Synthetic Turf/Recycled Tire Crumb Rubber Research

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

NTP Board of Scientific Counselors
June 20, 2018
Variety of Uses for Recycled Tires

From this...to this

(tire mulch)
Variety of Uses for Recycled Tires
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
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
**RESEARCH FOCUS:** What conditions in an experimental setting have the potential to result in systemic exposure to crumb rubber constituents?
RESEARCH FOCUS: What conditions in an experimental setting have the potential to result in systemic exposure to crumb rubber constituents?
• **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: Suramya 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

**Lead:** Suramya Waidyanatha, NIEHS
• **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

Lead: Suramya Waidyanatha, NIEHS
• **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
**In Vitro Evaluations**

**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

Lead: William Gwinn, NIEHS
**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

Lead: William Gwinn, NIEHS
• **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

- 14 Mesh
- 40 Mesh
- 80 Mesh
- 400 Mesh

Dermal

Feed

Bedding

Gavage

400 Mesh Only

Lead: Jamie Richey, Battelle
Feasibility to Conduct In Vivo Studies

• Results

Material was size fractionated

<table>
<thead>
<tr>
<th>Sieve Mesh</th>
<th>Size (µm)</th>
<th>% Total by Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-80</td>
<td>170 to &gt; 1410</td>
<td>&gt; 99</td>
</tr>
<tr>
<td>400</td>
<td>37 - 170</td>
<td>0.34</td>
</tr>
</tbody>
</table>

• 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

Lead: Jamie Richey, Battelle
• 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
**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
**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:**

![PCA plots](attachment:PCA_plots.png)
**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.

| Ion Mode | Oral Gavage |  | Feed |  | Mixed-Bedding |  |
|----------|-------------|----------------|-------|----------------|----------------|
|          | Plasma      | Urine          | Plasma| Urine          | Plasma          | Urine |
| Positive | 25          | 0              | 5     | 0              | 0               | 0     |
| Negative | 13          | 1              | 2     | 0              | 4               | 1     |

Number of tentatively identified compounds with a \textbf{p-value}<0.05 and a \textbf{fold change}>5 in treated versus controls.

Lead: Georgia Roberts, NIEHS
• **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

Define Hypotheses & Design a Testing Strategy

Data Mining

QSAR Profiling

Bioactivity Screening

In vitro Studies

Longer-term in vivo Tests

Short-term in vivo Tests

Fit for purpose products

Inform Public Health Decisions

Chemical Characterization

Suramya Weidyanatha

In Vitro Characterization

William Gwinn

Feasibility to Conduct In Vivo Studies

Jamie Richey

14-Day In Vivo Studies

Georgia Roberts
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

[Logos of OEHHA, ATSDR, ECHA, and RIVM]
Acknowledgements

NIEHS
Matt Bell
Abee Boyles
Michelle Cora
David Crizer
Mike DeVito
Darlene Dixon
Stephen Ferguson
William Gwinn
Stephanie Holmgren
Michelle Hooth
Jui-Hua Hsieh
Ruth Lunn
Dave Malarkey
Scott Masten
Dan Morgan
Arun Pandiri
Cynthia Rider
Matthew Stout
Erik Tokar
Suramya Waidyanatha
Nigel Walker
Mary Wolfe

Battelle
Barney Sparrow (PI, Toxicology)
Karen Elsass
Dawn Fallacara
Jamie Richey
Brian Burback (PI, Chemistry)
Tim Cristy

ICF International
Dave Burch (PI)
Audrey Turley (PM)
Susan Blaine
Kate Helmick

RTI International
Tim Fennell
EPL
Amy Brix

NIEHS Battelle
EPL
Questions