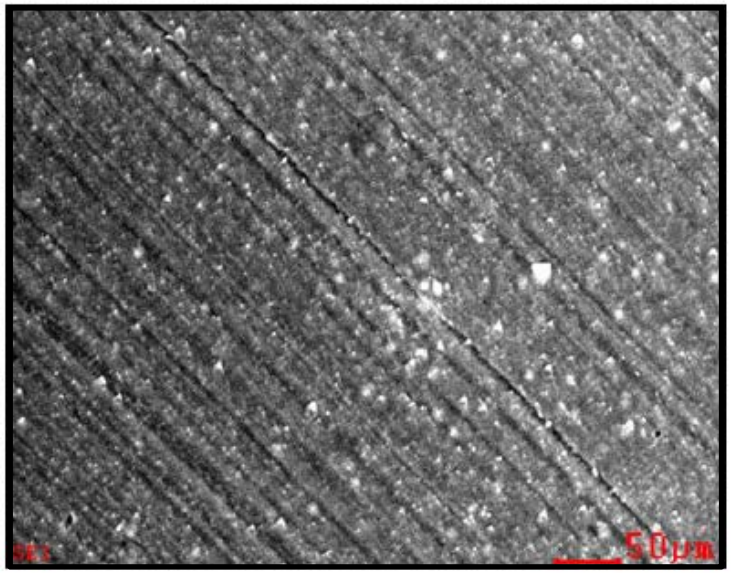
- Irregular particles with a wide range of sizes from <0.1 to 4 mm
- Light and dark particles, presence of fibers noted

**NTP Lead: Suramya Waidyanatha**

# NTP's Research Approach: Chemistry

## Morphology and Elemental Composition (SEM/EDS)



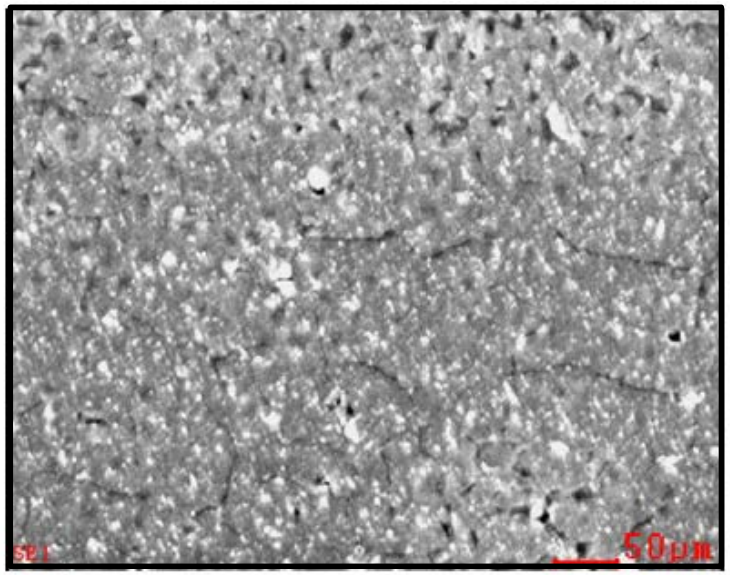



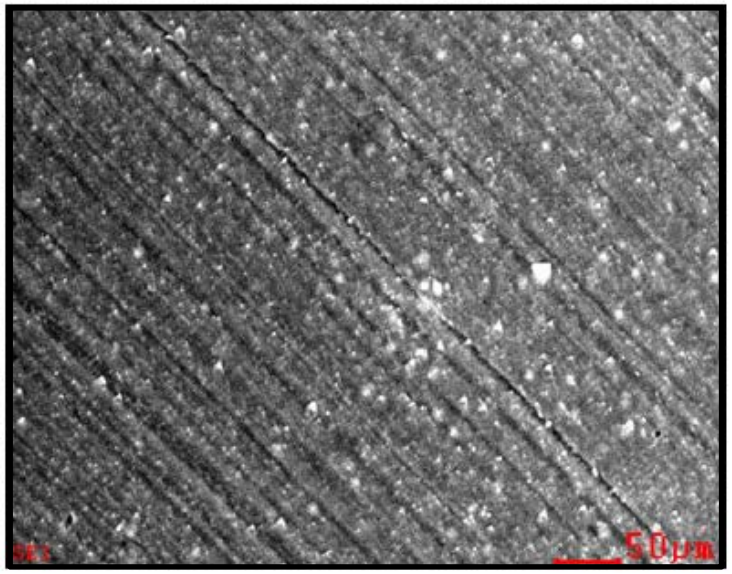

| Element | Average (Std. Dev) |
|---|---|
| C | 87.2 (6.6) |
| O | 3.5 (1.3) |
| Mg | 0.1 (0.1) |
| Al | 1.2 (2.5) |
| Si | 1.4 (2.2) |
| S | 3.5 (1.7) |
| Ca | 0.1 (0.2) |
| Zn | 3.0 (1.8) |
| Inorganics Total | 9.3 (5.4) |

**NTP Lead: Suramya Waidyanatha**

# NTP's Research Approach: Chemistry

## Morphology and Elemental Composition (SEM/EDS)

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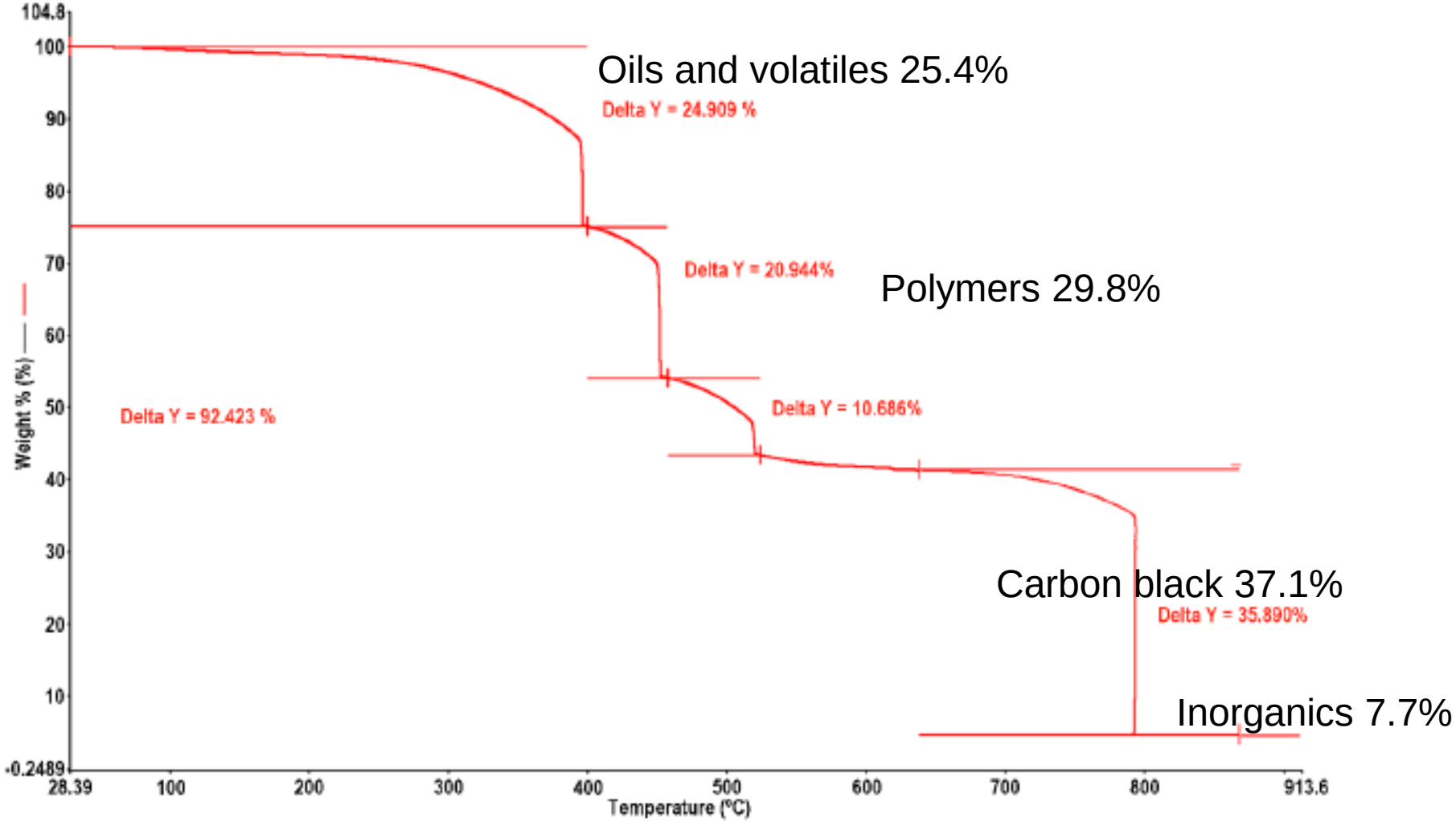
About 8% estimated by thermogravimetric
Only 3% identified as metals

**NTP Lead: Suramya Waidyanatha**

# NTP's Research Approach: Chemistry

## Morphology and Elemental Composition (Thermogravimetric)

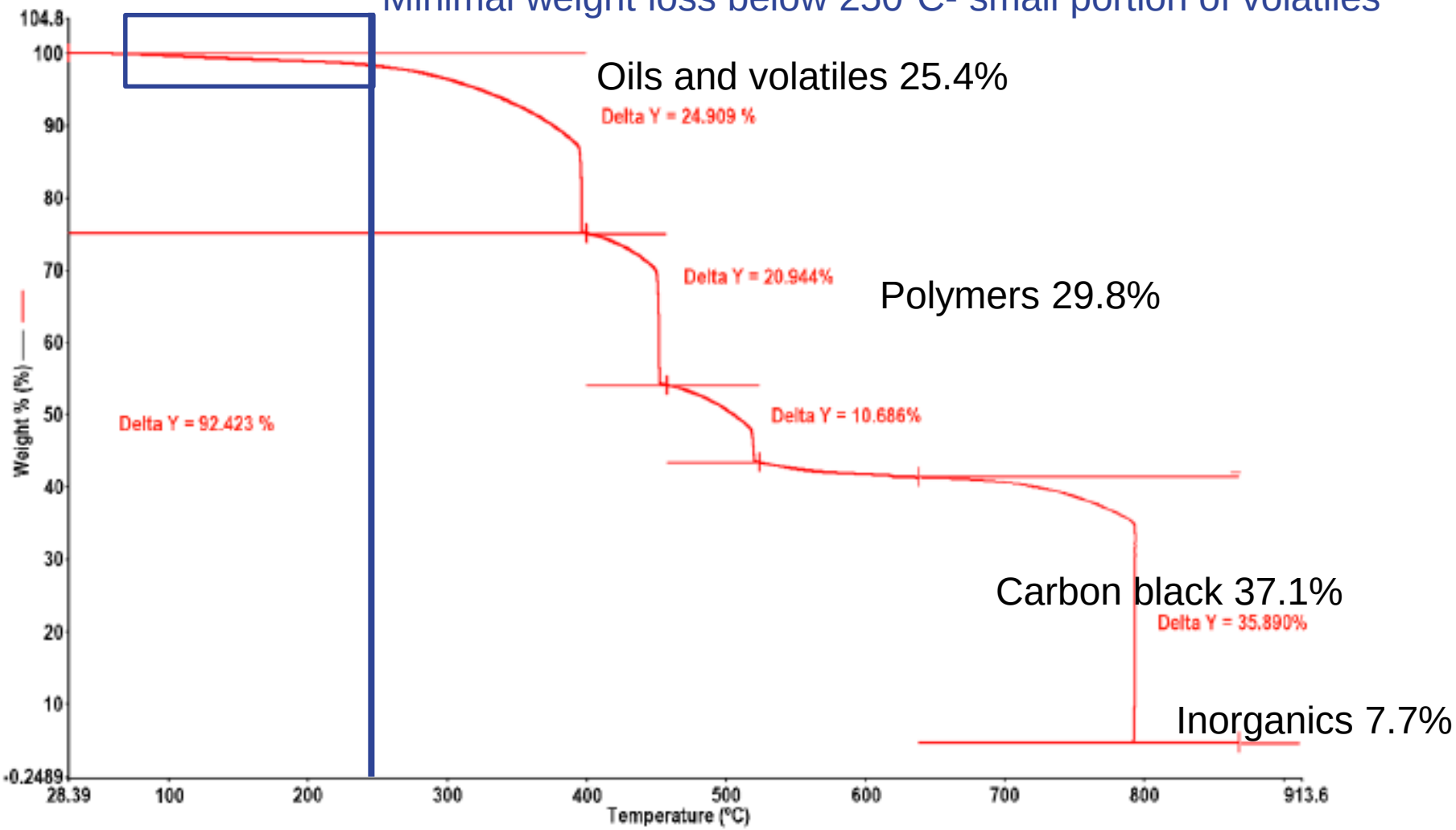



NTP Lead: Suramya Waidyanatha

# NTP's Research Approach: Chemistry

## Morphology and Elemental Composition (Thermogravimetric)

Minimal weight loss below 250ºC- small portion of volatiles



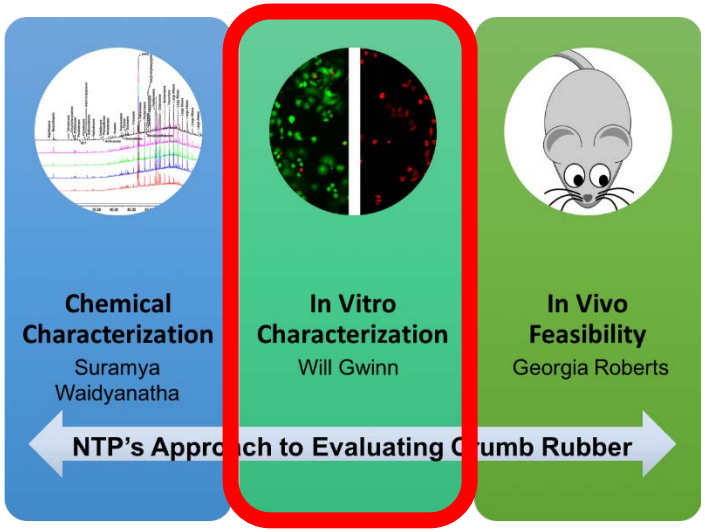

NTP Lead: Suramya Waidyanatha

## Summary

- Volatile and semivolatile organic compounds comprise approximately 0.0007%
- Inorganics comprise approximately 8-9%
  - Metals account for 3%

# NTP's Research Approach: In Vitro

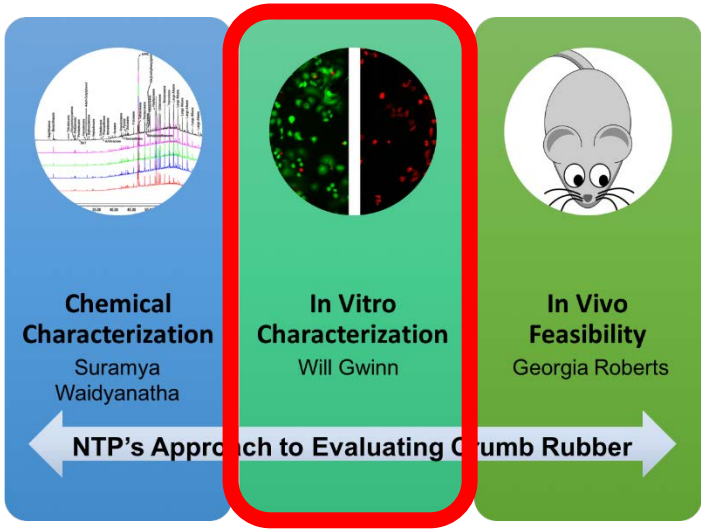
- In Vitro Evaluations
  - Evaluate the effect of bioaccessible crumb rubber constituents in human cell lines
  - Develop an assay that may be used for read-across of additional test materials




**NTP Lead: Will Gwinn**

# NTP's Research Approach: In Vitro

- Test System
  - Incubate crumb rubber in cell culture media as extraction method to produce 'conditioned' media
    - 0 (media only) or 100 mg/mL
    - 3 hours, 1, 4 or 7 days
    - Room temperature, 37ºC or 60ºC

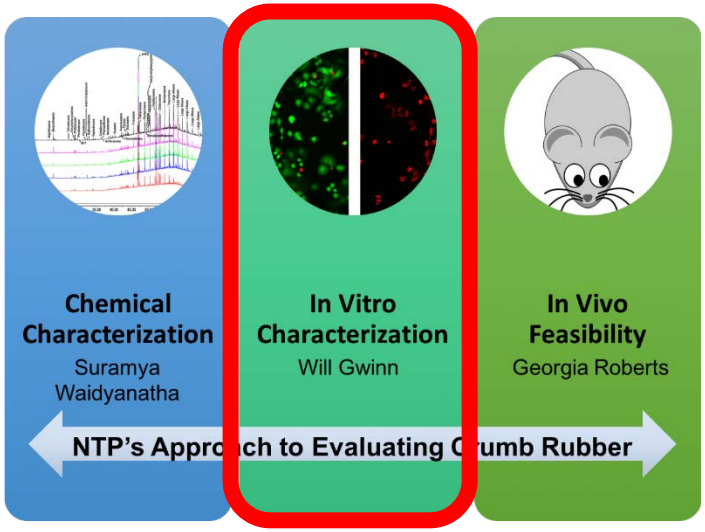



    - Crumb rubber (in media) vortexed every 1-2 hours for 12 hours/day
  - Remove crumb rubber by centrifugation and sterile filter supernatant to produce 'conditioned' media for cell exposures
    - Conduct serial dilutions of media 'conditioned' at 100 mg/mL (1X concentration) to produce 0.5, 0.25, 0.125 and 0.0625 concentrations

**NTP Lead: Will Gwinn**

# NTP's Research Approach: In Vitro

- Test System
  - Incubate crumb rubber in cell culture media as extraction method to produce 'conditioned' media
  - Expose human cells to 'conditioned' media for 24 or 72 hours

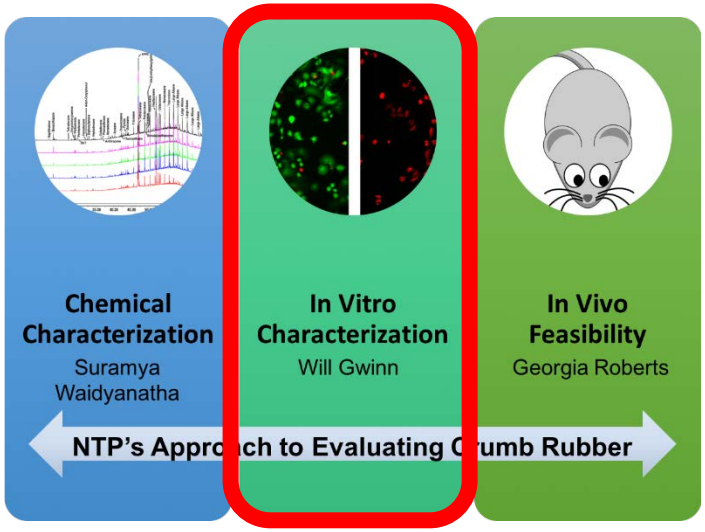



  - Immortalized, non-tumorigenic human cell lines
    - Keratinocytes (HaCaT) (dermal exposure)
    - Alveolar epithelial cells (HPL-1D) (inhalation exposure)

**NTP Lead: Will Gwinn**

# NTP's Research Approach: In Vitro

- Test System
  - Incubate crumb rubber in cell culture media as extraction method to produce 'conditioned' media
  - Expose human cells to 'conditioned' media
  - Evaluate cytotoxicity and other endpoints

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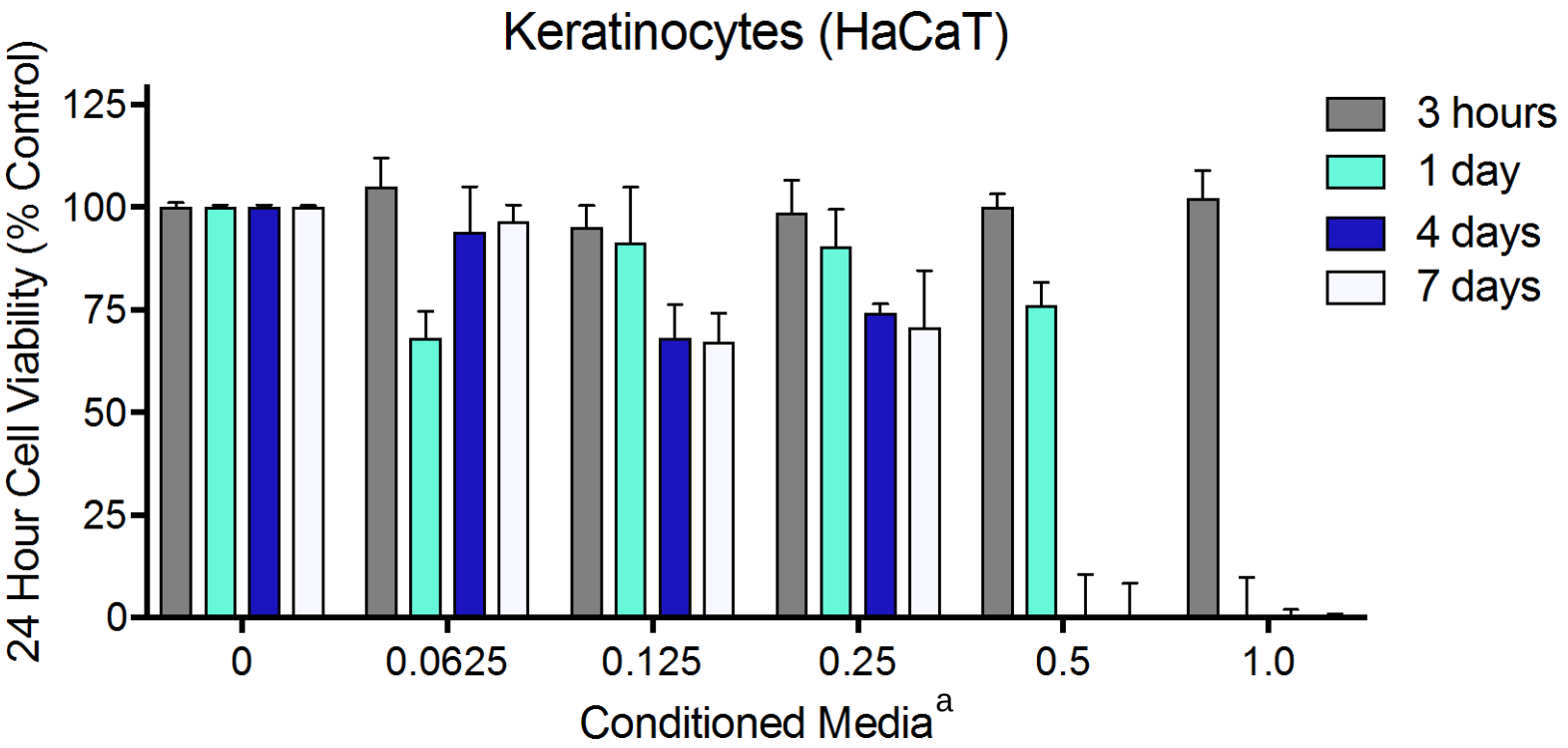
# NTP's Research Approach: In Vitro

## Media 'Conditioned' at 60ºC for 3 hours, 1, 4 or 7 days
## 24 Hour Cell Exposure




[a] Media was conditioned at 100 mg/mL (1X) and then serial diluted to produce the 0.5, 0.25, 0.125 and 0.0625 concentrations

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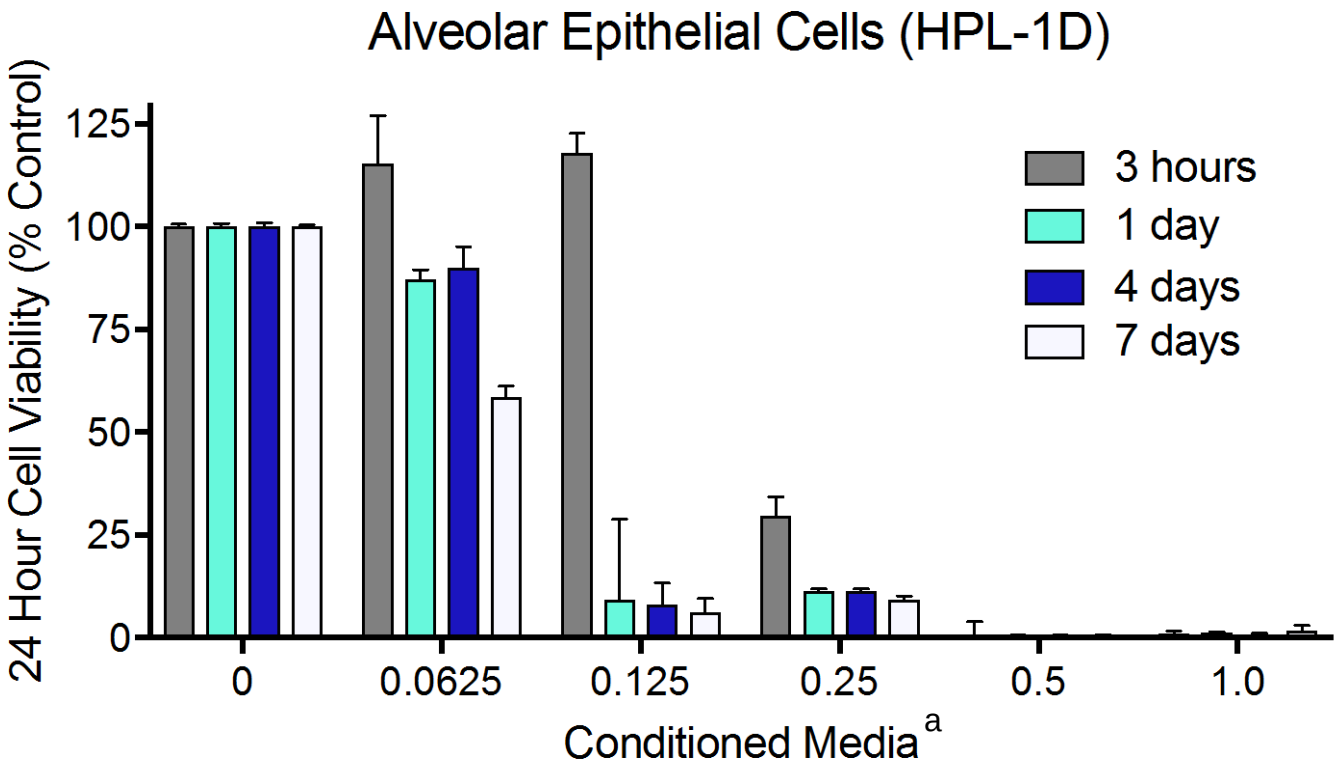
# NTP's Research Approach: In Vitro

## Media 'Conditioned' at 60ºC for 3 hours, 1, 4 or 7 days
## 24 Hour Cell Exposure

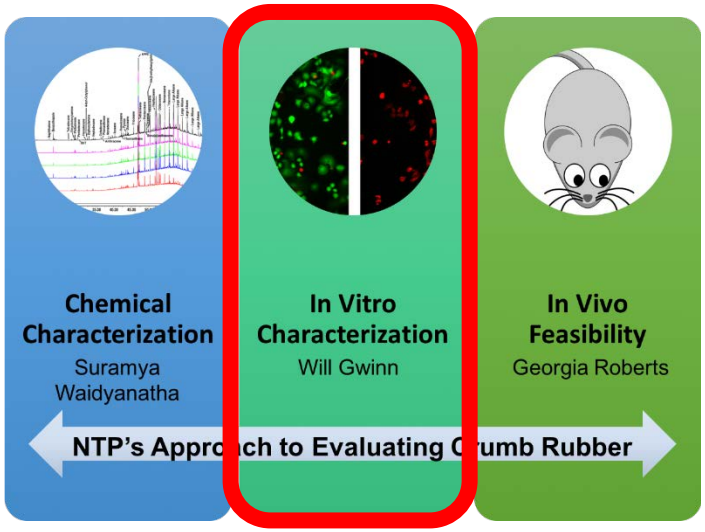



[a] Media was conditioned at 100 mg/mL (1X) and then serial diluted to produce the 0.5, 0.25, 0.125 and 0.0625 concentrations

**NTP Lead: Will Gwinn**

# NTP's Research Approach: In Vitro

## Summary



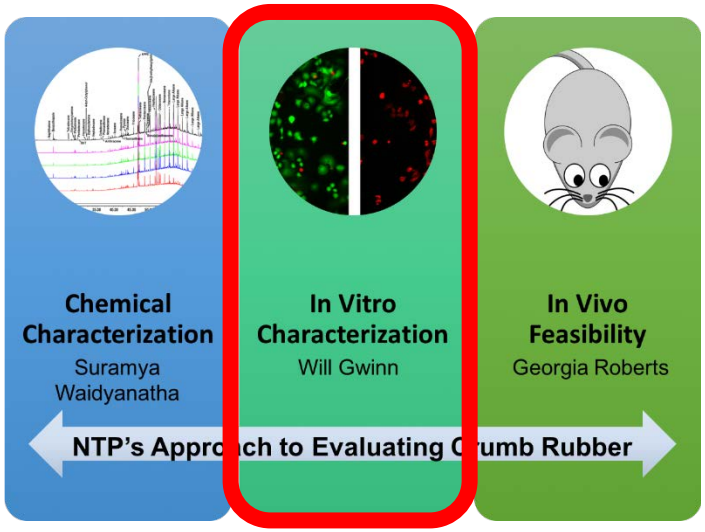

- Cytotoxic effects observed in keratinocytes (HaCaT) and alveolar epithelial cells (HPL-1D) when exposed to 'conditioned' media
  - Cytotoxicity more pronounced in alveolar epithelial cells
  - Cytotoxicity observed following exposure to 'conditioned' media from crumb rubber incubated at room temperature, 37ºC and 60ºC (only 60ºC data shown)
    - Increasing potency at higher incubation temperatures
  - Similar effects were observed with longer cell exposures (72 hours versus 24 hours) (only 24 hour data shown)
- Evaluated microbial contamination

**NTP Lead: Will Gwinn**

# NTP's Research Approach: In Vitro

## What's next?

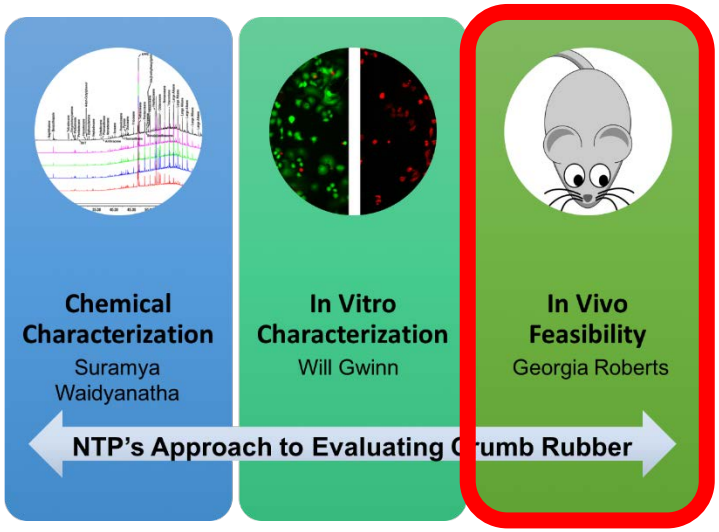
- Evaluate other endpoints
  - Oxidative stress
  - Genetic toxicity
- Analyze 'conditioned' media (LC-MS) to identify bioaccessible constituents
- Evaluate other human cell types
  - Intestinal epithelial cells (oral exposure)
  - Primary bone marrow-derived hematopoietic cells




**NTP Lead: Will Gwinn**

# NTP's Research Approach: In Vivo

- In vivo testing
  - Is in vivo testing feasible?
  - Provide information regarding feasibility and study design considerations for working with crumb rubber

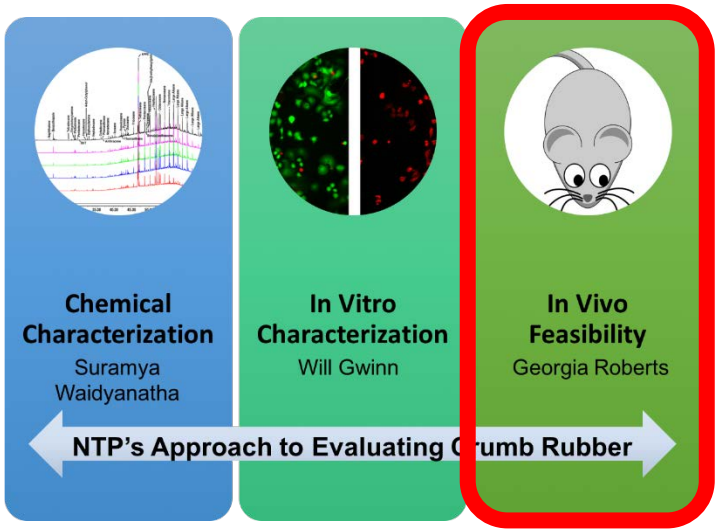



**NTP Lead: Georgia Roberts**

# NTP's Research Approach: In Vivo

- Benchtop feasibility
  - Determine what routes of exposure to pursue
- Test material was fractionated by mesh size to maximize opportunities to test various routes of exposure

| Sieve Mesh | Size (µm) | % Total by Weight |
|---|---|---|
| 14 | > 1410 | 70.56 |
| 40 | 420 - 1410 | 28.71 |
| 80 | 170 - 420 | 0.39 |
| 400 | 37 - 170 | 0.34 |

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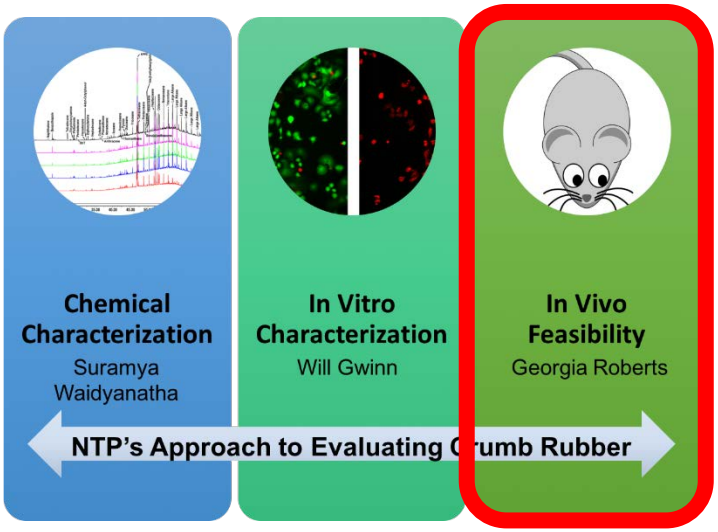


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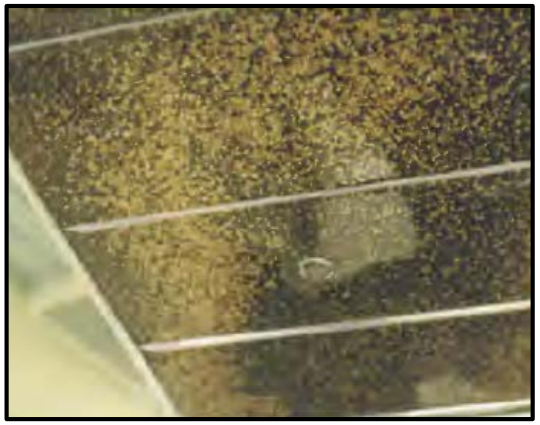
**Example of Benchtop Feasibility**
Crumb rubber in bedding
(50/50 weight)

14 mesh
(>1410 µm)
After day 4 of shaking



40 mesh
(420- 1410 µm)
Immediately settled

**NTP Lead: Georgia Roberts**

# NTP's Research Approach: In Vivo

- Benchtop feasibility
  - Determine what routes of exposure to pursue
  - Oral gavage, feed and mixed bedding have been conducted
  - On-going feasibility work for dermal and inhalation
- Mesh sizes being used:

| Sieve Mesh | Size (µm) | % Total by Weight |
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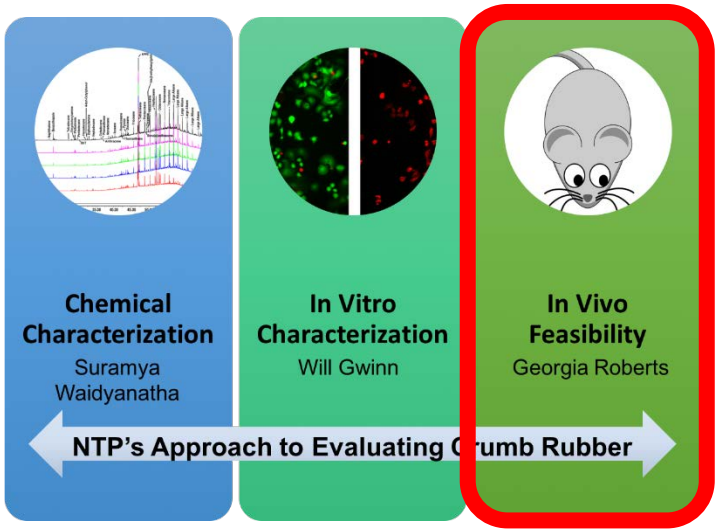


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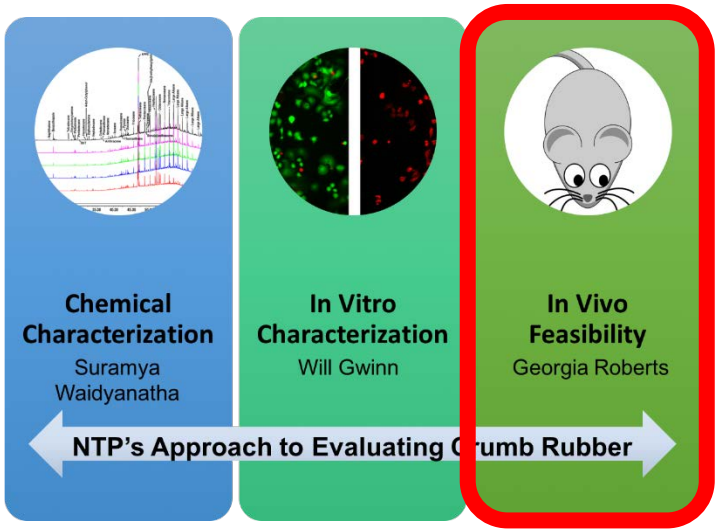


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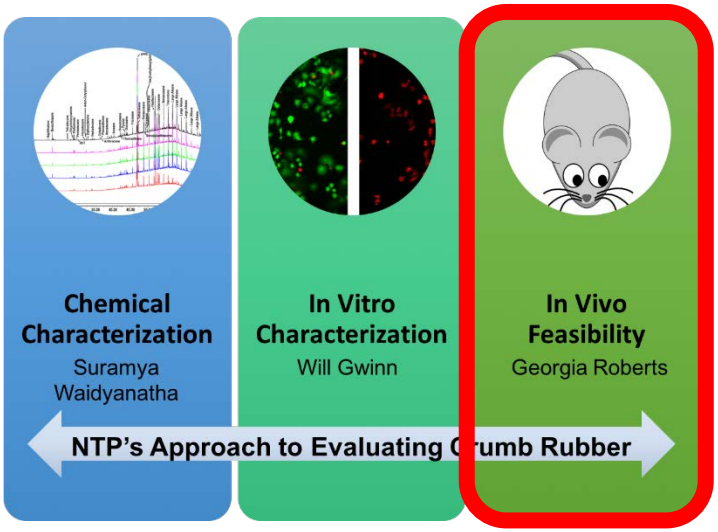
- Combined for feed and bedding
  - No homogeneity concerns for feed
  - Homogeneity issues with 40 mesh in bedding not of concern due to animal behavior and availability of settled material

**NTP Lead: Georgia Roberts**

# NTP's Research Approach: In Vivo

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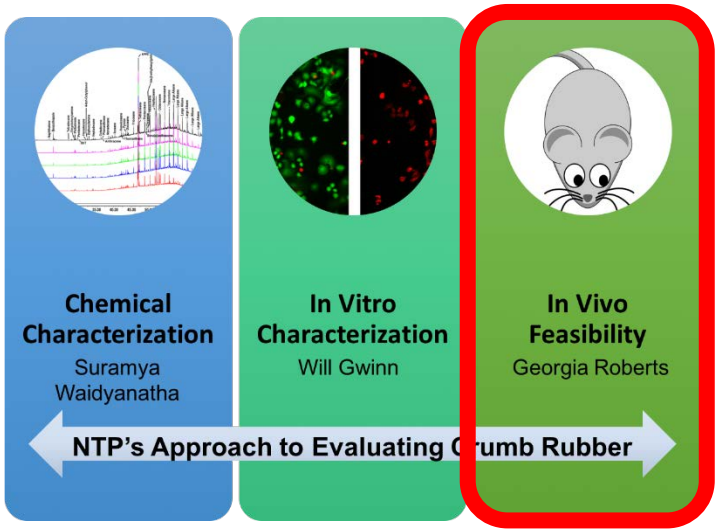
- Combined for feed and bedding
  - No homogeneity concerns for feed
  - Homogeneity issues with 40 mesh in bedding was not of concern due to animal behavior and availability of settled material
- Oral gavage
  - Only mesh size that met gavagability requirements

[Additional chemical characterization work to compare fractions to original test material]

**NTP Lead: Georgia Roberts**

# NTP's Research Approach: In Vivo

- Female B6C3F1/N mice
  - **Feed:** 50,000 ppm
  - **Bedding**: 50/50 by weight
  - **Oral gavage:** 1250 mg/kg
- 14 days
- Characterize exposure
  - Urine
  - Blood
  - Plasma
- Toxicity endpoints
  - Limited histopathology
  - Bone marrow cytology
  - Clinical pathology

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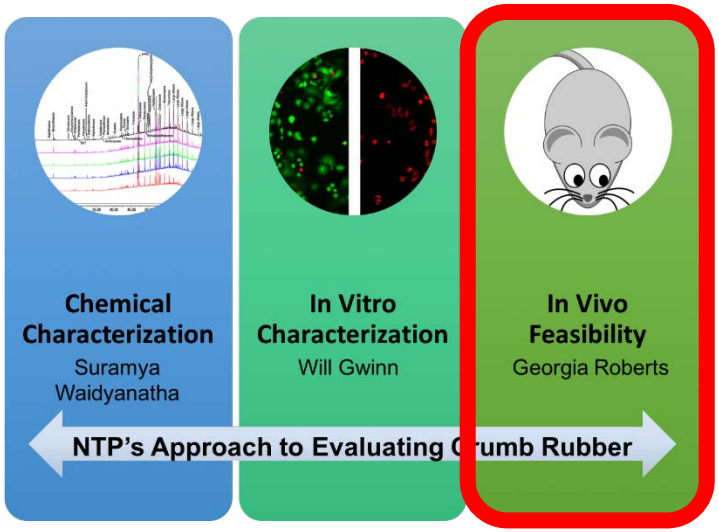


## Is there evidence of systemic exposure?

Are chemical constituents detected in biological fluids?

Is there evidence of biological effect?

**NTP Lead: Georgia Roberts**

# Timeline

- **November 2015**: OEHHA nominates synthetic turf crumb rubber to NTP (short term and in vitro testing)
- **June 2016:** Research Concept presented to BSC
- **June 2017:** Update to BSC
  - Chemical characterization work near completion
  - In vitro work near completion
  - In vivo
    - In-life completed for oral gavage, feed, bedding
    - Dermal and inhalation feasibility on-going
- **Late 2017/Early 2018:** Data release of completed work

# Acknowledgements


- Matt Bell
- Abee Boyles
- Amy Brix
- Michelle Cora
- Mike DeVito
- Darlene Dixon
- Stephen Ferguson
- Will Gwinn
- Stephanie Holmgren
- Jui-Hua Hsieh
- Ruth Lunn
- Dave Malarkey
- Scott Masten
- Rachel McIntosh-Kastrinsky
- Dan Morgan
- Arun Pandiri
- Cynthia Rider
- Matt Stout
- Erik Tokar
- Suramya Waidyanatha
- Nigel Walker

- Battelle Memorial Institute (Toxicology Contract)
  PI: Barney Sparrow
  Project Leads: Karen Elsass, Dawn Fallacara, Amit Gupta, Jamie Richey

- Battelle Memorial Institute (Chemistry Contract)

# Questions for BSC

- Please comment on whether NTP's research program appropriately addresses BSC and other inputs received at the June 2016 BSC meeting. Are there additional areas in which NTP should focus effort?
- Please comment on the scope of NTP's effort, specifically regarding the likelihood of meeting stated objectives in a reasonable time frame.
- Please comment on whether NTP's research program is addressing critical questions regarding public health significance of exposure to crumb rubber.
- Please comment on additional outreach efforts NTP might consider for engagement with key stakeholders regarding its crumb rubber research activities.