The National Toxicology Program Synthetic Turf/Recycled Tire Crumb Rubber Research: Characterization of a Crumb Rubber Lot for Use in in Vitro and in Vivo Studies

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Abstract

Artificial turf athletic fields have been growing in popularity due to decreased maintenance required relative to conventional fields. Crumb rubber (CR) used as an infill in artificial turf has brought public health concerns in recent years. The National Toxicology Program (NTP) is conducting research to improve the understanding of human health impacts following exposure to CR. As a part of the NTP research program, a lot of CR prepared by combining material from multiple commercial sources was analyzed using a variety of techniques to generate information on chemical and physical characteristics. Optical and scanning electron microscopy demonstrated that the lot consists of a range of particle sizes (0.1 to 4 mm) and types (dark and light rubber, visible inclusions, fibers). Thermogravimetric analysis revealed that the lot contains a minute fraction of volatile (VOCs) and semivolatile (SVOCs) organic compounds and ~8% inorganics by weight. Elemental analysis by inductively coupled plasma with atomic emission spectrometry or mass spectrometry (MS) identified zinc, aluminum, cobalt and other metals and metalloids totaling ~2.9% by weight. Analysis for VOCs and SVOCs by gas chromatography (GC) and MS with head space sampling detected a large number of constituents; 33 compounds were identified totaling ~0.0007% by weight in CR. Extraction of CR with multiple solvents covering different polarities showed that 0.6% and ~8% by weight, respectively, were extracted with water and methylene chloride, demonstrating that the majority of the extractable material consists of relatively non-polar organics. Analysis of methylene chloride extract by GC/MS identified 42 compounds with high confidence using authentic standards or reference library spectra, 7 of which were also identified in the VOC analysis, and 62 compounds with lower confidence using reference library spectra, 9 of which were also identified in the VOC analysis. An additional ~200 compounds previously reported to be in CR were investigated but were not detected in the extracts of the current lot. Analysis of solvent extracts of CR by liquid chromatography MS revealed few new analytes that were not previously detected by GC-MS. These data demonstrate that VOCs, SVOCs and metals constitute a very small fraction of the CR lot.

Introduction

- Synthetic turf fields are widely used in the United States and their use is continuing to expand.
- There is a rise in public health concern for playing on synthetic turf fields due to reported health effects in young adult soccer players in Washington State.
- Crumb rubber (CR) manufactured by shredding recycled automotive tires into rubber particles has been employed as the infill material in synthetic turf fields.
- CR is suspected to contain potential carcinogenic and toxic substances.
- Several federal and state research efforts are being undertaken to evaluate potential risks from crumb rubber exposure.
- California Office of Environmental Health Hazard Assessment (OEHHA) nominated synthetic turf/crumb rubber to the National Toxicology Program (NTP) to conduct short-term in vivo and in vitro studies to enhance understanding of potential health impacts of chemicals released from synthetic turf, with an emphasis on crumb rubber.
- This work focuses on characterizing a CR lot for use in NTP research activities.

Objective

To characterize a crumb rubber lot for use in NTP research activities.

Materials

CR LOT: Three lots of CR from 2 facilities manufactured either via ambient or cryogenic manufacturing process representing fresh crumb rubber were received through OEHHA. The three lots were combined to produce one test lot.

SIZE FRACTIONATION OF CR LOT: A weighed aliquot of CR lot was sieved using mesh sizes 14, 40, 80, and 400 and the weight % of each fraction was recorded. Small aliquots from 14-, 40- and 80-mesh sizes were removed for analysis and the remaining material was combined to generate one fraction (14-80-mesh).

STANDARDS: Over 160 compounds (or mixtures of compounds) reported as being present in crumb rubber in the literature, used in tire manufacture, or tentatively identified by mass spectrometry during method development were purchased from Sigma-Aldrich (St. Louis, MO), Alfa Aesar (Tewksbury, MA), and SPEX Certiprep (Metuchen, NJ). These included: Volatile organic compounds (VOCs); Phthalates; Polynuclear aromatic hydrocarbons (PAHs); Alkanes up to $C_{30;}$Vulcanization accelerators (various benzothiazoles and thiuram sulfides); Antioxidants and antiozonants (various diphenylamine and phenyldiamine derivatives and their decomposition product aniline, butylated hydroxytoluene, and butylated hydroxyanisole).

Standards for the volatiles analysis were prepared in either methanol or 2-phenoxyethanol (depending on boiling point so that the solvent would not interfere with the analyte). Standards for the solvent extract analysis were prepared in dichloromethane except for the alkanes mixture, which was diluted in hexane.

Methods and Instrumentation

Optical Microscopy: An Olympus SZX12 stereo microscope with an Olympus DP-71 camera (Tokyo, Japan).

Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectroscopy (EDS): JEOL 7600-F (JOEL, Tokyo, Japan) and Apollo X silicon drift detector (EDAX, Mahwah, NJ).

***Thermogravimetric Analysis*:** Pyris 1 (Perkin Elmer, Waltham, MA) thermal gravimetric analyzer

Analysis for Inorganics: Inductively coupled plasma (ICP) coupled with mass spectrometry (MS) or atomic emission spectrometry (AES)

Analysis for VOCs and SVOCs by Head Space Gas Chromatography-Mass Spectrometry: CTC Analytics CombiPAL autosampler (Leap Technologies, Carrboro, NC) coupled to a 6890 GC and a 5973 MSD (Agilent Technologies, Santa Clara, CA)

Estimation of Solvent-Extractable Constituents Fraction: by gravimetry/mass balance

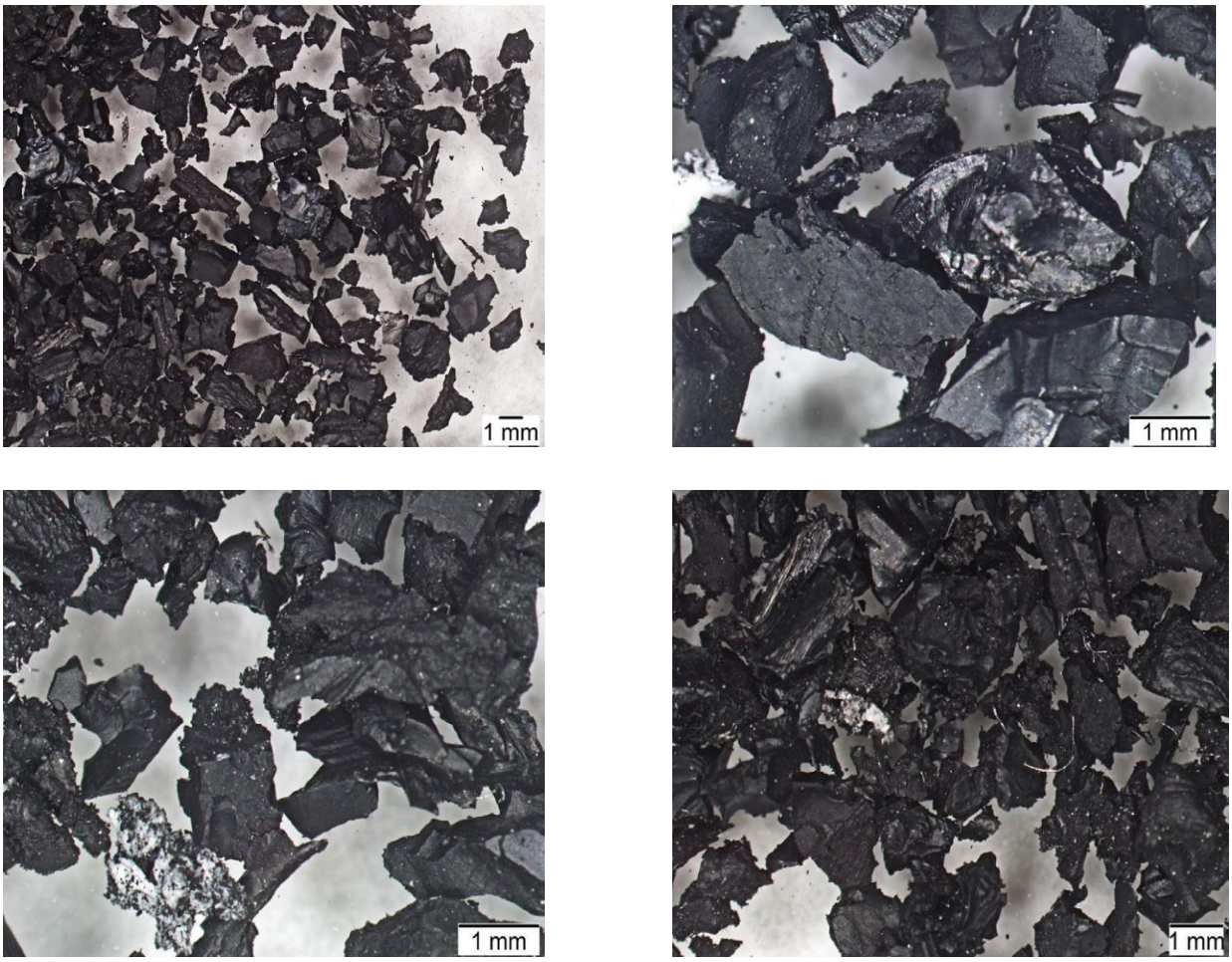
Analysis for Solvent-Extractable Constituents Fraction by Gas Chromatography-Mass Spectrometry: GC-MS using a 6890 GC coupled to a 5975 MSD (Agilent Technologies, Santa Clara, CA).

Analysis of Solvent-Extractable Constituents by Liquid Chromatography-Mass Spectrometry: Agilent 1100 LC (Agilent, Santa Clara, CA) and API 4000 QTrap MS (Sciex, Toronto, Canada) using electrospray (ESI) and atmospheric pressure chemical ionization (APCI) sources in both positive and negative ion modes

Size Fractionation of Crumb Rubber Lot and Analysis of Fractions by Head Space Gas Chromatography-Mass Spectrometry: CTC Analytics CombiPAL autosampler (Leap Technologies, Carrboro, NC) coupled to a 6890 GC and a 5973 MSD (Agilent Technologies, Santa Clara, CA)

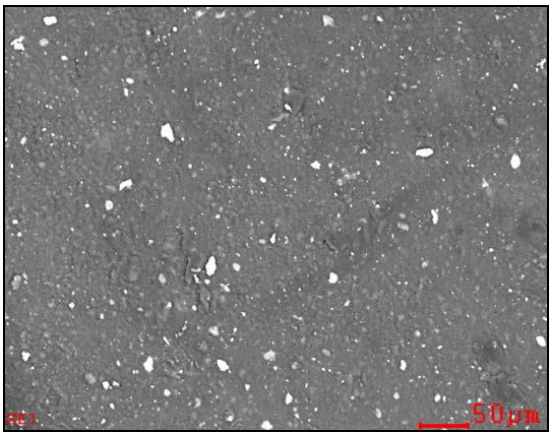
Results

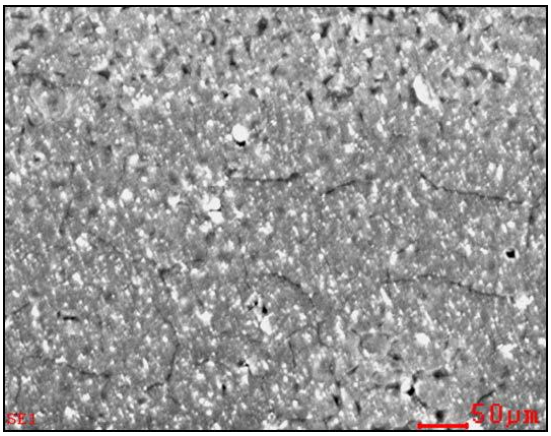
Optical Microscopy

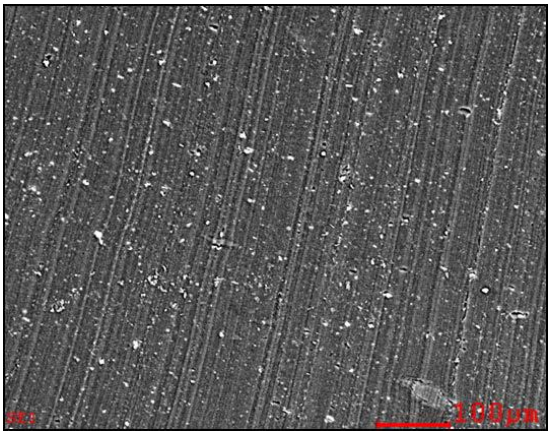


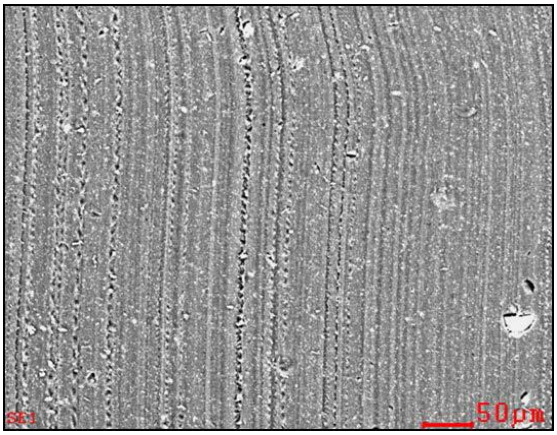
- Seven regions of CR lot were sampled and micrographed. Micrographs from 4 regions are shown above.
- The CR lot consists of irregular particles ranging from < 0.1 to 4 mm.
- There were light and dark particles and visible inclusions and fibers.

Scanning Electron Microscopy









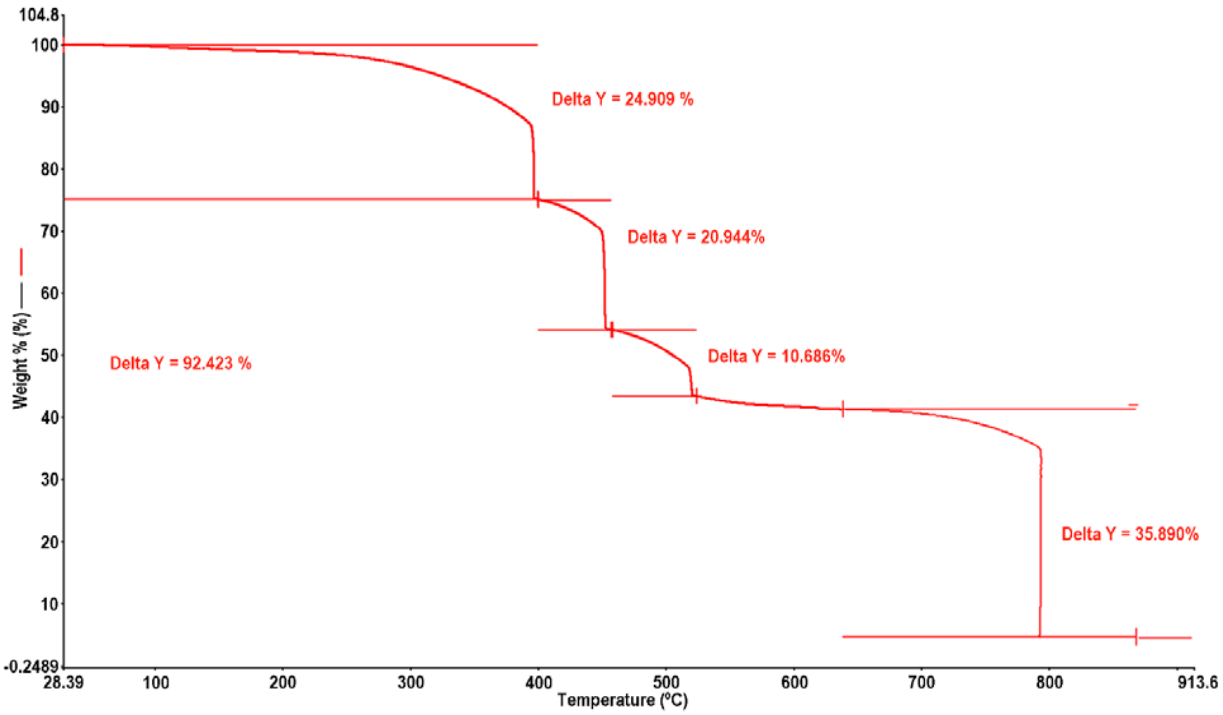
- Elemental composition of the analyzed areas for 6 grains are given in the table below.
- Data show the variability in composition between different CR grains.
- Carbon represents the major with sulfur, zinc, and oxygen likely coming from sulfur and zinc oxide used in the rubber vulcanization process.
- Elemental composition of the inclusions seen in CR was identified as likely clay (hydrated aluminum silicates), calcium carbonate, and talc (hydrated magnesium silicates) based on the levels of silicon, aluminum, calcium, magnesium, and oxygen detected in each inclusion.

Elemental Composition (%)

	Grain1	Grain2	Grain3	Grain4	Grain5	Grain6	Average	St Dev
C	**92.1**	**80.0**	**93.9**	**76.2**	**90.3**	**90.4**	**87.2**	**6.6**
O	**3.1**	**4.3**	**1.7**	**5.9**	**2.7**	**3.5**	**3.5**	**1.3**

Mg	**0.1**	**0.3**	**0.0**	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**
Al	**0.2**	**0.1**	**0.0**	**6.7**	**0.1**	**0.2**	**1.2**	**2.5**
Si	**0.5**	**1.0**	**0.2**	**6.3**	**0.1**	**0.3**	**1.4**	**2.2**
S	**2.4**	**7.2**	**3.1**	**2.2**	**2.9**	**3.1**	**3.5**	**1.7**
Ca	**0.0**	**0.5**	**0.0**	**0.2**	**0.0**	**0.0**	**0.1**	**0.2**
Ti	**0.0**	**0.0**	**0.0**	**0.2**	**0.0**	**0.0**	**0.0**	**0.1**
Zn	**1.7**	**6.5**	**1.0**	**2.2**	**3.9**	**2.4**	**3.0**	**1.8**
Total	**4.9**	**15.6**	**4.3**	**17.9**	**7.1**	**6.1**	**9.3**	**8.5**

Thermogravimetric Analysis



- The minimal weight loss < 200 ºC suggests that VOCs make up a small fraction.
- The significant weight loss up to ~ 400 ºC is likely due to extender oils.
- The two step weight loss between ~ 400 ºC to ~ 520 ºC is likely due to presence of two types of polymers.
- Based on this assessment, the crumb rubber lot contains approximately 25% total VOCs, SVOCS and extender oils, 29.9% polymeric material, 37.1% carbon black, and 7.7% inorganics.

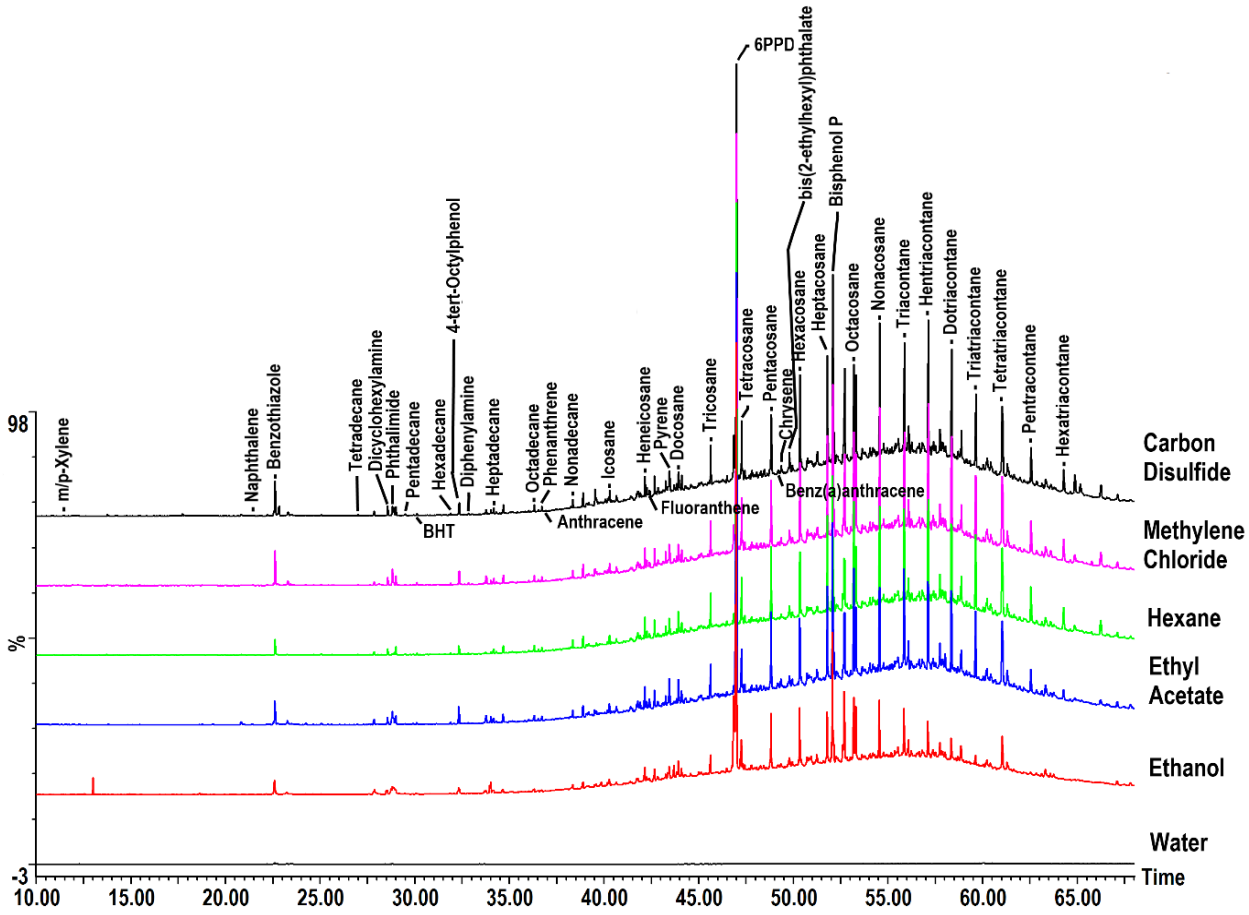
Analysis for Inorganics

Element	Replicate 1	Replicate 2	Average
Zinc (%)	**1.62**	**1.74**	**1.68**
Silicon (%)	**0.898**	**0.966**	**0.932**
Aluminum (%)	**0.104**	**0.108**	**0.106**
Potassium (ppm)	**473**	**438**	**456**
Iron (ppm)	**523**	**340**	**432**
Magnesium (ppm)	**367**	**321**	**344**
Sodium (ppm)	**304**	**300**	**302**
Cobalt (ppm)	**153**	**136**	**145**
Copper (ppm)	**51.6**	**37.5**	**44.6**
Lead (ppm)	**12.1**	**12.9**	**12.5**
Nickel (ppm)	**4.53**	**7.32**	**5.93**
Manganese (ppm)	**5.78**	**5.99**	**5.89**
Barium (ppm)	**4.95**	**5.48**	**5.22**
Tin (ppm)	**1.51**	**2.39**	**1.95**
Arsenic (ppm)	**0.929**	**0.680**	**0.805**
Cadmium (ppm)	**0.715**	**0.582**	**0.649**
Chromium (ppm)	**BLOQ[a]**	**BLOQ**	**BLOQ**

- Only zinc (1.68%), silicon (0.932%), and aluminum (0.106%) was detected above 0.1%.
- All other analytes were below 0.1%.
- The level of chromium was below quantitation limit of the method (13 ppm).

Analyses of Solvent Extractable Constituents by GC- AND LC-MS

- The % extracted was estimated by mass balance and are shown in the table below.
- Ethyl acetate, methylene chloride, hexane, and carbon disulfide yielded the highest extractable % suggesting majority of the extractables are relatively non-polar organic compounds.
- Extracts were analyzed by GC-MS and the profiles and analytes identified in methylene chloride extract with high confidence are shown below.
- Analysis of the ethanol extracts by LC-MS showed fewer peaks than the GC-MS, but did confirm some of the same constituents seen in the GC-MS.



Solvent	Average (%) Extracted
Water	0.60
Ethanol	4.19
Ethyl Acetate	7.21
Hexane	7.39
Methylene Chloride	7.98
Carbon Disulfide	8.35

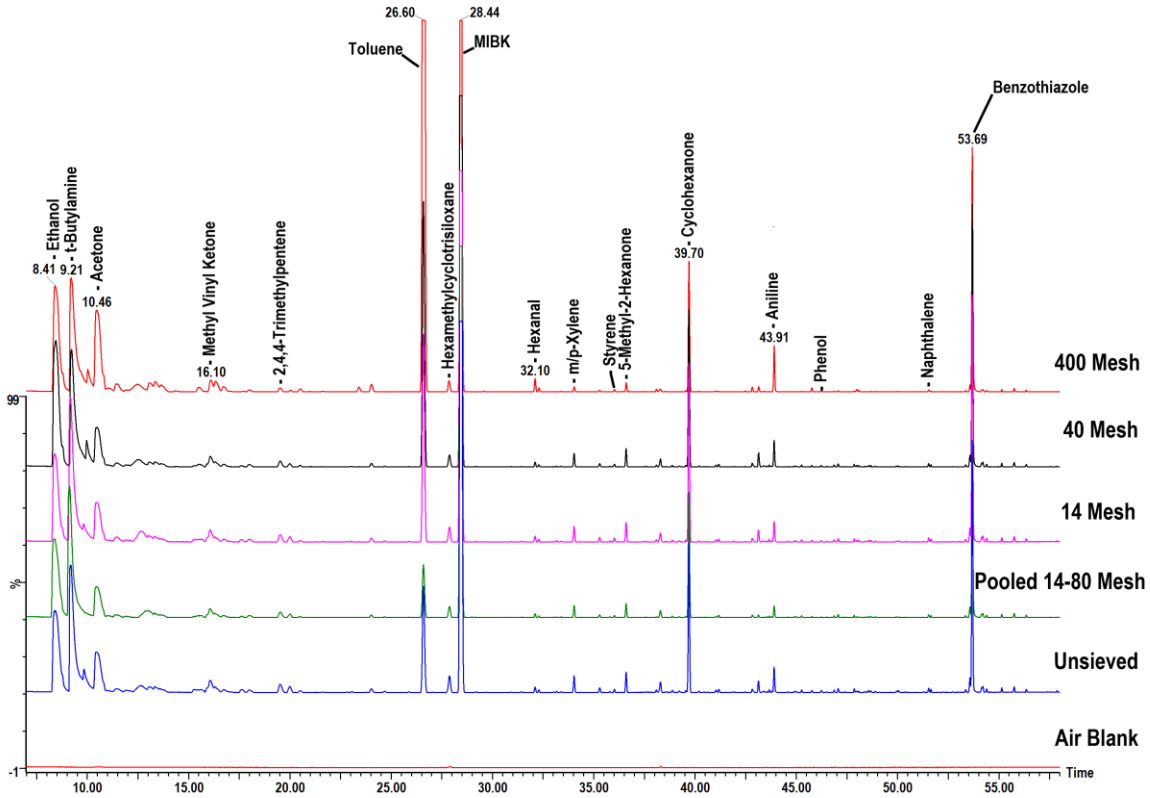
Analyses of Volatiles (PPM) by Headspace GC-MS

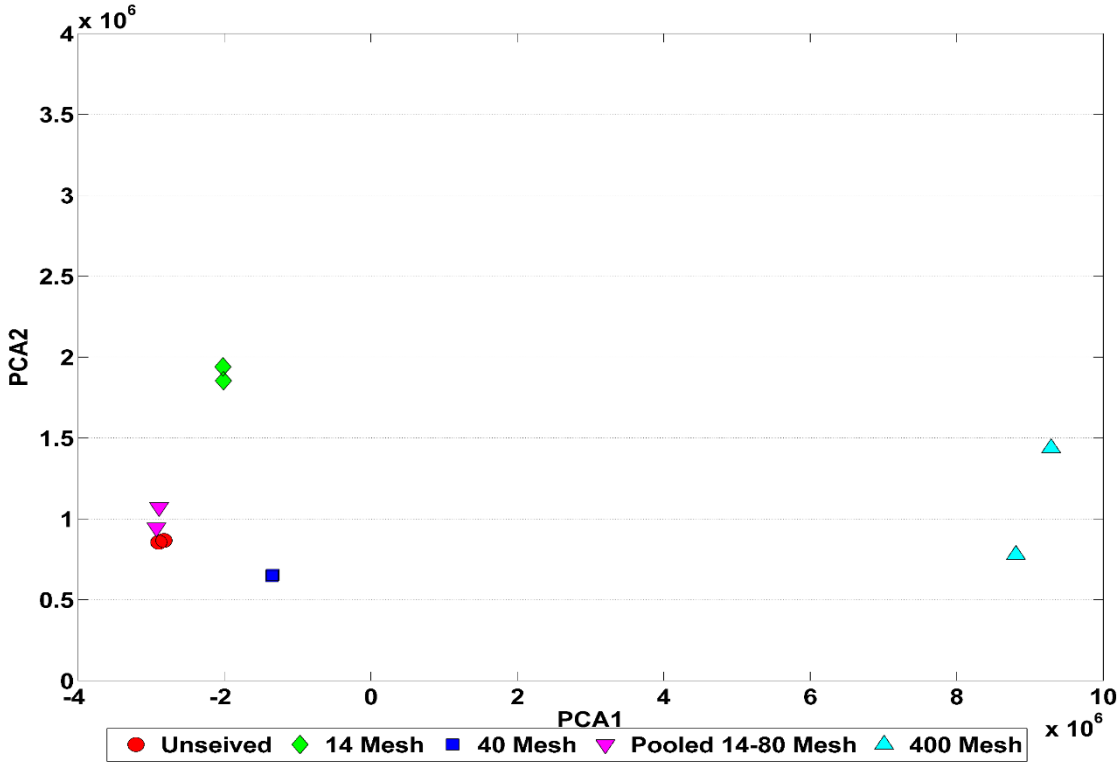
Compound	Replicate 1	Replicate 2	Average	Match Score[a](%)
Methyl Isobutyl Ketone	3.48	3.21	3.35	98.6
Aniline	0.980	1.12	1.05	99.6
Benzothiazole	0.753	0.684	0.719	94.0
Toluene	0.441	0.415	0.428	100.0
Cyclohexanone	0.387	0.344	0.366	99.3
2,4,4-Trimethylpentene	0.125	0.116	0.120	96.5
Methyl Vinyl Ketone	0.113	0.0990	0.106	85.2[b]
Hexamethylcyclotrisiloxane	0.105	0.0954	0.100	99.6
5-Methyl-2-hexanone	0.0968	0.0773	0.0870	98.0
Phenol	0.0758	0.0876	0.0817	87.0
Trichloroethylene	0.0681	0.0648	0.0665	99.7
Butyraldehyde	0.0313	0.0293	0.0303	26.6[b]
Hexanal	0.0198	0.0180	0.0189	90.9
m/p-Xylene	0.0171	0.0163	0.0167	99.1
3-Methylhexane	0.00882	0.0118	0.0103	89.5
1-Methylnaphthalene	0.00994	0.00814	0.00904	93.6
Naphthalene	0.00689	0.00599	0.00644	99.9
Styrene	0.00603	0.00661	0.00632	80.2
Furfural	0.00516	0.00561	0.00539	52.7[b]
1,2,4-Trimethylbenzene	0.00577	0.00483	0.00520	83.6
2-Methylnaphthalene	0.00535	0.00487	0.00511	88.8
Ethanol	0.00451	0.00538	0.00495	99.6
Pentadecane	0.00410	0.00497	0.00454	64.8[c]
Decane	0.00394	0.00360	0.00377	58.8[c]
Tetradecane	0.00379	0.00364	0.00371	74.4[c]
Butylated Hydroxytoluene	0.00294	0.00205	0.00250	84.6
Tridecane	0.00198	0.00166	0.00182	63.7[c]

3-Ethyltoluene	**0.00141**	**0.00130**	**0.00135**	**87.7**
t-Butylamine	**0.00147**	**0.00118**	**0.00132**	**99.5**
o-Xylene	**0.00115**	**0.00131**	**0.00123**	**68.4[b]**
Ethylbenzene	**0.00118**	**0.00098**	**0.00108**	**83.1**
Acetone	**0.000502**	**0.000495**	**0.000499**	**98.6**
Heptane	**0.000249**	**0.000207**	**0.000228**	**96.4**

- Methyl isobutyl ketone (the most prevalent volatile compound) is a precursor in the synthesis of the phenyldiamine antiozonants used in rubber. Aniline (the second most prevalent volatile compound) is a decomposition product of the phenyldiamine antiozonants. The phenyldiamine antiozonant, 6PPD, was the highest concentration compound seen in the solvent extracts.
- Other high concentration constituents are benzothiazole (antioxidant), toluene (used as a solvent for adhesives used in tire manufacture and a rubber softener during grinding), and cyclohexanone (used in nylon manufacturing).
- The only PAHs detected in the volatile and semivolatiles analysis were naphthalene and methyl naphthalenes, which are the most volatile PAHs.

Comparison of Fractions Following Sieving





- The pattern of chromatographic profiles were similar between different size fractions.
- PCA score plots generated using peaks in profiles show that 14- and 40-mesh fractions were similar to bulk, pooled 14-80 mesh fraction was less similar likely due to inclusion of 80-mesh and 400-mesh was very different from bulk. The difference is likely stemming from the higher levels of toluene and methyl isobutyl ketone in smaller size fraction (400-mesh).

Conclusion

- The CR lot consisted of a very small fraction of volatile chemicals (~ 0.0075%). The minimal weight loss at loss < 200 ºC in TGA confirms this. Thirty three of these compounds were identified using authentic standards.
- Metals and metalloids constitutes ~2.9% by weight of the lot.
- Extraction of CR lot with multiple solvents covering different polarities showed that 0.6% and ~8% by weight, respectively, were extracted with water and methylene chloride. 42 of compounds in methylene chloride extract were identified with high confidence and additional 62 were identified with lower confidence.
- Size fractionation shows that 400-mesh is different from the other fractions as well as CR bulk.

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Acknowledgements

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