



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

# NTP RESEARCH REPORT ON THE CHEMICAL AND PHYSICAL CHARACTERIZATION OF RECYCLED TIRE CRUMB RUBBER

NTP RR 11

JULY 2019

# **NTP Research Report on the Chemical and Physical Characterization of Recycled Tire Crumb Rubber**

Research Report 11  
National Toxicology Program

July 2019

National Toxicology Program  
Public Health Service  
U.S. Department of Health and Human Services  
ISSN: 2473-4756

Research Triangle Park, North Carolina, USA

## Table of Contents

About This Report.....	v
Peer Review .....	vii
Publication Details .....	viii
Abstract .....	ix
Introduction.....	1
Materials and Methods.....	3
Materials.....	3
Optical Microscopy.....	3
Scanning Electron Microscopy and Energy Dispersive X-Ray Spectroscopy.....	3
Thermogravimetric Analysis.....	3
Inorganic Analysis .....	4
Headspace Gas Chromatography–Mass Spectrometry Analysis for Volatile and Semivolatile Organic Compounds.....	4
Estimation of Solvent-Extractable Constituents Fraction .....	5
Gas Chromatography–Mass Spectrometry Analysis of Solvent-Extractable Constituents Fraction .....	5
Liquid Chromatography–Mass Spectrometry Analysis of Solvent-Extractable Constituents .....	6
Size Fractionation of Crumb Rubber Lot and Gas Chromatography–Mass Spectrometry Profiling of Fractions .....	6
Energy Dispersive X-Ray Spectroscopy of Crumb Rubber Fractions.....	7
Bioaccessibility In Vitro .....	7
Results.....	9
Optical Microscopy.....	9
Scanning Electron Microscopy and Energy Dispersive X-Ray Spectroscopy.....	9
Thermogravimetric Analysis.....	9
Inorganics Analysis.....	9
Headspace Gas Chromatography–Mass Spectrometry Analysis for Volatile and Semivolatile Organic Compounds.....	10
Gas Chromatography–Mass Spectrometry Analysis of Solvent-Extractable Constituents Fraction .....	10
Liquid Chromatography–Mass Spectrometry Analysis of Solvent-Extractable Constituents Fraction.....	11
Gas Chromatography–Mass Spectrometry Analysis of Crumb Rubber Fractions .....	11
Energy Dispersive X-Ray Spectroscopy of Crumb Rubber Fractions.....	13
Bioaccessibility In Vitro .....	13
Conclusions.....	14
References.....	15
Appendix A. Individual and Mixtures of Standards Procured to Identify Crumb Rubber Constituents.....	A-1

Appendix B. Potential Crumb Rubber Constituents Identified in the Literature and Initial Screening Analyses .....	B-1
Appendix C. Additional Constituents of Crumb Rubber Identified by Various Techniques .....	C-1
Appendix D. Settings Used in XCMS Software.....	D-1
Appendix E. Bioaccessibility Studies .....	E-1

## Tables

Table 1. Elemental Composition <sup>a</sup> (Wt %) of Six Individual Crumb Rubber Grains by Energy Dispersive Spectroscopy .....	17
Table 2. Estimated Levels of Metals in Crumb Rubber Lot <sup>a</sup> .....	17
Table 3. Estimated Levels of Volatile and Semivolatile Organic Compounds in Crumb Rubber Lot by Headspace GC–MS <sup>a</sup> .....	18
Table 4. Percent of Extractable Crumb Rubber in Various Solvents <sup>a</sup> .....	19
Table 5. Constituents of Crumb Rubber in Methylene Chloride Extract Identified by GC–MS with High Confidence <sup>a</sup> .....	20
Table 6. Constituents of Crumb Rubber in Ethanolic Extract Identified by LC–MS.....	21
Table 7. Average Estimated Levels of Volatile and Semivolatile Organic Compounds in Various Crumb Rubber Fractions by Headspace GC–MS .....	22
Table 8. Elemental Composition (Wt %) of Crumb Rubber Fractions by Energy Dispersive Spectroscopy <sup>a</sup> .....	23
Table 9. Estimated Blank Biofluid Concentration (Equivalent $\mu\text{g}$ in Extract per g of Crumb Rubber Extracted) <sup>a</sup> .....	24
Table 10. Estimated Bioaccessible Constituent Concentration ( $\mu\text{g}$ in Extract per g of Crumb Rubber Extracted) <sup>a</sup> .....	26

## Figures

Figure 1. Representative Optical Micrographs of Two Crumb Rubber Regions .....	28
Figure 2. Representative Scanning Electron Micrographs of the Cut Surfaces of Four Dissected Crumb Rubber Grains.....	29
Figure 3. Representative Thermogram from Thermo Gravimetric Analysis of Crumb Rubber .....	30
Figure 4. Comparison of GC–MS Chromatograms at Varying Incubation Temperatures in Headspace GC–MS Analysis: (A) Full Scale (B) Expanded Scale.....	31
Figure 5. GC–MS Spectra for Methyl Isobutyl Ketone: (A) Standard Spectrum, (B) Crumb Rubber Sample Spectrum, and (C) Library Spectrum .....	32
Figure 6. Comparison of GC–MS Chromatograms of Crumb Rubber Following Extraction with Various Solvents: (A) Full- and (B) Expanded-Scale Chromatograms, and (C) Blank Solvents.....	34
Figure 7. GC–MS Chromatogram of Crumb Rubber Following Extraction with Water .....	35
Figure 8. GC–MS Spectra for the n-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD): (A) Standard Spectrum, (B) Crumb Rubber Sample Spectrum, and (C) Library Spectrum .....	36

Figure 9. LC–MS Total Ion Chromatograms of Ethanol Extract of Crumb Rubber: Comparison of Blank-Subtracted Extract of Crumb Rubber, (A) Positive Ion Mode and (B) Negative Ion Mode .....	37
Figure 10. Comparison of Headspace GC–MS Chromatograms of Various Crumb Rubber Fractions and Unsieved Bulk Material Following Incubation at 100°C: (A) Full- and (B) Expanded-Scale Chromatograms.....	38
Figure 11. Cloud Plot of GC–MS Non-Targeted Data Showing 177 Significantly Different Features Between Various Crumb Rubber Fractions .....	39
Figure 12. Principal Component Analysis Scores Plots of GC–MS Non-Targeted Data Comparing Various Crumb Rubber Fractions: (A) Using Mass Fragments (177 Features) and (B) Using Individual Peaks.....	40
Figure 13. Hierarchical Cluster Analysis of GC–MS Non-Targeted Data Comparing Various Crumb Rubber Fractions Using Individual Peaks .....	41
Figure 14. Headspace GC–MS Chromatograms at 100°C Incubation for Crumb Rubber Extracts Using Simulated Ingestion Fluid Extract and Corresponding Method Blanks .....	42
Figure 15. GC–MS Liquid Injection Chromatograms for Crumb Rubber Extracts Using Simulated Ingestion Fluid Extract and Corresponding Method Blanks.....	43
Figure 16. LC–MS Chromatograms for Crumb Rubber Extracts Using Simulated Ingestion Fluid Extract and Corresponding Method Blank.....	44

## About This Report

National Toxicology Program<sup>1</sup>

<sup>1</sup>Division of the National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA

### Collaborators

Suramya Waidyanatha, Georgia Roberts, Scott Masten, Timothy Cristy

**Division of the National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA**

*Designed study, evaluated and interpreted study findings*

Suramya Waidyanatha, Ph.D.

*Designed study*

Scott Masten, Ph.D.

Georgia Roberts, Ph.D.

**Battelle Memorial Institute, Columbus, Ohio, USA**

*Conducted study, evaluated and interpreted study findings*

Timothy Cristy, B.A.

### Contributors

**Division of the National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA**

*Reviewed research report*

Brad Collins, M.S.

David Crizer, Ph.D.

Will Gwinn, Ph.D.

Michelle Hooth, Ph.D., DABT

Esra Mutlu, Ph.D.

Matthew Stout, Ph.D., DABT

Nigel Walker, Ph.D., DABT

*Provided overall programmatic guidance and review*

Abee Boyles, Ph.D.

John Bucher, Ph.D.

Michelle Cora, D.V.M., DACVP

Michael DeVito, Ph.D.

Darlene Dixon, Ph.D.

Dave Malarkey, Ph.D., D.V.M.

Cynthia Rider, Ph.D., DABT

Chemical and Physical Characterization of Recycled Tire Crumb Rubber

*Provided oversight of external peer review*

Mary Wolfe, Ph.D.

**National Exposure Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA**

*Provided internal peer review of draft report*

Kent Thomas, B.S.P.H.

**ICF, Durham, North Carolina, USA**

*Prepared reports and conducted peer review*

David Burch, M.E.M., Principal Investigator

Susan Blaine, B.A.

Natalie Blanton, M.P.H.

Jeremy Frye, M.S.L.S

Lindsey Green, M.P.H.

Tara Hamilton, M.S.

Katherine Helmick, M.P.H.

Penelope Kellar, M.S.

Whitney Mitchell, B.S.

## Peer Review

The draft research report, *NTP Research Report on the Chemical and Physical Characterization of Recycled Tire Crumb Rubber*, was evaluated by the reviewers listed below. These reviewers served as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determined if the design and conditions of these NTP studies were appropriate and ensured that this NTP Research Report presented the experimental results and conclusions fully and clearly.

### Peer Reviewers

**Joshua Kellogg, Ph.D.**

Postdoctoral Research Fellow  
University of North Carolina at Greensboro  
Greensboro, North Carolina, USA

**Robert J. Strife, Ph.D.**

Senior Scientist, Consultant (Retired)  
Procter & Gamble, Research and Development  
West Chester, Ohio, USA



## Publication Details

Publisher: National Toxicology Program

Publishing Location: Research Triangle Park, NC

ISSN: 2473-4756

DOI: <https://doi.org/10.22427/NTP-RR-11>

Report Series: NTP Research Report Series

Report Series Number: 11

*Official citation:* National Toxicology Program (NTP). 2019. NTP research report on the chemical and physical characterization of recycled tire crumb rubber. Research Triangle Park, NC: National Toxicology Program. Research Report 11.

## Abstract

Public health concern for playing on synthetic turf fields with crumb rubber infill has increased in recent years. Crumb rubber manufactured from recycled automobile tires contains potential carcinogenic and toxic substances, and, with over 12,000 synthetic turf fields in the United States, the potential for exposure is widespread. The National Toxicology Program (NTP) conducted research to improve the understanding of potential human exposure to crumb rubber and its biological activity. As a part of the NTP research program, a crumb rubber lot, 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 consisted of a range of particle sizes (0.1–4 mm) and types (dark and light rubber, visible inclusions, fibers). Thermogravimetric analysis revealed that the lot contains a minute fraction of volatile organic compounds (VOCs) 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 by gas chromatography (GC) and MS with headspace sampling detected a large number of constituents; 33 compounds were identified totaling ~0.0007% by weight in crumb rubber.

Extraction of crumb rubber with multiple solvents covering different polarities showed that 0.6% and ~8% by weight, respectively, were extracted with water and methylene chloride, demonstrating that most 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 crumb rubber were investigated but were not detected in the extracts of the current lot under the conditions used. Analysis of ethanol extracts of crumb rubber by liquid chromatography coupled with MS did not reveal any new analytes not previously detected by GC–MS.

These data demonstrate that VOCs and metals constitute a very small fraction of the crumb rubber lot. In vitro bioaccessibility studies to mimic various routes of exposure showed that only a limited number of constituents are present in these fluids, regardless of the biofluid or the analytical method used.

## Introduction

Synthetic turf fields are widely used in the United States, and their use is expanding. Although such fields historically have been installed in professional sports complexes, they are becoming increasingly popular in community recreational areas, including schools and public parks. These expanded applications include areas across the United States that are used by people of all ages, including young children. In a synthetic turf field, infill materials are spread between the “grass” fibers to provide cushioning and traction. Today, most commonly, the infill consists of granulated rubber pellets referred to as crumb rubber. Crumb rubber is manufactured by shredding used or recycled automobile tires. Recycled tires contain numerous, potentially carcinogenic and toxic substances, either as components of the original tire rubber or accumulated during normal use. While information is known about tire manufacturing, many variables are unknown, including persistence of byproducts, chemical changes during vulcanization, and adsorption of environmental chemicals, which might affect the chemical composition of the ultimate crumb rubber product. A recent literature review of crumb rubber chemical characterization studies outlines some chemicals of concern; using ADMET Predictor™, this study identified 197 predicted carcinogens, of which 52 have been previously classified as carcinogens by the U.S. Environmental Protection Agency (EPA) or European Chemicals Agency (ECHA)<sup>1</sup>. Other chemicals of concern as well as more information about crumb rubber manufacturing and synthetic turf construction and standards, are described in the 2016 EPA Federal Research Action Plan<sup>2</sup>.

Public concerns about potential health impacts associated with the use of synthetic turf fields have risen dramatically in recent years due to the chemical composition of crumb rubber and the opportunity for widespread, frequent, and long-term exposure, particularly for young athletes. Considering that information to evaluate whether there are adverse health effects from playing on synthetic turf fields is currently limited, several governmental organizations at the international, federal, and state level recently launched research efforts to better understand human exposure and human health hazards focusing on crumb rubber. Internationally, ECHA<sup>3</sup> and the National Institute for Public Health and the Environment (RIVM)<sup>4</sup> of the Netherlands have evaluated the health risks of playing sports on synthetic turf with crumb rubber. The Federal Research Action Plan<sup>5-7</sup>, which involves several U.S. federal agencies, aims to determine and fill important knowledge gaps (FRAP 2016, Appendix B), characterize constituents of recycled tire crumb, and identify ways that people are exposed to tire crumb rubber through typical field use activities. The California Office of Environmental Health Hazard Assessment (OEHHA)<sup>8</sup> is evaluating exposure scenarios, characterizing new and in-field crumb rubber, and developing biomonitoring protocols.

In 2015, OEHHA nominated synthetic turf/crumb rubber to the National Toxicology Program (NTP) for short-term in vivo and in vitro studies to generate information and improve understanding of potential health impacts of chemicals released from synthetic turf, with an emphasis on crumb rubber. The NTP research program<sup>9</sup> developed in response to this request used in vitro and in vivo systems to address uncertainties about potential human exposure to hazardous components of crumb rubber. The NTP research approach included both in vivo and in vitro studies and considered the most likely routes of human contact with crumb rubber (e.g., ingestion, dermal contact, and inhalation). The study objective was to investigate which exposure

conditions could have biological effects, with a focus on characterizing the bioaccessibility and systemic exposure of crumb rubber constituents.

The results of the NTP studies on crumb rubber are communicated in a series of Research Reports<sup>10-13</sup>. The crumb rubber used in the NTP studies was provided by OEHHA specifically for research purposes, and consisted of fresh recycled tire crumb rubber obtained from manufacturing facilities. The physical and chemical characteristics of the material were evaluated using a variety of analytical methods<sup>10</sup>. The characterization work provided an understanding of the material used in the NTP studies and helped interpret chemical analyses in the *in vivo* and *in vitro* studies. Characterization of the NTP material will allow interpretation of NTP study findings in relation to crumb rubber evaluated as part of the Federal Research Action Plan (FRAP) and OEHHA research efforts, including samples from new and weathered material, and samples from indoor and outdoor fields. For *in vitro* testing, multiple cell lines (representing skin, lung, and small intestine) were used to evaluate the extractability of crumb rubber and to look for evidence of biological activity of crumb rubber constituents via measurements of cytotoxicity<sup>11</sup>. For *in vivo* studies, feasibility testing was conducted to determine what routes of exposure were possible<sup>12</sup>. On the basis of this testing, 14-day *in vivo* studies were performed<sup>13</sup> using oral exposure and exposure in bedding. Dermal studies were not conducted on the basis of feasibility work<sup>12</sup>, and feasibility testing for particle and vapor inhalation studies is ongoing.

This report focuses on characterizing a crumb rubber lot for use in NTP research activities. A composite lot of crumb rubber was prepared from commercial material obtained from multiple sources via OEHHA (see [Materials and Methods](#)). The lot may or may not represent what is found from other sources or on synthetic turf fields of different ages. The lot was analyzed using a variety of techniques to generate information on its chemical and physical characteristics. The lot was sieved to generate different size fractions to be used in animal toxicology studies via different exposure scenarios<sup>12; 13</sup>. *In vitro* bioaccessibility studies were conducted to mimic various routes of exposure to investigate potential crumb rubber constituents bioaccessible under these conditions.

## Materials and Methods

### Materials

Three lots of fresh crumb rubber, two from one facility manufactured by an ambient or a cryogenic process and one from one facility manufactured by an ambient process were received in multiple 1-L glass jars via OEHHA. First, the material from one facility and one type of processing was combined to produce three individual lots representing each original lot; each lot was approximately 5 kg. Subsequently, all material was combined into one homogeneous lot. All equipment and containers used during handling were cleaned with ASTM Type 1 water and dried in an oven at 150°C before use. The homogenized material was assigned lot number CRM06092016, repackaged into 1-L amber glass bottles with Teflon-lined lids, and stored in the refrigerator.

Individual and mixtures of standards of potential crumb rubber constituents were procured from Alpha Aesar (Ward Hill, MA), Sigma-Aldrich (Milwaukee, WI) or Spex Certiprep, Metuchen, NJ (Appendix A).

### Optical Microscopy

Optical micrographs of crumb rubber were obtained using an Olympus SZX12 stereo microscope with an Olympus DP-71 camera (Tokyo, Japan). The software used was PAX-it! (MIS, Villa Park, IL, version 8.0.0). A sample of crumb rubber was obtained from the bulk lot. Seven regions of this sample were micrographed to determine variations in size and coloration.

### Scanning Electron Microscopy and Energy Dispersive X-Ray Spectroscopy

Selected crumb rubber grains were cut with a clean razor blade to generate a flat surface required for this analysis and to expose the interior surface and any inclusions in rubber grains. The cut grains were mounted onto carbon tabs with the cut surfaces up, and the mounts were lightly coated with Au-Pd target. The mounts were examined using a JEOL 7600-F (JOEL, Tokyo, Japan) scanning electron microscope (SEM), and images were obtained using secondary and back-scattered electrons. Elemental analysis of the crumb rubber mounts, including inclusions, was obtained by energy dispersive spectroscopy (EDS) using an Apollo X silicon drift detector (EDAX, Mahwah, NJ). Spectra of six particles were analyzed and weight percent of the elements detected was estimated.

### Thermogravimetric Analysis

The crumb rubber (~18 mg per analysis) was analyzed in duplicate on a Pyris 1 (PerkinElmer, Waltham, MA) thermal gravimetric analyzer with software package v. 11.0.3.0470. Samples were heated from 30°C to 700°C in a nitrogen (inert) atmosphere and then from 700°C to 900°C in an air (oxidative) atmosphere to combust any remaining organic material. The temperature was increased at a rate of 20°C per minute. An auto stepwise software method was used to improve resolution between differing weight loss events. This method allows for the definition of a set of parameters that, when met, will switch the constant temperature scan to an isothermal hold at the current temperature when a significant weight loss event is detected. Once the weight

loss slows, the temperature scan resumes. This technique is especially useful in analyzing crumb rubber, as the oil and polymer components tend to have similar weight loss onsets.

## **Inorganic Analysis**

Inorganic constituents were determined by Galbraith Laboratories, Inc. (Knoxville, TN), using inductively coupled plasma (ICP)–mass spectrometry (MS) or ICP–atomic emission spectrometry (AES). Briefly, duplicate samples of 150–300 mg of crumb rubber were analyzed for arsenic, lead, barium, manganese, nickel, cadmium, and tin by ICP–MS (Galbraith method ME-30) using a Sciex Elan 6000, 6100, or 9000 (PerkinElmer, Waltham, MA) and for aluminum, cobalt, chromium, copper, iron, potassium, magnesium, sodium, silicon, and zinc by ICP–AES (Galbraith method ME-70) using a PerkinElmer Optima 4300 (PerkinElmer, Waltham, MA).

## **Headspace Gas Chromatography–Mass Spectrometry Analysis for Volatile and Semivolatile Organic Compounds**

Crumb rubber was analyzed using a headspace gas chromatography (GC)–MS method to determine volatile (VOC) and semivolatile (SVOC) organic compounds. The method was based on EPA method 8620B, which is designed to analyze for volatile organic compounds with boiling points less than 200°C in solid matrices by GC–MS. The system used was a CTC Analytics CombiPAL autosampler (Leap Technologies, Carrboro, NC), coupled to a 6890 GC and a 5973 MSD (Agilent Technologies, Santa Clara, CA). Duplicate crumb rubber samples (2 g) were weighed into 10-mL headspace vials and capped (Supelco, Bellefonte, PA). Vials were incubated at varying temperatures (ambient, 50°C or 100°C) for 40 minutes. One mL was sampled from the vial headspace and injected at 500  $\mu\text{L}/\text{second}$  into the GC injector port at 240°C in splitless mode. An RTX-VMS column (60 m  $\times$  0.25 mm, 1.4- $\mu\text{m}$  film thickness, Restek, Bellefonte, PA) was used with the following oven temperature program: 40°C held for 5 minutes, increased at 2°C per minute to 80°C, followed by increases at 4°C per minute to 245°C, and held for 15 minutes. The mass spectrometer conditions were ionization mode, electron ionization; auxiliary temperature, 240°C; ion source temperature, 250°C; quadrupole temperature, 150°C; and scan range,  $m/z$  35 to 500. Total run time was ~81 minutes.

A single concentration of standards of potential crumb rubber constituents (~130), a subset of those given in Appendix A, except the alkanes mix (see below), was prepared in either 2-phenoxyethanol or methanol. The concentration of individual standards ranged from 0.1 to 160  $\mu\text{g}/\text{mL}$ , to cover the expected range in crumb rubber, based on preliminary experiments. The compounds with low boiling points were prepared in the late-eluting 2-phenoxyethanol and compounds with higher boiling points were prepared in the early-eluting methanol to prevent interference of the solvent with the standard during analysis. Because the alkanes mix was procured in hexane, it was diluted with hexane. The standards were spiked (25  $\mu\text{L}$ ) into 10-mL headspace vials approximately half filled with 1-mm glass beads (Sigma-Aldrich, St. Louis, MO). Method blanks were prepared by using glass beads or glass beads spiked with 25  $\mu\text{L}$  of the respective solvent used for the standards that were prepared for analysis.

Constituents in crumb rubber were identified using a combination of retention times of analytical standards and their mass spectra and library matches of mass spectra using the combined NIST 2014 and Wiley 11th Edition Mass Spectral Library (NIST/Wiley Library) (Wiley, Hoboken,

NJ). For the alkanes larger than decane, the abundance of the higher mass fragments ( $>m/z$  100) was too low to be detected in the samples (and sometimes in the standards with higher concentration), so the identity was based on the pattern of low mass fragments (serial losses of  $\text{CH}_2$ ) and retention time match with the standard. Although straight-chain alkane standards were used for identification, the identified peaks could be branched isomers of other alkanes. Because the profiles of the duplicate samples were nearly identical, peaks were identified using one replicate only.

Under the experimental conditions mentioned above, the level of each compound in the samples that matched with a standard was estimated using the response factor of a single concentration of standard. This estimate assumes the compounds volatilized similarly in the crumb rubber samples and standards spiked on glass beads. Meta- and para-xylene were not separated by this method and have nearly identical mass spectra. Therefore, the concentration for the peak was estimated using the m-xylene response factor given that commercial xylenes are predominantly m-xylene.

## **Estimation of Solvent-Extractable Constituents Fraction**

To determine the solvent-extractable fraction, 0.5-g portions of crumb rubber were extracted, in duplicate, by rotation overnight at ambient temperature at  $\sim 70$  rpm with 10 mL of water, ethanol, ethyl acetate, hexane, methylene chloride, or carbon disulfide. Samples were centrifuged for 5 minutes at 3,000 rpm and the supernatant removed. The residual crumb rubber was dried at  $60^\circ\text{C}$  for 2 days and weighed to determine the percentage extracted.

## **Gas Chromatography–Mass Spectrometry Analysis of Solvent-Extractable Constituents Fraction**

All solvent extracts from above were analyzed by GC–MS using a 7890 GC coupled to a 5975 MSD (Agilent Technologies, Santa Clara, CA). One  $\mu\text{L}$  of extract was injected into the GC injector port at  $300^\circ\text{C}$  in splitless mode. A DB-5MS column ( $60\text{ m} \times 0.25\text{ mm}$ ,  $0.25\text{-}\mu\text{m}$  film thickness, Agilent, Santa Clara, CA) was used with a helium carrier gas flow at 1 mL per minute. The initial oven temperature was  $40^\circ\text{C}$ , which was ramped to  $330^\circ\text{C}$ , at  $5^\circ\text{C}$  per minute and held for 5 minutes. The mass spectrometer conditions were ionization mode, electron ionization; auxiliary temperature,  $300^\circ\text{C}$ ; ion source temperature,  $250^\circ\text{C}$ ; quadrupole temperature,  $200^\circ\text{C}$ ; and scan range,  $m/z$  35 to 650. Total run time was  $\sim 68$  minutes.

Chromatograms from methylene chloride extract were used to identify crumb rubber constituents. Standards for some potential crumb rubber constituents (listed in Appendix A) that are expected to elute after the solvent were prepared in methylene chloride (except the alkanes mix) or hexane (alkanes mix). All standards were prepared at a sufficiently high concentration ( $40\text{ }\mu\text{g/mL}$ ) to be able to obtain mass spectra with sufficient fragments above the detection limit to allow for identification. Blanks containing either the solvent used for the extraction and the standards also were prepared for analysis.

To identify as many constituents as possible, the following criteria were used: (1) comparison with the retention time and spectra of analytical standards followed by comparison of mass spectra to the NIST/Wiley library (Wiley, Hoboken, NJ) (targeted analysis); (2) search for potential crumb rubber constituents (Appendix B) in the chromatogram using extracted ion

chromatograms for the parent and significant unique fragment ions such as halides, followed by comparison of the mass spectrum to the NIST/Wiley library (suspect screening); and (3) comparison of mass spectra of unknown peaks with area percent greater than 0.1% in the total chromatogram with the NIST/Wiley library (non-targeted analysis).

## Liquid Chromatography–Mass Spectrometry Analysis of Solvent-Extractable Constituents

An ethanol extract (prepared as described above, except using 2 g crumb rubber) was analyzed by LC–MS using an Agilent 1100 Liquid Chromatograph (Agilent, Santa Clara, CA) coupled to an API 4000 QTrap MS (Sciex, Toronto, Canada) using electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) sources in both positive and negative ion modes. The data system used was Analyst (1.6.1), ACD (Toronto, Canada) Spectrus (2017.1.2). The mass spectrometer was operated in enhanced MS mode with dynamic ion trap fill time, 1000 Da/sec ion trap readout rate, and scanning from  $m/z$  50 to 450. A low wavelength UV (210 nm) chromatogram also was collected. Analyte separation was achieved on a Phenomenex (Torrance, CA) Kinetex Biphenyl column (150 × 3.0 mm, 2.6- $\mu$ m particle size). Mobile phases, 0.1% formic acid in water (A), and 0.1% formic acid in acetonitrile (B), were used at a linear gradient of 10% B to 13.4% B over 3 minutes, followed by 100% B over 77 minutes with a flow rate of 300  $\mu$ L/minute.

Standards of several of the largest constituents observed in the GC–MS analyses of VOCs, SVOCs, and solvent extracts were prepared in ethanol at 10 and 100  $\mu$ g/mL. The standards and blank ethanol were analyzed using the same methods used for the samples.

To identify constituents in crumb rubber, the following approach was used: (1) combination of retention times of analytical standards and corresponding spectra (targeted analysis), (2) search for molecular weight of the constituents identified in the GC–MS analysis and in the *Handbook for the Chemical Analysis of Plastic and Polymer Additives*<sup>14</sup> (suspect screening), and (3) comparison of the mass spectra of unknown peaks against the Massbank (<http://www.massbank.jp>) library (non-targeted analysis).

## Size Fractionation of Crumb Rubber Lot and Gas Chromatography–Mass Spectrometry Profiling of Fractions

To generate different sized fractions for use in NTP research, samples of the crumb rubber lot were sieved using mesh sizes 14 ( $\geq 1.41$  mm), 40 (0.420–1.41 mm), 80 (0.177–0.420 mm), and 400 (0.037–0.177 mm), and the weight % of each fraction was recorded (note: theoretical particle sizes are given in parentheses). Small aliquots from 14-, 40-, and 80-mesh sizes were removed for analysis, and the remaining material was combined to generate one fraction (14- to 80-mesh). The 400-mesh was saved separately. Duplicate samples from mesh fractions 14-, 40-, 80-, 14- to 80-, and 400-mesh were analyzed, along with the original bulk material, by the headspace GC–MS method as described above using 100°C as the incubation temperature. Concentrations of selected VOCs and SVOCs were estimated as described above for the unsieved bulk material.

The spectral data from duplicate analyses were imported into XCMS software (<https://xcmsonline.scripps.edu>) for non-targeted analysis using multivariate statistics and



settings used are given in Appendix D. The software was set to find features that differed between samples at the 95% confidence level and had at least a 3:1 signal-to-noise ratio to find the most significant differences. The data were presented as a cloud plot and principal component analysis (PCA) score plot using both spectral features and peaks.

Solo v8.5.1 software (Eigenvector Research, Manson, WA) was used to perform PCA analysis and hierarchical cluster analysis using Ward's method using peaks in the profiles (instead of spectral features). This technique compares the overall profile of peaks rather than extracted ion chromatograms for every mass.

## **Energy Dispersive X-Ray Spectroscopy of Crumb Rubber Fractions**

The bulk material was size fractionated (mesh sizes 400, 80, 40, and 14) and the three larger particle size fractions (mesh sizes 80, 40, and 14) were combined, resulting in two fraction lots [Lot No. CRM12052016 (combined material) and Lot No. CRM09132016 (400-mesh<sup>12</sup>)]. EDS were done on both these lots using an Apollo X silicon drift detector (EDAX, Mahwah, NJ). Spectra of 10 particles from each lot were analyzed and the weight percent of the elements detected were estimated.

## **Bioaccessibility In Vitro**

To determine whether crumb rubber constituents are bioaccessible, crumb rubber was extracted with simulated biofluids to mimic dermal, inhalation, and oral (ingestion) routes of exposures. The simulated sweat, lung fluid, saliva, gastric fluid, and intestinal fluid, and their combination in ingestion pathway fluid, were prepared as described by Pavilonis et al. (2016)<sup>15</sup>; the composition of each fluid are given in Appendix E. Briefly, for simulated dermal exposure, 400-mg aliquots of crumb rubber were incubated in 20 mL of simulated sweat medium at 37°C for approximately 1 hour. For simulated inhalation exposure, 200 mg of crumb rubber were incubated in 10 mL of simulated lung fluid for approximately 24 hours. For simulated ingestion exposure, 8 mL of simulated saliva were added to 2 g of crumb rubber and shaken for ~30 seconds. After adding 100 mL of simulated gastric fluid, the sample was incubated at 37°C for approximately 2 hours. Approximately 50 mL of the extract was pipetted into another bottle to serve as the gastric phase-only sample. To the remaining sample (containing the crumb rubber) 100 mL of simulated intestinal fluid was added (final pH 6.5) and incubated at 37°C for approximately 2 hours. All extracts from the above-mentioned incubations were filtered through 0.45- $\mu$ m syringe filters to remove any crumb rubber particles and analyzed as described below.

The headspace GC–MS analysis of samples was conducted as described previously using a 300  $\mu$ L of each extract in duplicate in 10-mL vials containing ~2 g of glass beads. For the analysis by liquid injection GC–MS under the conditions described previously, 4 mL of each crumb rubber extract prepared as described above were rotated end over end with 4 mL of methylene chloride for approximately 1 hour. The samples were centrifuged for 5 minutes at 1,800 g, and the methylene chloride layers were transferred to autosampler vials for analysis. For the LC–MS analysis, the simulated biofluid extracts and blanks were analyzed as is. Method blanks were prepared similarly for all analysis methods except for the addition of crumb rubber. Instruments and the chromatographic methods used were the same as those described above except for the following: Both headspace and liquid injection GC–MS analyses were performed

for constituents identified in crumb rubber above using selected ion monitoring (SIM) where one ion (generally the  $m/z$  of the molecular ion) was used as the quantitation ion and a second ion as a confirmation ion that must be present to confirm identity. To keep the mass spectrometer data acquisition cycle time short enough to generate a sufficient number of data points to improve sensitivity, the samples and blanks were injected three times with a different set of SIM ions monitored each time. The grouping of ions was based on mass and retention time. Compounds with similar masses or retention times were assigned into different groups to prevent any potential interferences from closely eluting compounds. The LC–MS analyses were performed using full scan ( $m/z$  50 to 450), and extracted ion chromatograms for the  $[M+H]^+$  ion of each compound were generated. An estimated concentration of each compound found in the analyses was calculated using the response factor of the standard.

Stock standards of 32 selected compounds (based on the analytes found in crumb rubber and previous screening; see [Results](#) section) were prepared at 2 mg/mL in methylene chloride (except 2-methylnaphthalene and 1-methylnaphthalene, which were purchased as a 1-mg/mL and 2-mg/mL solution in methanol). The stock standards were combined into three intermediate standard mixes containing approximately 100  $\mu\text{g/mL}$  of each compound in methylene chloride. For the headspace GC–MS analysis, 50  $\mu\text{L}$  of each intermediate standard mix was diluted to 10 mL in ASTM Type 1 water to produce standards at 500 ng/mL. As with the samples, 300  $\mu\text{L}$  of each standard mix was pipetted into 10-mL headspace vials containing ~2 g of glass beads. For the liquid injection GC–MS analysis, 50  $\mu\text{L}$  of each intermediate standard mix was diluted to 10 mL in methylene chloride to produce standards at 500 ng/mL. For LC–MS analysis, stock standards were combined into three standard mixes containing approximately 20  $\mu\text{g/mL}$  of each compound in ethanol. The standards were prepared at a higher concentration than the GC–MS due to the lower sensitivity of the full-scan analysis.

## Results

### Optical Microscopy

Seven regions of a sample of the crumb rubber lot were micrographed. Micrographs from two selected regions are shown in Figure 1. The crumb rubber lot consists of irregular particles in a wide range of sizes, from approximately <0.1 to 4 mm. Particles consisting of both dark and light rubber and visible non-rubber inclusions and fibers were observed.

### Scanning Electron Microscopy and Energy Dispersive X-Ray Spectroscopy

SEM images for six individual crumb rubber grains were obtained, four of which are shown in Figure 2 as images representative of the variations between different grains. Elemental composition of the analyzed areas for six grains are given in Table 1. These data represent the elemental composition of the analyzed areas of different rubber grains and not the composition of bulk crumb rubber. The data demonstrate variability in composition between crumb rubber grains, which may have derived from different tires or different parts of the same tire. As would be expected of material consisting primarily of polymer, carbon ( $87.2 \pm 6.6\%$ ) makes up most of the crumb rubber with much lower percentages of sulfur ( $3.5 \pm 1.3\%$ ), zinc ( $3.0 \pm 1.8\%$ ), and oxygen ( $3.5 \pm 1.3\%$ ), likely derived from the sulfur and zinc oxide used in the vulcanization process. A few other elements were detected at <1% (Table 1). Elemental composition of inclusions observed in crumb rubber also was measured and identified as likely clay (aluminum silicates), calcium carbonate, and talc (magnesium silicates) based on high levels of silicon, calcium, oxygen, aluminum, and magnesium detected in these inclusions.

### Thermogravimetric Analysis

Duplicate samples were analyzed to estimate the composition of the crumb rubber lot. Samples were heated from 30°C to 700°C in a nitrogen (inert) atmosphere and then from 700°C to 900°C in an ambient air (oxidative) atmosphere to combust any remaining organic material. According to the thermogram (Figure 3), a minimal weight loss occurs below 200°C suggesting that VOCs and SVOCs comprise a very small fraction of the crumb rubber lot. The significant weight loss up to ~400°C is likely due to extender oils such as paraffinic, naphthenic, or aromatic oils<sup>16; 17</sup>. The weight loss between ~400°C and ~520°C may be due to decomposition of polymers with two steps indicating likely presence of two types of polymers. The last step is combustion of any uncombusted carbon-containing material including carbon black. The residue remaining after ~800°C represents the total inorganics present in the material. Based on this assessment, the crumb rubber lot contains a very small fraction of VOCs and SVOCs, approximately 25%, and extender oils; 29.9% polymeric material; 37.1% carbon black (arising likely from a combination of uncombusted material and original carbon black present in crumb rubber); and 7.7% inorganics.

### Inorganics Analysis

Inorganics were determined in duplicate using ICP-MS or ICP-AES, shown in Table 2. Only zinc (1.68%), silicon (0.932%), and aluminum (0.106%) were detected above 0.1%. All other

analytes were below 0.1% (Table 2). The level of chromium was below the limit of quantitation for the method.

## Headspace Gas Chromatography–Mass Spectrometry Analysis for Volatile and Semivolatile Organic Compounds

Duplicate samples of crumb rubber were analyzed using headspace GC–MS to determine VOCs and SVOCs using varying incubation temperatures. The temperatures were selected to cover a potential range of temperatures on playing fields including worst case scenarios<sup>18-20</sup>. Figure 4 shows a comparison of chromatograms from ambient temperature, 50°C, and 100°C. Very few analytes were present in chromatograms incubated at ambient temperature; as the temperature increased, the number and level of constituents in chromatograms increased. For example, the approximate number of peaks increased from ~7 at ambient temperature to >150 at 100°C. Therefore, samples from 100°C incubation were selected for identifying and quantifying constituents using the conditions described under methods. Of the large number of peaks detected (~150), 33 were identified using a combination of retention times of analytical standards (targeted analysis) and library matches of mass spectra; as an example, comparison of spectra leading to identification of methyl isobutyl ketone is shown in Figure 5. The identified peaks constituted a majority (~86%) of the total peak area. The remaining peaks had low confidence library matches. Using the conditions described under methods, the estimated levels of the 33 compounds identified are given in Table 3, totaling ~0.0007% by weight in crumb rubber.

## Gas Chromatography–Mass Spectrometry Analysis of Solvent-Extractable Constituents Fraction

Duplicate samples of the crumb rubber were extracted with different solvents across the polarity range from water to hexane. The average estimated extractable material, expressed as a percentage, is given in Table 4. Ethyl acetate, methylene chloride, hexane, and carbon disulfide yielded similar amounts of extractable material, and ethanol extracted approximately half as much material. Using water as the solvent yielded less than 1%. This observation indicates most extractable materials from crumb rubber are relatively non-polar organic compounds.

All samples were analyzed by GC–MS, the chromatograms for which are given in Figure 6 including for blank solvents. Under these conditions, the pattern of profiles is similar except for the water extract, where very few discernible peaks were observed (Figure 7). No peaks were detected in corresponding solvent blanks (data not shown).

The chromatogram for the methylene chloride extract was selected for identification of peaks using the criteria given under methods. Compounds identified with high confidence are presented in Table 5. To qualify for high confidence identification, the peak in the extract had to have either a matching retention time and mass spectrum of a standard or a >85% match with a library reference spectrum, or both. As an example, comparison of spectra leading to identification of *n*-(1,3-dimethylbutyl)-*n*'-phenyl-*p*-phenylenediamine (6PPD) is shown in Figure 8. Compounds that did not meet the criteria for the first category were assigned medium confidence. For these compounds, matching retention time and mass spectrum with a standard was difficult due to co-eluting or small peaks, or the match with the library spectrum was lower (30–84%); these data are presented in Appendix C, Table C-1. In cases of multiple, very similar isomers (such as the xylenes), standards would be required to determine which isomers might be

present. Appendix C, Table C-2 lists potential crumb rubber constituents based on the literature (Appendix A), which were investigated but not found in the current lot under these instrumental and test conditions.

## **Liquid Chromatography–Mass Spectrometry Analysis of Solvent-Extractable Constituents Fraction**

Ethanol extract was analyzed by LC–MS using positive and negative ion modes of ESI and APCI sources. The APCI source resulted in better ionization and detection of more peaks. Similarly, positive ionization resulted in more peaks. Because extensive investigation was conducted using GC and the LC profiles had fewer peaks, only the chromatograms from APCI positive ion mode were used for analyte identification (although this may have limited the identification of unique compounds that ionize only in negative ion mode). Total ion chromatograms following analyses of samples by positive and negative ion modes are shown in Figure 9. The blank ethanol chromatograms were subtracted from the sample chromatograms to produce the blank-subtracted total ion chromatograms. A list of analytes identified under the conditions mentioned above is given in Table 6.

The largest peak observed in both the positive and negative ion modes (29.7 minutes) the matched retention time and mass spectrum with the standard for 6PPD, which was also the largest peak observed in the GC–MS analysis of solvent extracts. The 21.6-minute peak matched retention time and mass spectrum with the standard for benzothiazole, which was also one of the major peaks observed in the volatiles analysis. The Massbank library identified the 19.8-minute peak as 2-hydroxybenzothiazole and the 22.5-minute peak as hexa(methoxymethyl)melamine. Several remaining unknown peaks matched molecular weight with either previously observed crumb rubber constituents or known polymer additives, but library spectra were not available and standards for those compounds had not been included in the analysis, so their identities could not be confirmed.

## **Gas Chromatography–Mass Spectrometry Analysis of Crumb Rubber Fractions**

Duplicate samples of crumb rubber from different mesh sizes were analyzed along with the bulk material to determine differences, if any, between different mesh sizes. Figure 10 shows a comparison of chromatographic profiles between samples; duplicates were similar and hence the chromatogram from only one replicate is shown in the figure. In general, the pattern of profiles was similar in each sample. Visually, most of the peaks show little or no change except the peak at 26.60 minutes, which was identified as toluene. As the particle size decreases, the intensity of toluene peak increases, with the 400-mesh fraction showing a significantly elevated peak compared to the unsieved bulk crumb rubber.

Spectral data from samples were compared to each other using multivariate statistics. Each peak in chromatogram is associated with a mass-to-charge ratio, retention time, fold change between different samples, p-value, and relative intensity. A cloud plot, which is a visualization of this multidimensional data to facilitate compound identification and data interpretation, identifying 177 significantly different spectral features is shown in Figure 11. The cloud plot shows all chromatograms overlaid along the x-axis based on the retention time and red circles at each mass-to-charge ratio (y-axis) that met the significance criteria (with size of the circle showing the

relative difference between the samples). Because the mass spectrum of each compound contains multiple fragments, it is possible to have several circles over one chromatographic peak (for example, as seen for the toluene peak labeled 4 on the plot). Upon closer examination of the data, a significant number of these features were found to arise from changes in levels of toluene and methyl isobutyl ketone in these fractions (Figure 11). Although several small peaks such as xylenes also met the criteria for significance, the large cyclohexane and benzothiazole peaks did not. Other small peaks that also met the criteria for significance included 2,4,4-trimethylpentene (~19.5 minutes), naphthalene (~51.5 minutes), methyl vinyl ketone (~16.1 minutes), and acetone (~10.5 minutes) (Figure 11).

PCA score plots, generated using the features described above from mass fragments, are shown in Figure 12A. This analysis shows that the 14-mesh and 40-mesh fractions were similar to the bulk crumb rubber lot; the pooled 14- to 80-mesh fraction was less similar to the bulk lot, likely due to inclusion of the 80-mesh fraction. Individual 80-mesh samples were not analyzed due to limitation in available material. The 400-mesh fraction differed markedly from the bulk lot and all other mesh sizes analyzed. As mentioned above, this difference between the bulk lot and mesh sizes likely stems from the changes in levels of toluene and methyl isobutyl ketone in these fractions. Because using mass fragments potentially overemphasizes the contribution from compounds such as toluene and methyl isobutyl ketone that have multiple fragments, the PCA analysis also was performed using peaks in the chromatographic profiles (Figure 12B). The outcome was similar, with the 400-mesh differing from the others.

Hierarchical cluster analysis was conducted on individual replicates using Ward's method; the corresponding dendrogram is shown in Figure 13. The replicates for each sample were closest to each other, demonstrating the reproducibility in sample analysis. For the different mesh-size fractions, all except the 400-mesh fraction fell in one branch of the dendrogram. The unsieved material and 14-mesh fraction clustered, and the 40-mesh and pooled 14- to 80-mesh fractions clustered.

In conclusion, GC-MS data show differences between the bulk and sieved materials. The unsieved, 14-mesh, 40-mesh, and pooled 14- to 80-mesh fractions are more similar to each other than any of them are to the 400-mesh fraction.

Concentrations of selected VOCs and SVOCs, based on those estimated previously in the bulk crumb rubber lot, were estimated for different mesh fractions, under the experimental conditions described. The estimated values are shown in Table 7 along with the bulk crumb rubber. Percent change observed for different size fractions with respect to the bulk sample was estimated as shown in Table 7. Toluene showed the greatest differences between the fractions, with the amount increasing as particle size decreased. The 400-mesh fraction contained 15 times as much toluene as the unsieved material. The second largest relative difference was with hexanal, where the 400-mesh sample had 2.5 times as much as the unsieved material. Benzothiazole and cyclohexanone, although some of the largest constituents, showed relatively little difference (<25%) between the samples.

## Energy Dispersive X-Ray Spectroscopy of Crumb Rubber Fractions

The weight percent of the elements detected in the two fractions are given in Table 8. The composition of elements between the fractions, in general, are similar to each other and consistent with the data observed for the bulk material (Table 1).

## Bioaccessibility In Vitro

During development of a method, crumb rubber was extracted using simulated biofluids from ISO Method 17294<sup>21</sup> (which is based the Bioaccessibility Research Group of Europe Unified Bioaccessibility Method) and Pavilonis et al. (2016)<sup>15</sup>. The ISO method biofluids, which were more complex mixtures, were found to have much higher background interferences with common crumb rubber constituents than the simpler biofluid formulation used by Pavilonis et al (2016)<sup>15</sup>. The EPA proposed simulated biofluids<sup>22</sup> are even more complex than the ISO method; for example, the gastric fluid alone contains 37 ingredients and hence was expected to have similar or higher background interferences. Hence, the Pavilonis et al. (2016)<sup>15</sup> method, which has a simpler formula, was used in the current assessment.

Chromatograms of combined SIM ions for the headspace GC–MS, liquid injection GC–MS, and LC–MS from simulated ingestion fluid samples, and corresponding blanks are shown respectively, in Figure 14, Figure 15, and Figure 16 as examples. SIM ion Sets 1 through 3 denote multiple injections needed to cover multiple SIM ions as described in methods.

Due to its high carbon black composition, crumb rubber can act as a sorbent, in addition to leaching its own constituents. In many of the simulated biofluids, this phenomenon is evident, as many of the peaks in the blank biofluids are reduced or eliminated in the crumb rubber extracts. The method blanks had small background peaks (generally less than 10 ng/mL) for many of the compounds, so the detection limit for this analysis was set to three times the response in the blanks. The concentrations of the analytes observed in the blanks (calculated as equivalent  $\mu\text{g/g}$  in the crumb rubber based on the amount of crumb rubber extracted and volume of the extracts) are shown in Table 9. Any results for the samples where the blanks contained a large background (greater than 20 ng/mL) were marked with an I (for interference) in the results tables (calculated values for the samples are shown only if the sample response was at least three times that of interference peak). More of the peaks in the liquid injection GC–MS and the LC–MS analyses showed interference than in the headspace GC–MS.

The peaks identified and corresponding estimated concentration in simulated biofluids using each technique are shown in Table 10. Overall, a limited number of constituents present in crumb rubber were observed in these extracts, regardless of the biofluid or the analytical method used. In addition, hexa(methoxymethyl)melamine was identified by LC–MS in the simulated inhalation extract by library match (~80% match), but no standard was available to confirm the identity.

## Conclusions

A crumb rubber lot 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 consisted of a range of particle sizes (<0.1–4 mm). Data from a combination of analyses demonstrate that VOCs, SVOCs, and metals constitute a very small fraction of the crumb rubber lot. Size fractionation of crumb rubber showed that chemical profiles are fairly similar between different size fractions except the 400-mesh fraction. Bioaccessibility studies conducted in vitro to mimic various routes of exposure showed that only a few constituents present in crumb rubber are present in these extracts under the conditions used, regardless of the biofluid or the analytical method used.



## References

1. Perkins AN, Inayat-Hussain SH, Deziel NC, Johnson CH, Ferguson SS, Garcia-Milian R, Thompson DC, Vasiliou V. Evaluation of potential carcinogenicity of organic chemicals in synthetic turf crumb rubber. *Environ Res.* 2019; 169:163-172.  
<https://doi.org/10.1016/j.envres.2018.10.018>
2. United States Environmental Protection Agency (U.S. EPA). Federal research action plan (FRAP) on recycled tire crumb used on playing fields and playgrounds. 2016. EPA/600/R-16/364. [https://www.epa.gov/sites/production/files/2016-12/documents/federal\\_research\\_action\\_plan\\_on\\_recycled\\_tire\\_crumb\\_used\\_on\\_playing\\_fields\\_and\\_playgrounds\\_status\\_report.pdf](https://www.epa.gov/sites/production/files/2016-12/documents/federal_research_action_plan_on_recycled_tire_crumb_used_on_playing_fields_and_playgrounds_status_report.pdf) [Accessed: 3 Oct, 2017]
3. European Chemicals Agency (ECHA). Annex XV Report: An evaluation of the possible health risks of recycled rubber granules used as infill in synthetic turf sports fields. 2017.  
[https://echa.europa.eu/documents/10162/13563/annex-xv\\_report\\_rubber\\_granules\\_en.pdf/dbcb4ee6-1c65-af35-7a18-f6ac1ac29fe4](https://echa.europa.eu/documents/10162/13563/annex-xv_report_rubber_granules_en.pdf/dbcb4ee6-1c65-af35-7a18-f6ac1ac29fe4)
4. Rijksinstituut voor Volksgezondheid en Milieu [Netherlands National Institute for Public Health and the Environment] (RIVM). Evaluation of health risks of playing sports on synthetic turf pitches with rubber granulate. 2017. Report Number: 2017-0016.  
<https://www.rivm.nl/dsresource?objectid=a397bdf3-7aa7-490a-85bd-5992b78bcb42&type=pdf&disposition=inline>
5. United States Environmental Protection Agency (U.S. EPA). Federal research on recycled tire crumb used on playing fields. U.S. Environmental Protection Agency; 2016.  
<https://www.epa.gov/chemical-research/federal-research-recycled-tire-crumb-used-playing-fields> [Accessed: 2017]
6. Agency for Toxic Substances and Disease Registry (ATSDR). Federal research action plan on recycled tire crumb used on playing fields and playgrounds. 2016.  
<https://www.atsdr.cdc.gov/frap/index.html> [Accessed: 2017]
7. Consumer Product Safety Commission (CPSC). Crumb rubber information center. 2016.  
<https://www.cpsc.gov/Safety-Education/Safety-Education-Centers/Crumb-Rubber-Safety-Information-Center> [Accessed: 2017]
8. Office of Environmental Health Hazard Assessment (OEHHA). Synthetic turf studies. 2017.  
<https://oehha.ca.gov/risk-assessment/synthetic-turf-studies> [Accessed: 2017]
9. National Toxicology Program (NTP). Synthetic turf/recycled tire crumb rubber. 2017.  
<https://ntp.niehs.nih.gov/results/areas/syntheticurf/index.html> [Accessed: 2017]
10. National Toxicology Program (NTP). NTP research report on chemical and physical characterization of crumb rubber. Research Triangle Park, NC: National Toxicology Program; 2019. Research Report 11. <https://doi.org/10.22427/NTP-RR-11>
11. National Toxicology Program (NTP). NTP research report on synthetic turf/recycled tire crumb rubber: Characterization of the biological activity of crumb rubber in vitro. Research

Triangle Park, NC: National Toxicology Program; 2019. Research Report 12.

<https://doi.org/10.22427/NTP-RR-12>

12. National Toxicology Program (NTP). NTP research report on synthetic turf/recycled tire crumb rubber: Feasibility study in support of non-inhalation in vivo exposures of synthetic turf/recycled tire crumb rubber. Research Triangle Park, NC: National Toxicology Program; 2019. Research Report 13. <https://doi.org/10.22427/NTP-RR-13>

13. National Toxicology Program (NTP). NTP research report on synthetic turf/recycled tire crumb rubber: 14-day exposure characterization studies of crumb rubber in female mice housed on mixed bedding or dosed via feed or oral gavage. Research Triangle Park, NC: National Toxicology Program; 2019. Research Report 14. <https://doi.org/10.22427/NTP-RR-14>

14. Bolgar MH, J; Groeger, J; Meronek, S. Handbook for the Chemical Analysis of Plastic and Polymer Additives, Second Edition. Boca Raton, FL: CRC Press; 2015.

15. Pavilonis BW, CP; Buckley, B; Lioy, PJ,. Bioaccessibility and risk of exposure to metals and SVOCs in artificial turf field fill materials and fibers. Risk Anal. 2014; 34(1):44-55.

<http://doi.org/10.1111/risa.12081>

16. PerkinElmer. Compositional Analysis of Tire Elastomers Using AutoStepwise TGA. 2012.

17. Cambridge Polymer Group. CPGAN #025 Car Tire Composition Analysis by TGA-FTIR. 2014.

18. McNitt A, Petrunak D, Serensits T. Temperature amelioration of synthetic turf surfaces through irrigation. University Park, PA: The Pennsylvania State University.

<https://plantscience.psu.edu/research/centers/ssrc/documents/temperature-irrigation.pdf>

19. Williams C, Pulley G. Synthetic surface heat studies. Provo, UT: Brigham Young University.

<https://aces.nmsu.edu/programs/turf/documents/brigham-young-study.pdf>

20. AST temps and TPG temps. 2016. <http://www.synturf.org/images/AST-Temps-TPG-Temps-Rev1-20161007.pdf>

21. International Organization for Standardization (ISO). (International Organization for Standardization). Soil quality - Assessment of human exposure from ingestion of soil and soil material - Procedure for the estimation of the human bioaccessibility/bioavailability of metals in soil. 2016. ISO/DIS 17924:2016(E).

22. United States Environmental Protection Agency (U.S. EPA). (U.S. Environmental Protection Agency). Research Protocol: Collections Related to Synthetic Turf Fields with Crumb Rubber Infill. 2016. [https://www.epa.gov/sites/production/files/2016-08/documents/tcrs\\_research\\_protocol\\_final\\_08-05-2016.pdf](https://www.epa.gov/sites/production/files/2016-08/documents/tcrs_research_protocol_final_08-05-2016.pdf)

**Table 1. Elemental Composition<sup>a</sup> (Wt %) of Six Individual Crumb Rubber Grains by Energy Dispersive Spectroscopy**

Element	%
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)
Ti	0.0 (0.1)
Zn	3.0 (1.8)

<sup>a</sup>Data given are for the analyzed area of individual grains to show the composition difference between different grains and do not represent composition of the bulk crumb rubber.

**Table 2. Estimated Levels of Metals in Crumb Rubber Lot<sup>a</sup>**

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	473 ppm	438 ppm	456 ppm
Iron	523 ppm	340 ppm	432 ppm
Magnesium	367 ppm	321 ppm	344 ppm
Sodium	304 ppm	300 ppm	302 ppm
Cobalt	153 ppm	136 ppm	145 ppm
Copper	51.6 ppm	37.5 ppm	44.6 ppm
Lead	12.1 ppm	12.9 ppm	12.5 ppm
Nickel	4.53 ppm	7.32 ppm	5.93 ppm
Manganese	5.78 ppm	5.99 ppm	5.89 ppm
Barium	4.95 ppm	5.48 ppm	5.22 ppm
Tin	1.51 ppm	2.39 ppm	1.95 ppm
Arsenic	0.929 ppm	0.680 ppm	0.805 ppm
Cadmium	0.715 ppm	0.582 ppm	0.649 ppm
Chromium	<LOQ <sup>b</sup>	<LOQ	<LOQ

<sup>a</sup>Analyses were conducted either by ICP-MS or ICP-AES. Data are shown for duplicate analyses. Data are organized based on the levels estimated with highest shown on top.

<sup>b</sup>LOQ = limit of quantitation. For chromium, LOQ was ~13 ppm.

**Table 3. Estimated Levels of Volatile and Semivolatile Organic Compounds in Crumb Rubber Lot by Headspace GC–MS<sup>a</sup>**

Compound	Replicate 1 (ppm in Rubber)	Replicate 2 (ppm in Rubber)	Average (ppm in Rubber)	Match Score <sup>b</sup> (%)
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 <sup>c</sup>
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 <sup>c</sup>
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 <sup>c</sup>
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 <sup>d</sup>
Decane	0.00394	0.00360	0.00377	58.8 <sup>d</sup>
Tetradecane	0.00379	0.00364	0.00371	74.4 <sup>d</sup>
Butylated Hydroxytoluene	0.00294	0.00205	0.00250	84.6
Tridecane	0.00198	0.00166	0.00182	63.7 <sup>d</sup>
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 <sup>c</sup>
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

<sup>a</sup>The data presented are following incubation at 100 °C and under conditions described in methods.

<sup>b</sup>The compounds in this table were identified by matching retention time and mass spectra with standards. The match score with a library spectrum is included as a confirmation.

<sup>c</sup>The match score with the library spectrum was low because the peak in the sample was poorly resolved from neighboring peaks and its mass spectrum contained fragments contributed by those peaks.

<sup>d</sup>For the larger alkanes, the response in the samples was too low to detect the low abundance of higher mass fragments, but the peak did match retention time and lower mass fragments with the corresponding standard.

**Table 4. Percent of Extractable Crumb Rubber in Various Solvents<sup>a</sup>**

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

<sup>a</sup>The amount extracted by solvent under the conditions described in the method was calculated by subtracting the weight of the dried residual crumb rubber from the starting crumb rubber weight. The percent extracted was calculated by dividing the extracted weight by the starting crumb rubber weight.

**Table 5. Constituents of Crumb Rubber in Methylene Chloride Extract Identified by GC–MS with High Confidence<sup>a</sup>**

Name	CAS Number	ID by Match with Standard <sup>a</sup>	ID by Match with Library <sup>b</sup>
Naphthalene	91-20-3	Yes	Yes
Acenaphthene	83-32-9	Yes	Yes
Phenanthrene	65996-93-2	Yes	Yes
Fluoranthene	206-44-0	Yes	Yes
Pyrene	129-00-0	Yes	Yes
2-Methylnaphthalene	91-57-6	No	Yes
1-Methylnaphthalene	90-12-0	No	Yes
1,2-Dihydro-2,2,4-trimethylquinoline	147-47-7	No	Yes
Benzothiazole	95-16-9	Yes	Yes
Butylated Hydroxytoluene	128-37-0	Yes	Yes
4-Tert-octylphenol	140-66-9	Yes	Yes
Heptadecane	629-78-7	Yes	Yes
Octadecane	593-45-3	Yes	Yes
Nonadecane	629-92-5	Yes	Yes
Icosane	112-95-8	Yes	Yes
Heneicosane	629-94-7	Yes	Yes
Docosane	629-97-0	Yes	Yes
Tricosane	638-67-5	Yes	Yes
Tetracosane	646-31-1	Yes	Yes
Pentacosane	629-99-2	Yes	Yes
Hexacosane	630-01-3	Yes	Yes
Heptacosane	593-49-7	Yes	Yes
Octacosane	630-02-4	Yes	Yes
Nonacosane	630-03-5	Yes	Yes
Triacontane	638-68-6	Yes	Yes
Hentriacontane	630-04-6	No	Yes
Dotriacontane	544-85-4	No	Yes
Tritriacontane	630-05-7	No	Yes
Pentatriacontane	630-07-9	No	Yes
Phthalimide	85-41-6	Yes	Yes
Cyclopropyl Methyl Ketone	765-43-5	Yes	Yes
Toluene	108-88-3	Yes	Yes
4-Methyl-2-pentanone (MIBK)	108-10-1	No	Yes

Chemical and Physical Characterization of Recycled Tire Crumb Rubber

Name	CAS Number	ID by Match with Standard <sup>a</sup>	ID by Match with Library <sup>b</sup>
n-(1,3-Dimethylbutyl)-n'-phenyl-p-phenylenediamine (6PPD)	793-24-8	Yes	Yes
N-Isopropyl-N'-phenyl-p-phenylenediamine (IPPD)	101-72-4	No	Yes
3,5-Di-tert-butyl-4-hydroxybenzaldehyde	1620-98-0	No	Yes
Methyl Stearate	112-61-8	No	Yes
Bis(2-ethylhexyl) Phthalate	117-81-7	Yes	Yes
Cyclopentanone, 2-Methyl-	1120-72-5	No	Yes
Trichloroethane	71-55-6/79-00-5	No	Yes
1-Hexene, 3,5,5-Trimethyl-	4316-65-8	No	Yes
3-[(E)-2-(4-Hexoxyphenyl)ethenyl]phenol	Unknown	No	Yes

<sup>a</sup>Identification of constituents was done with retention time and mass spectral match with a standard, a >85% match, or both with a library reference spectrum.

<sup>b</sup>≥85% match with a library spectrum.

**Table 6. Constituents of Crumb Rubber in Ethanolic Extract Identified by LC–MS**

Retention Time (Minutes)	Name	CAS No.	Basis for Identification
21.6	Benzothiazole	95-16-9	Retention time and mass spectrum match with a standard and a library spectrum.
29.7	n-(1,3-Dimethylbutyl)-n'-phenyl-p-phenylenediamine (6PPD)	793-24-8	Retention time and mass spectrum match with a standard.
19.8	2-Hydroxybenzothiazole	934-34-9	Mass spectrum match with a library spectrum. Seen in crumb rubber in the literature.
22.5	Hexa(methoxymethyl)melamine	68002-20-0	Mass spectrum match with a library spectrum. Seen in crumb rubber in the literature.

Chemical and Physical Characterization of Recycled Tire Crumb Rubber

**Table 7. Average Estimated Levels of Volatile and Semivolatile Organic Compounds in Various Crumb Rubber Fractions by Headspace GC–MS**

Compound	Bulk Lot <sup>a</sup>	Pooled 14–80-Mesh (ppm)	14-Mesh <sup>b</sup> (ppm)	40-Mesh (ppm)	400-Mesh (ppm)
Methyl Isobutyl Ketone	4.34	3.50	4.05	3.54	2.73
Toluene	0.454	0.218	0.875	1.12	6.80
Aniline	1.28	0.689	1.04	1.22	2.00
Benzothiazole	0.629	0.474	0.615	0.633	0.563
Cyclohexanone	0.464	0.361	0.438	0.422	0.347
Diphenylamine	0.0502	0.0343	0.0667	0.0606	0.0280
2,4,4-Trimethylpentene	0.0779	0.0470	0.0699	0.0537	0.0363
Hexamethylcyclotrisiloxane	0.207	0.125	0.179	0.144	0.133
5-Methyl-2-hexanone	0.330	0.201	0.274	0.265	0.139
Phenol	0.0696	0.0196	0.0367	0.0315	0.0311
Hexanal	0.128	0.0830	0.119	0.100	0.316
m/p-Xylene	0.0795	0.0566	0.0703	0.0615	0.0256
1-Methylnaphthalene	0.00352	0.00219	0.00313	0.00311	0.00139
Napthalene	0.0121	0.00727	0.0104	0.00923	0.00531
1,2,4-Trimethylbenzene	0.0150	0.00993	0.0124	0.0116	0.00210
2-Methylnaphthalene	0.00598	0.00408	0.00539	0.00514	0.00289
Butylated Hydroxytoluene	0.00298	0.00123	0.00314	0.00178	0.000503
Ethylbenzene	0.00423	0.00280	0.00389	0.00338	0.00242
Styrene	0.0169	0.0129	0.0192	0.0160	0.0126
3-Ethyltoluene	0.00390	0.00231	0.00335	0.00315	0.000279

<sup>a</sup>The bulk lot values were obtained in a different analysis using slightly different methodology and the duplicate samples analyzed differed by as much as 30%, so differences between samples of less than 2× are unlikely to be significant.

<sup>b</sup>The mesh size is the size sieve the crumb rubber was collected on, so the samples contain particles between the mesh size of the sieve above it in the stack and the mesh size of the sieve that the material did not go through. The 14-mesh sample (the largest sieve used) contained all of the particles larger than 1.41 mm. The 40-mesh sample contained the particles between 0.420 mm and 1.41 mm. The 80-mesh sample (not analyzed) contained particles between 0.177 and 0.420 mm. The 400-mesh sample contained particles between 0.037 mm and 0.177 mm. The pooled 14- to 80-mesh sample would, therefore, contain all particles greater than 0.177 mm.



**Table 8. Elemental Composition (Wt %) of Crumb Rubber Fractions by Energy Dispersive Spectroscopy<sup>a</sup>**

Element	Lot CRM12052016 <sup>b</sup>	Lot CRM09132016 <sup>c</sup>
C	80.0 (5.2)	81.9 (1.9)
O	10.5 (1.1)	9.67 (1.86)
Mg	0.14 (0.06)	0.16 (0.07)
Al	0.39 (0.65)	0.11 (0.15)
Si	3.60 (5.22)	0.17 (0.15)
S	2.53 (0.67)	3.90 (1.21)
Cl	0.26 (0.30)	0.33 (0.13)
Ca	0.35 (0.64)	0.07 (0.08)
Ti	0.01 (0.02)	0.00 (0.01)
Zn	2.23 (1.21)	3.64 (1.71)

<sup>a</sup>Average (standard deviation) for 10 particles is shown.

<sup>b</sup>Crumb rubber lot made from combined mesh sizes 14, 40, and 80.

<sup>c</sup>Crumb rubber lot made from mesh size 400.

**Table 9. Estimated Blank Biofluid Concentration (Equivalent  $\mu\text{g}$  in Extract per g of Crumb Rubber Extracted)<sup>a</sup>**

Simulated Fluid Analytical Technique	Dermal GC-MS Headspace	Dermal GC-MS Liquid	Dermal LC-MS	Inhalation GC-MS Headspace	Inhalation GC-MS Liquid	Inhalation LC-MS	Gastric GC-MS Headspace	Gastric GC-MS Liquid	Gastric LC-MS	Ingestion GC-MS Headspace	Ingestion GC-MS Liquid	Ingestion LC-MS
2,4,4-Trimethylpentene	0.368	0.00590	–	0.625	0.0141	–	0.483	0.00627	–	0.717	0.0360	–
Ethylbenzene	0.00192	0.108	–	0.00142	0.122	–	0.00191	0.0286	–	0.00238	0.124	–
1,2,4-Trimethylbenzene	0.00367	0.0218	–	0.00442	0.0253	–	0.00310	0.0124	–	0.00506	0.0404	–
Aniline	0.475	0.0159	–	0.408	0.0273	–	0.411	0.0469	–	0.789	0.0937	–
Naphthalene	0.00604	0.0569	–	0.00254	0.0629	–	0.00274	0.0226	–	0.00573	0.0649	–
1-Methylnaphthalene	0.00451	0.0195	–	0.00130	0.0207	–	0.00100	0.00730	–	0.00249	0.0291	–
BHT	0.00815	0.0830	0.576	0.00355	0.164	0.585	0.00275	0.129	0.125	0.00509	0.234	1.20
2-Mercaptobenzothiazole	–	1.70	0.00364	–	1.12	0.00151	–	0.222	0.00290	–	11.2	0.00557
Pyrene	–	0.168	0.102	–	0.291	0.0331	–	0.0166	0.135	–	0.187	0.323
Bis-2-ethylhexylphthalate	–	ND	1.11	–	ND	ND	–	ND	4.09	–	ND	1.29
Toluene	0.0458	ND	–	0.0327	ND	–	0.0440	16.1	–	0.477	ND	–
Hexamethylcyclotrisiloxane	2.69	3.91	–	3.24	3.65	–	3.29	3.87	–	3.80	6.68	–
m-Xylene	0.00568	0.0360	–	0.00350	0.0558	–	0.00497	0.0357	–	0.00754	0.107	–
Styrene	0.00389	0.446	–	0.00194	0.481	–	0.0323	0.205	–	0.00729	0.667	–
3-Ethyltoluene	ND	0.0243	–	ND	0.0327	–	ND	0.0157	–	ND	0.134	–
Benzothiazole	0.216	69.1	ND	0.129	63.7	ND	0.137	29.0	38.1	0.186	69.9	0.853
Dicyclohexylamine	–	ND	0.322	–	ND	0.266	–	0.0908	59.4	–	ND	23.5
Diphenylamine	0.0364	0.0987	0.546	0.0154	0.136	0.211	0.0309	0.0193	0.142	0.0739	0.132	0.509
4-Tert-octylphenol	–	ND	–	–	ND	–	–	ND	–	–	ND	–
6PPD	–	0.336	ND	–	0.973	ND	–	ND	ND	–	0.254	ND
Bisphenol P	–	3.86	0.883	–	2.42	1.61	–	ND	1.45	–	ND	6.68
MIBK	0.0977	0.0383	–	0.0567	ND	–	0.0814	ND	–	0.232	0.0478	–
Hexanal	0.520	0.253	–	0.557	1.71	–	1.19	0.238	–	2.34	0.320	–
Cyclohexanone	0.402	3.44	ND	0.457	3.53	ND	0.699	4.38	1.02	6.73	5.06	ND

## Chemical and Physical Characterization of Recycled Tire Crumb Rubber

Simulated Fluid Analytical Technique	Dermal GC-MS Headspace	Dermal GC-MS Liquid	Dermal LC-MS	Inhalation GC-MS Headspace	Inhalation GC-MS Liquid	Inhalation LC-MS	Gastric GC-MS Headspace	Gastric GC-MS Liquid	Gastric LC-MS	Ingestion GC-MS Headspace	Ingestion GC-MS Liquid	Ingestion LC-MS
5-Methyl-2-hexanone	ND	0.0150	–	ND	0.0211	–	ND	0.00700	–	ND	0.00506	–
Phenol	11.5	0.699	–	46.4	1.14	–	28.9	0.796	–	68.5	0.991	–
2-Methylnaphthalene	0.00564	0.0262	–	0.00902	0.0293	–	0.00241	0.0232	–	0.0134	0.0339	–
Phthalimide	–	2.53	0.876	–	2.00	0.214	–	2.72	279	–	2.73	31.9
Fluorene	0.00158	0.0608	ND	0.00114	0.0602	ND	ND	0.0216	ND	0.00165	0.0584	ND
2-Hydroxybenzothiazole	–	10.8	0.00454	–	12.3	ND	–	12.1	0.0975	–	9.12	0.0628
Tetracosane	–	30.0	ND	–	40.7	0.421	–	7.82	ND	–	25.0	ND
Tetratriacontane	–	13.5	–	–	39.4	–	–	7.68	–	–	13.2	–

<sup>a</sup>Data shown are those estimated under different experimental conditions used as described under methods.

<sup>b</sup>ND = No peak detected; – = Standard not seen on this method.

Chemical and Physical Characterization of Recycled Tire Crumb Rubber

**Table 10. Estimated Bioaccessible Constituent Concentration ( $\mu\text{g}$  in Extract per g of Crumb Rubber Extracted)<sup>a</sup>**

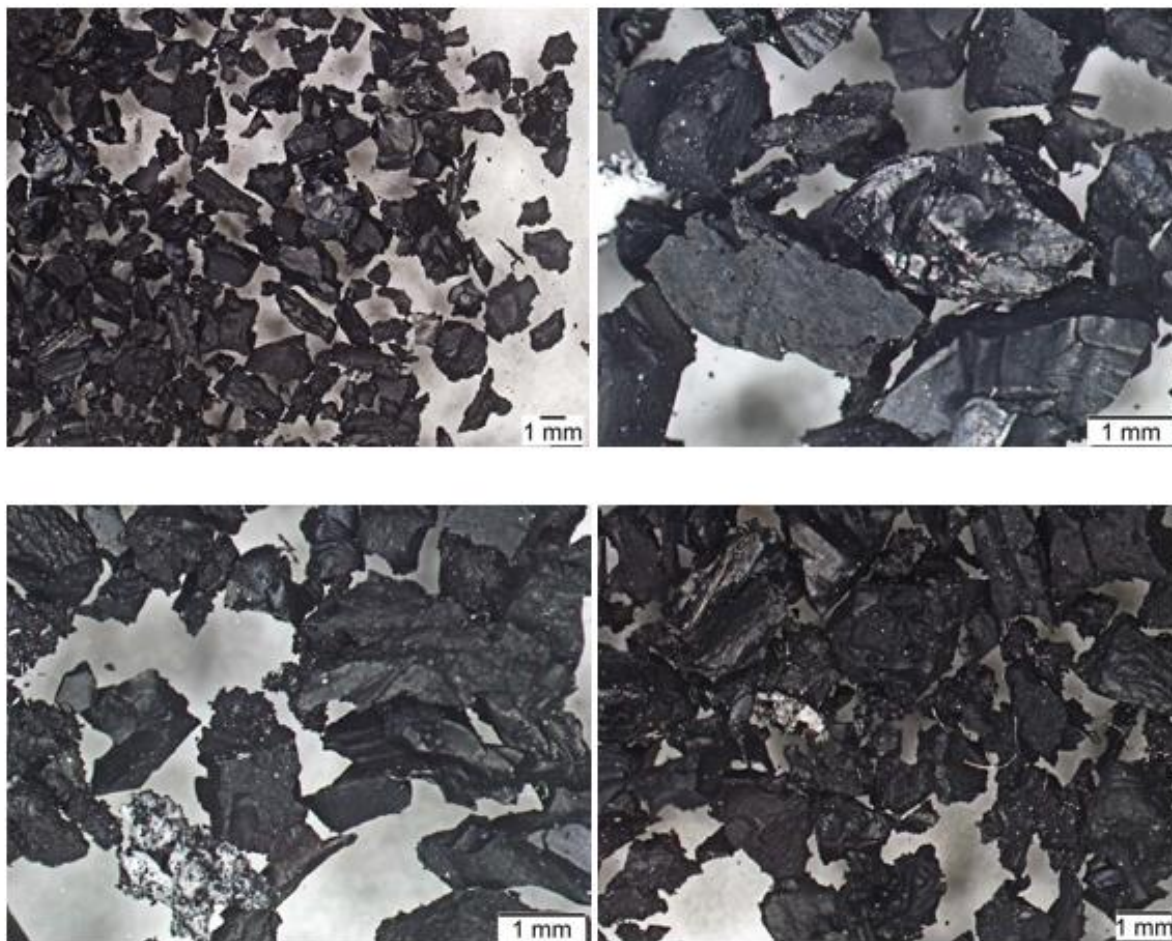
Simulated Fluid Analytical Technique	Dermal GC-MS Headspace	Dermal GC-MS Liquid	Derma ILC-MS	Inhalation GC-MS Headspace	Inhalation GC-MS Liquid	Inhalation LC-MS	Gastric GC-MS Headspace	Gastric GC-MS Liquid	Gastric LC-MS	Ingestion GC-MS Headspace	Ingestion GC-MS Liquid	Ingestion LC-MS
2,4,4-Trimethylpentene	ND <sup>b</sup>	ND	–	ND	ND	–	ND	ND	–	ND	ND	–
Ethylbenzene	ND	ND	–	ND	ND	–	ND	ND	–	ND	ND	–
1,2,4-Trimethylbenzene	ND	ND	–	ND	ND	–	ND	0.0772 I	–	ND	ND	–
Aniline	4.23 I	2.80	–	14.1	2.07	–	1.54 I	1.06	–	56.0	39.2	–
Naphthalene	ND	ND	–	ND	ND	–	ND	ND	–	ND	ND	–
1-Methylnaphthalene	ND	ND	–	ND	ND	–	ND	0.0386 I	–	ND	ND	–
BHT	ND	ND	ND	ND	ND	ND	ND	ND	0.727 I	0.0254 I	ND	ND
2-Mercaptobenzothiazole	–	11.0 I	ND	–	17.6	0.503	–	55.0	5.51	–	41.2 I	0.615
Pyrene	–	ND	ND	–	ND	ND	–	ND	ND	–	ND	ND
Bis-2-ethylhexylphthalate	–	ND	NDI	–	ND	1.90	–	ND	ND	–	ND	ND
Toluene	0.246 I	ND	–	0.278 I	2.17	–	0.362 I	NDI	–	ND	3.77	–
Hexamethylcyclotrisiloxane	NDI	NDI	–	NDI	NDI	–	NDI	NDI	–	NDI	NDI	–
m-Xylene	ND	ND	–	ND	ND	–	ND	ND	–	ND	ND	–
Styrene	ND	ND	–	ND	ND	–	ND	ND	–	ND	ND	–
3-Ethyltoluene	ND	ND	–	ND	ND	–	ND	ND	–	ND	ND	–
Benzothiazole	14.5	NDI	34.7	29.6	NDI	77.9	26.6	NDI	380 I	27.8	NDI	171
Dicyclohexylamine	–	ND	47.4	–	18.7	218	–	ND	387 I	–	7.26	385
Diphenylamine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-tert-Octylphenol	–	ND	–	–	ND	–	–	ND	–	–	ND	–
6PPD	–	ND	ND	–	ND	ND	–	26.3	ND	–	1.79 I	ND
Bisphenol P	–	NDI	ND	–	NDI	NDI	–	ND	NDI	–	ND	NDI
MIBK	22.0	2.83	–	8.52	3.40	–	0.496 I	3.28	–	1.63 I	2.32	–
Hexanal	ND	ND	–	ND	NDI	–	NDI	ND	–	NDI	ND	–
Cyclohexanone	6.09	NDI	ND	3.91 I	NDI	ND	11.4	NDI	NDI	NDI	NDI	12.2

Chemical and Physical Characterization of Recycled Tire Crumb Rubber

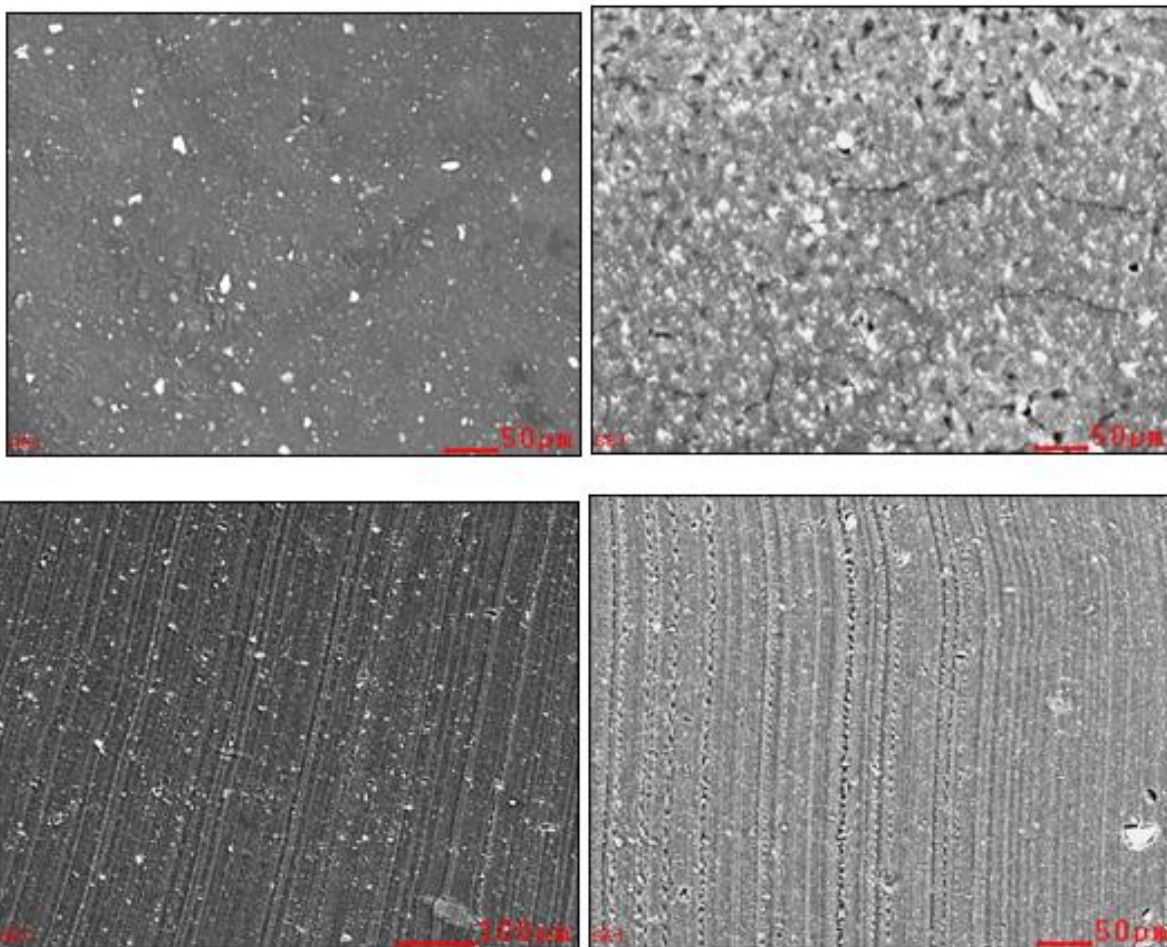
Simulated Fluid Analytical Technique	Dermal GC-MS Headspace	Dermal GC-MS Liquid	Derma ILC-MS	Inhalation GC-MS Headspace	Inhalation GC-MS Liquid	Inhalation LC-MS	Gastric GC-MS Headspace	Gastric GC-MS Liquid	Gastric LC-MS	Ingestion GC-MS Headspace	Ingestion GC-MS Liquid	Ingestion LC-MS
5-Methyl-2-Hexanone	0.328	0.0974 I	–	0.117	0.118 I	–	ND	0.121	–	ND	0.0939	–
Phenol	NDI	ND	–	NDI	NDI	–	NDI	ND	–	NDI	ND	–
2-Methylnaphthalene	ND	ND	–	ND	ND	–	ND	ND	–	ND	ND	–
Phthalimide	–	18.1 I	5.46	–	6.46 I	ND	–	19.9 I	NDI	–	17.7 I	NDI
Fluorene	ND	ND	ND	ND	ND	ND	ND	ND	0.491	ND	ND	0.981
2-Hydroxybenzothiazole	–	NDI	3.12	–	NDI	118	–	NDI	137	–	NDI	1510
Tetracosane	–	NDI	ND	–	NDI	ND	–	NDI	1.03	–	NDI	65.8
Tetratriacontane	–	NDI	–	–	NDI	–	–	NDI	–	–	NDI	–

<sup>a</sup>Data shown are those estimated under different experimental conditions used as described under methods.

<sup>b</sup>ND = <3 × blank and blank response small (<20 ng/mL); NDI = <3 × blank and blank response large (>20 ng/mL); Number and I = >3 × blank but blank response large (>10% on Sample Response); – = Standard not seen on this method.



**Figure 1. Representative Optical Micrographs of Two Crumb Rubber Regions**



**Figure 2. Representative Scanning Electron Micrographs of the Cut Surfaces of Four Dissected Crumb Rubber Grains**

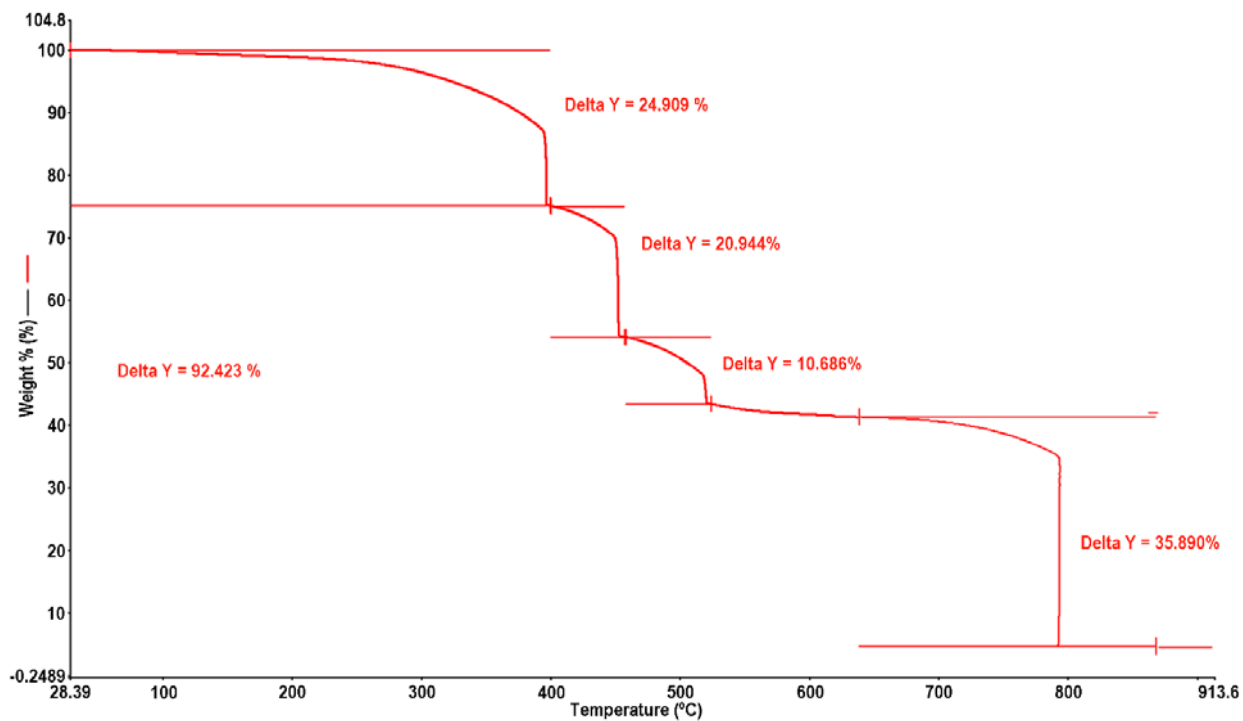


Figure 3. Representative Thermogram from Thermo Gravimetric Analysis of Crumb Rubber



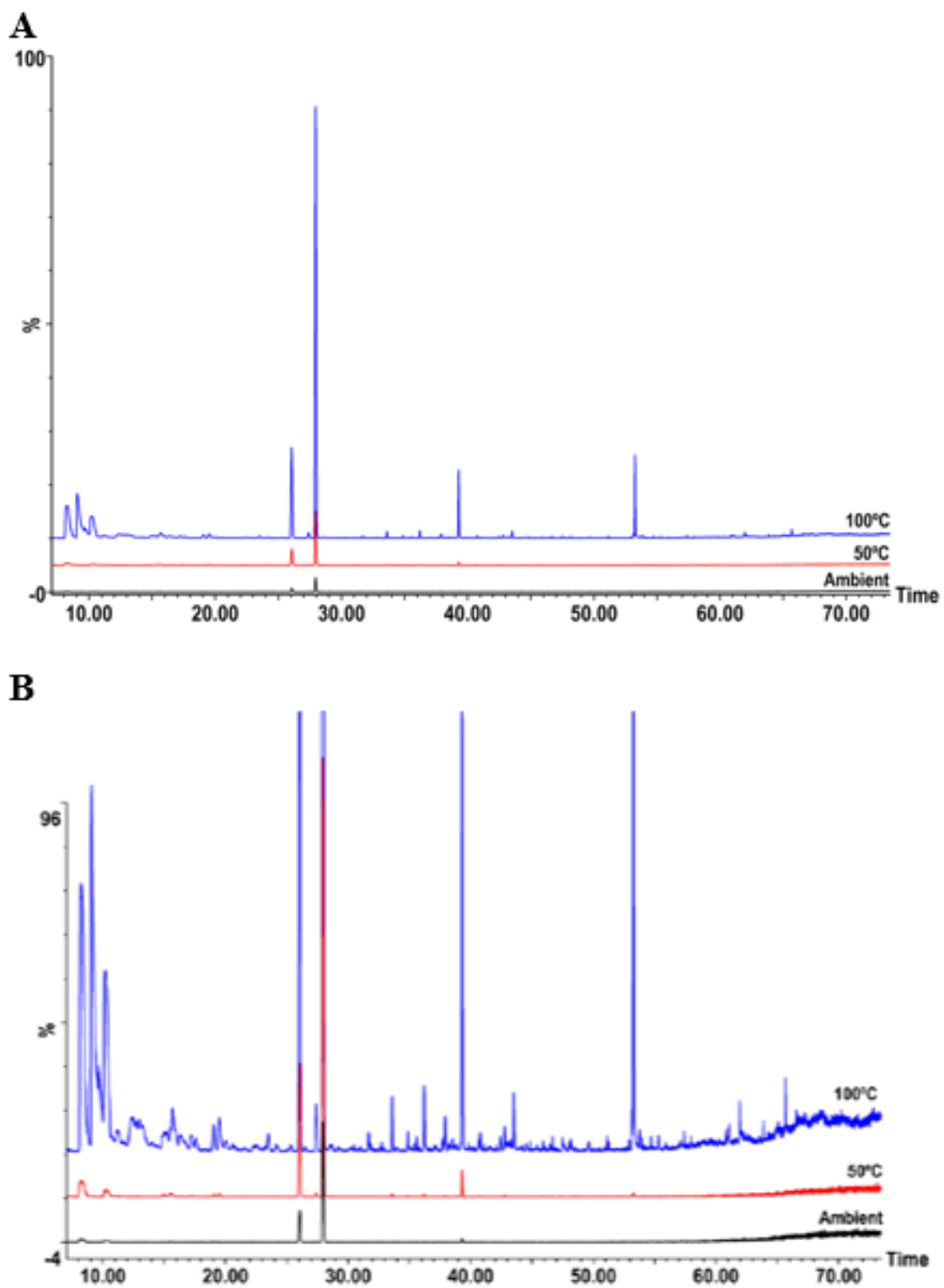


Figure 4. Comparison of GC-MS Chromatograms at Varying Incubation Temperatures in Headspace GC-MS Analysis: (A) Full Scale (B) Expanded Scale

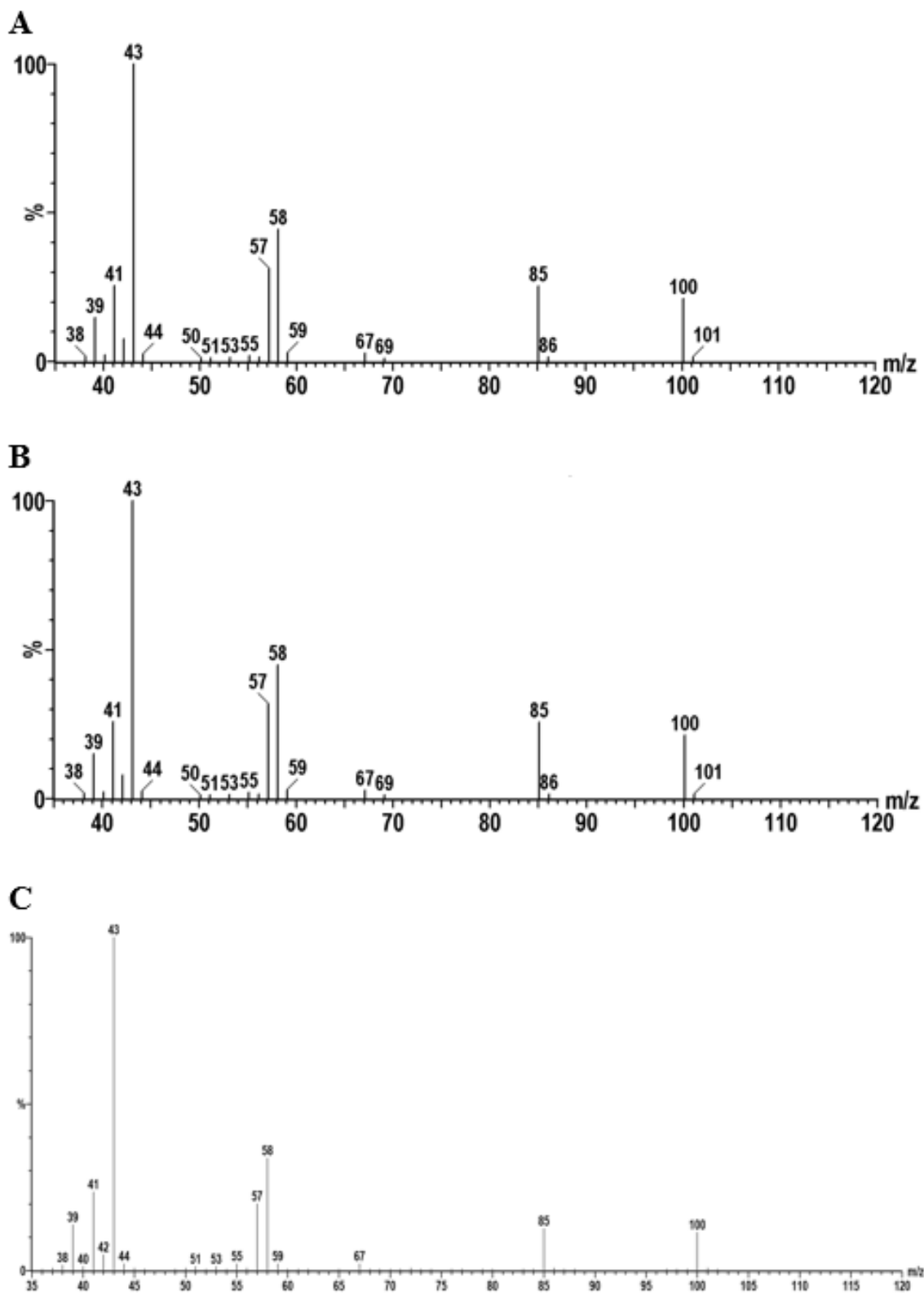
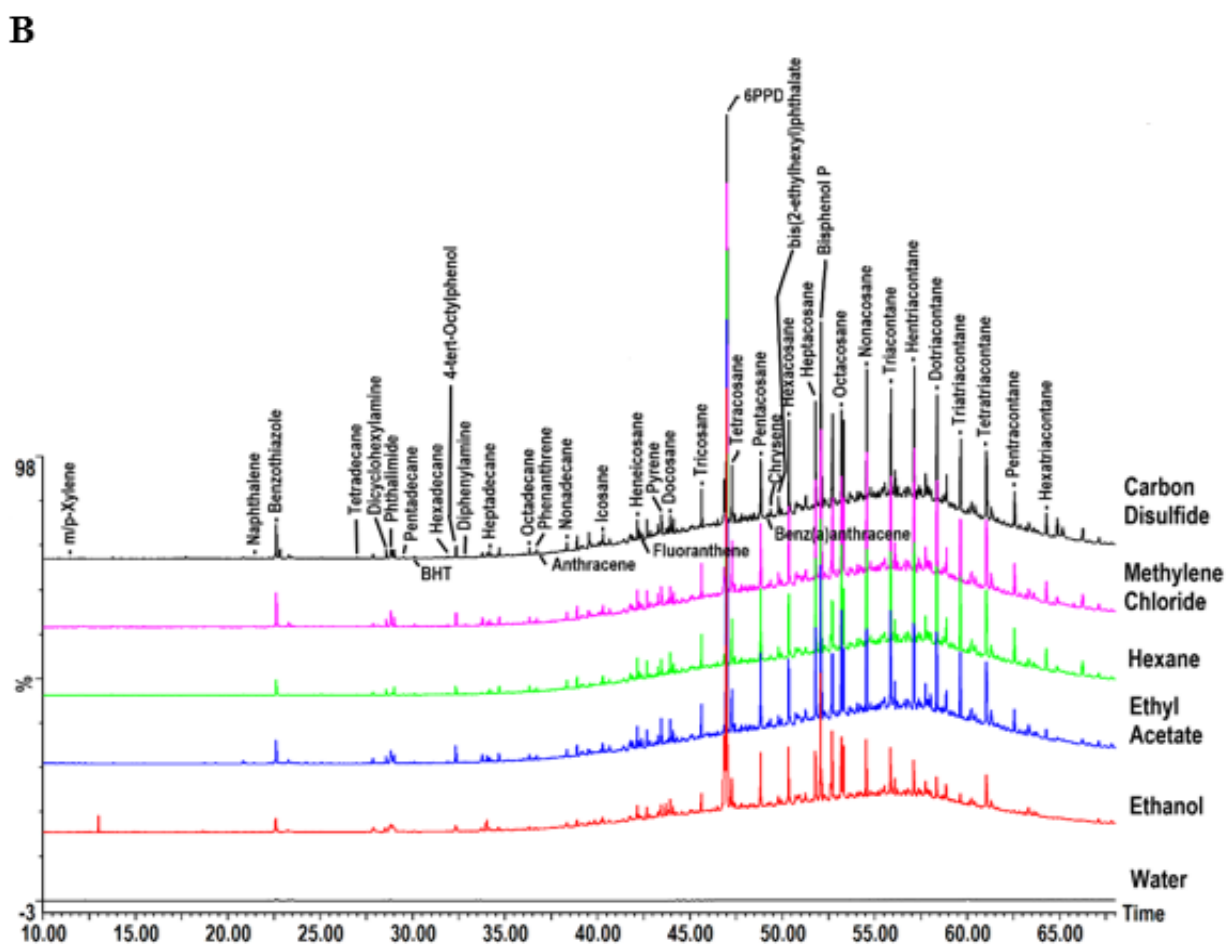
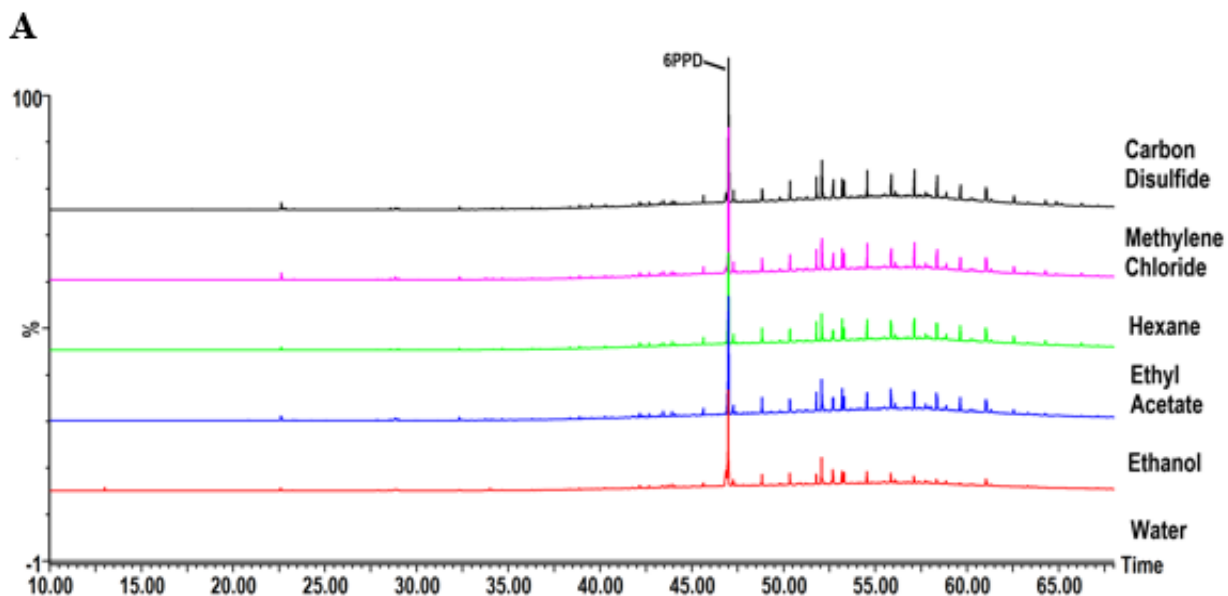
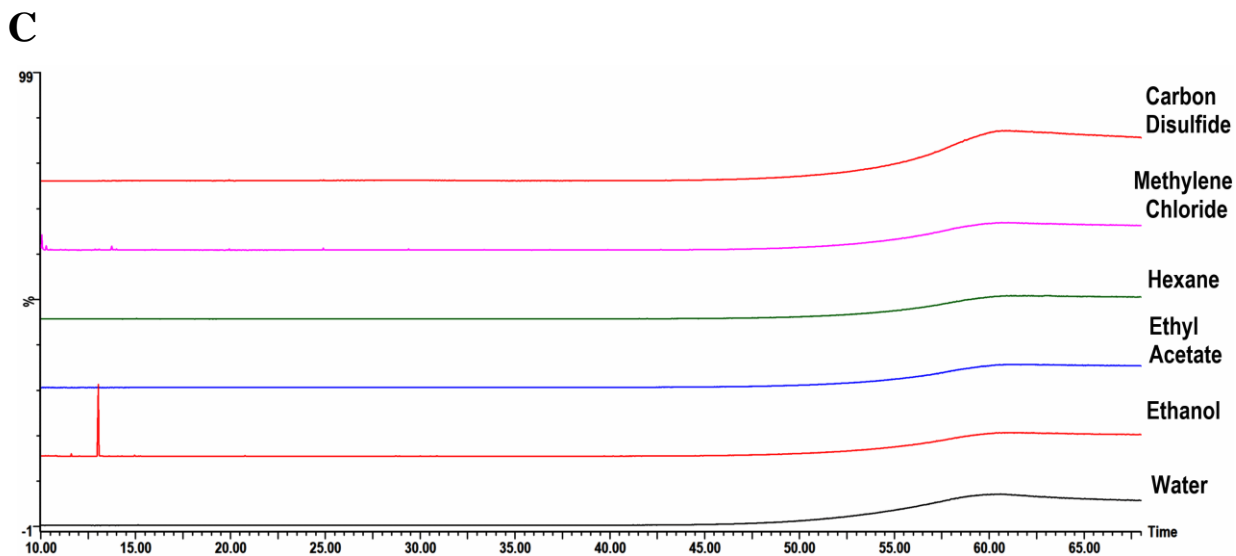


Figure 5. GC–MS Spectra for Methyl Isobutyl Ketone: (A) Standard Spectrum, (B) Crumb Rubber Sample Spectrum, and (C) Library Spectrum

# Chemical and Physical Characterization of Recycled Tire Crumb Rubber





**Figure 6. Comparison of GC–MS Chromatograms of Crumb Rubber Following Extraction with Various Solvents: (A) Full- and (B) Expanded-Scale Chromatograms, and (C) Blank Solvents**

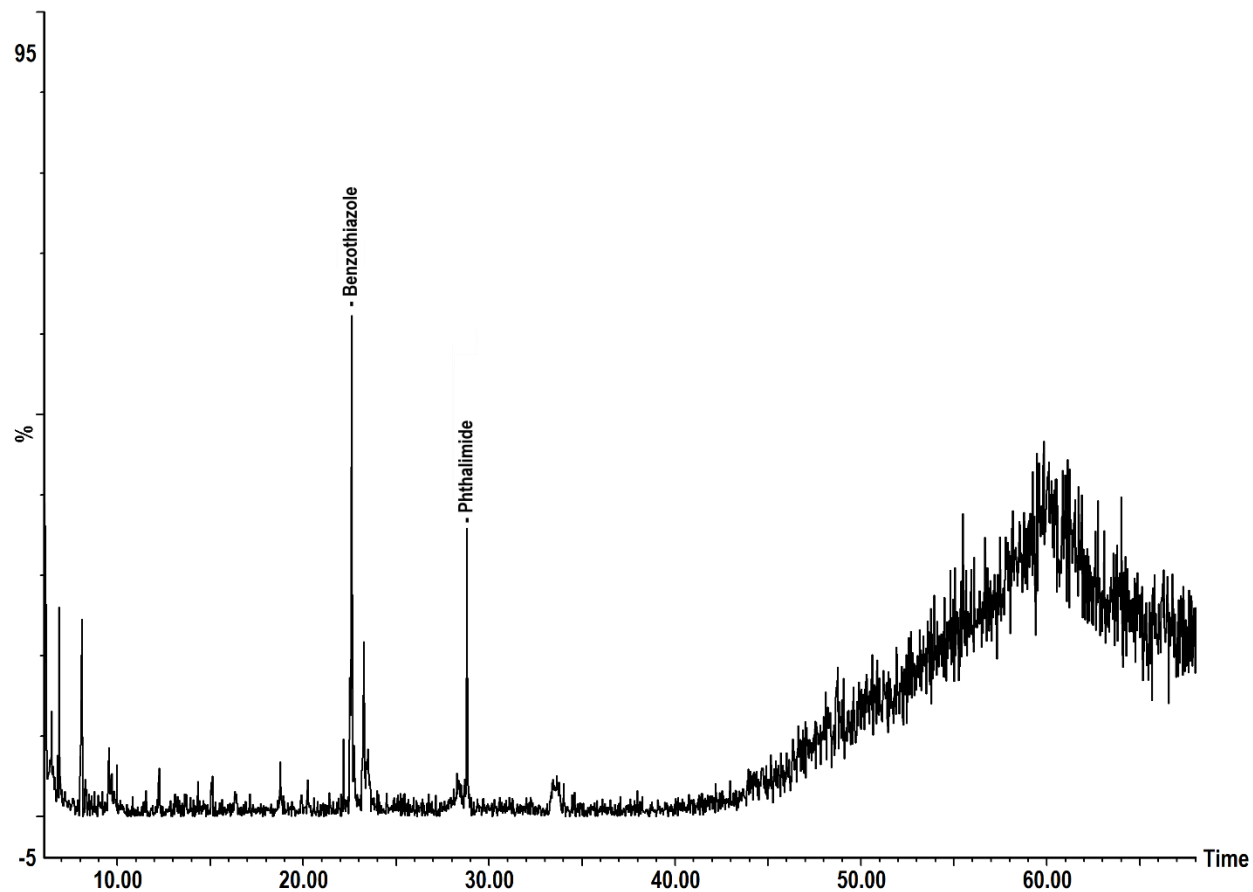
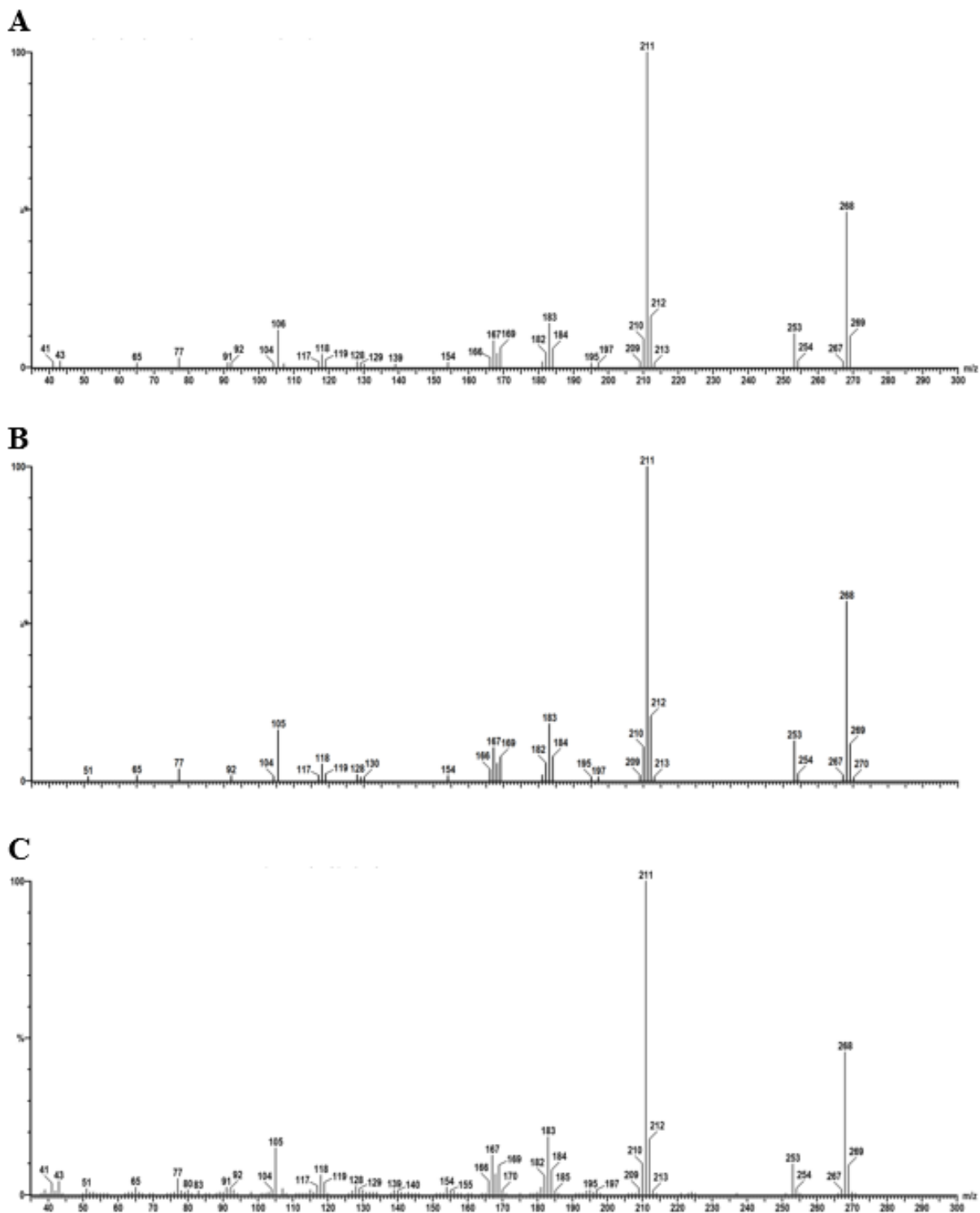


Figure 7. GC-MS Chromatogram of Crumb Rubber Following Extraction with Water



**Figure 8. GC–MS Spectra for the n-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD): (A) Standard Spectrum, (B) Crumb Rubber Sample Spectrum, and (C) Library Spectrum**

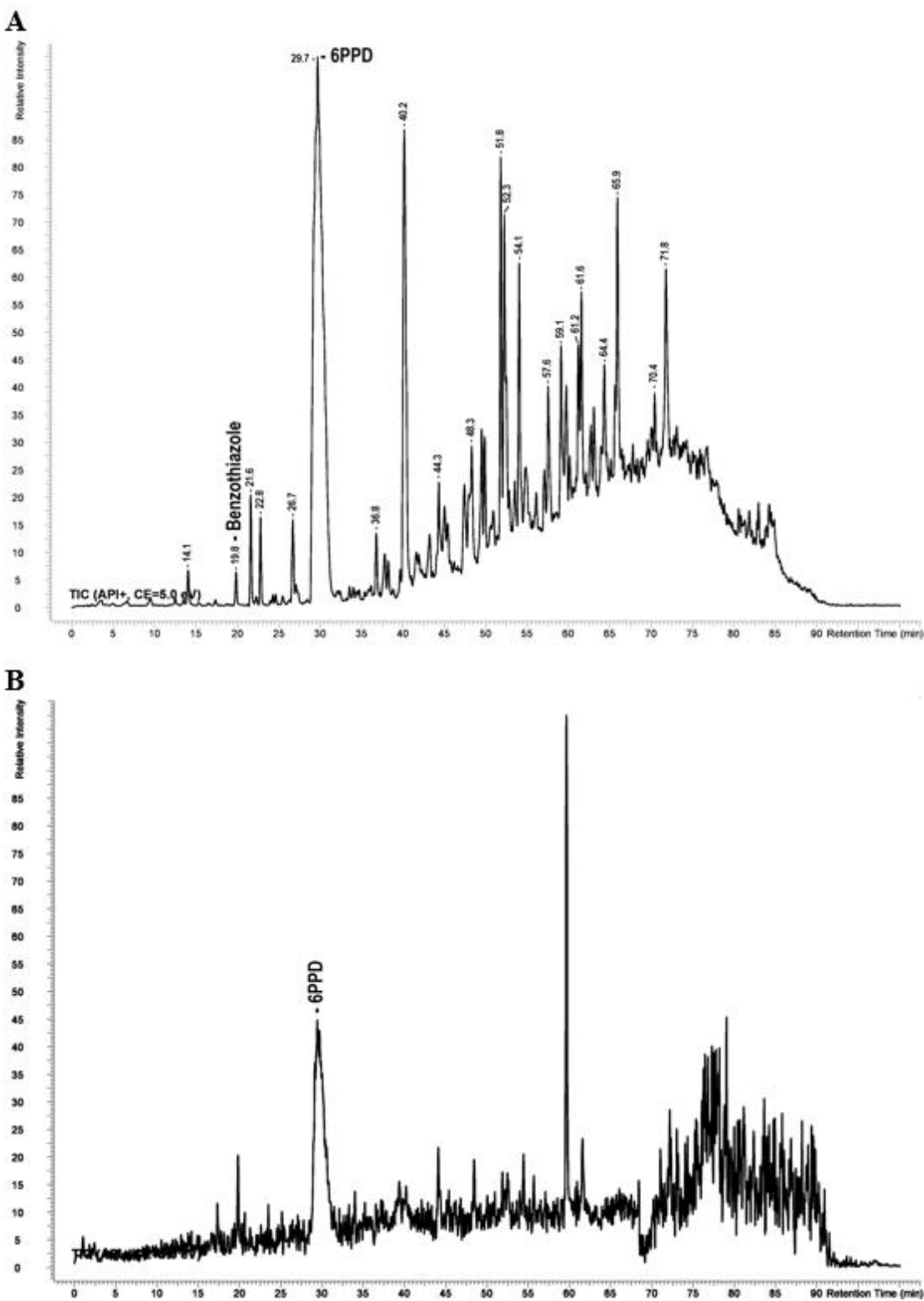
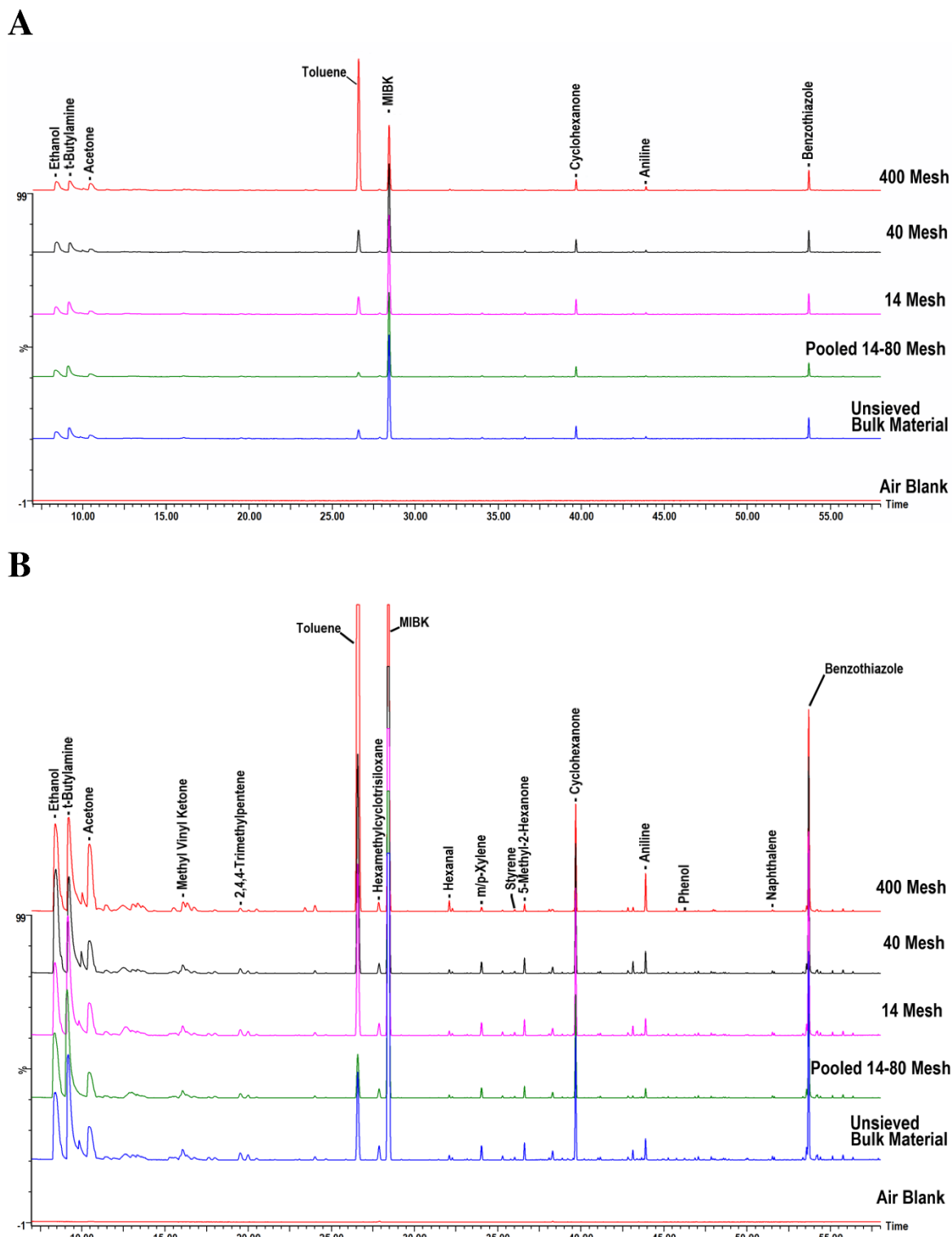
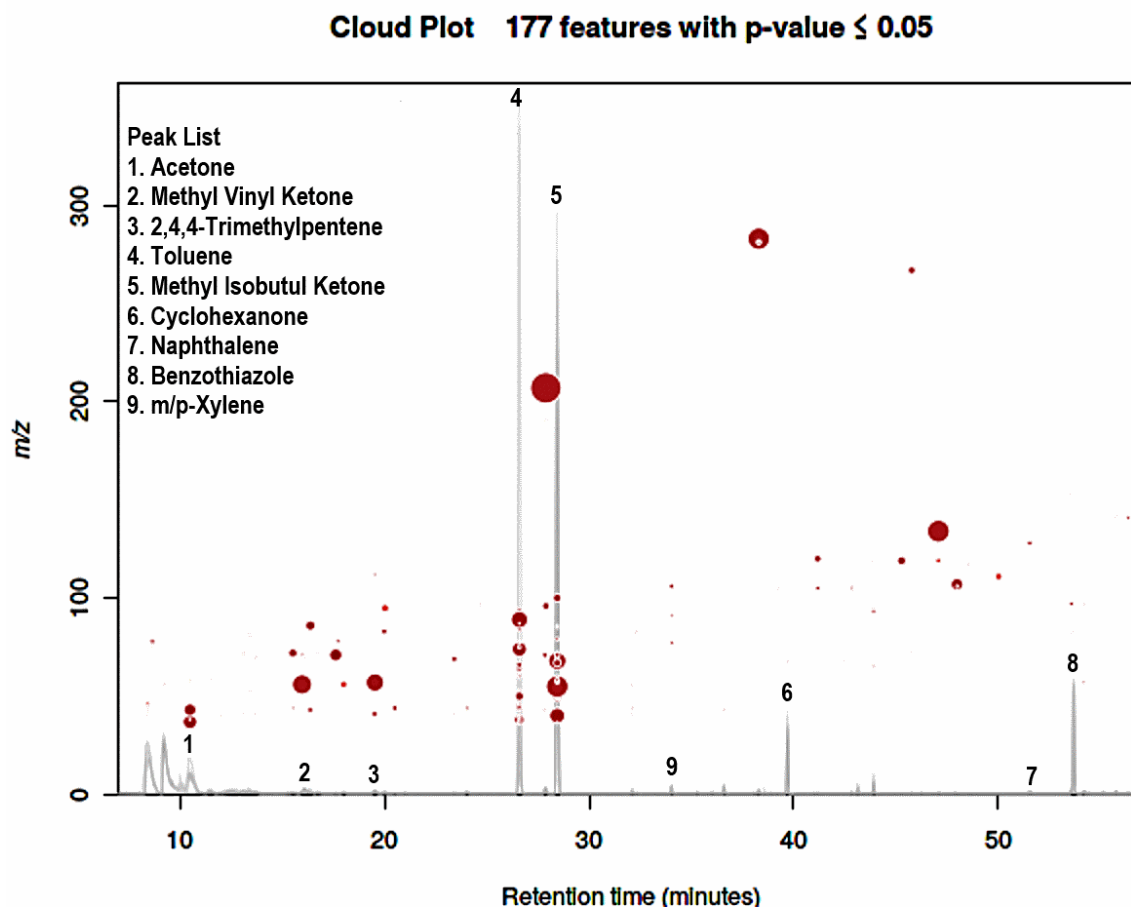


Figure 9. LC-MS Total Ion Chromatograms of Ethanol Extract of Crumb Rubber: Comparison of Blank-Subtracted Extract of Crumb Rubber, (A) Positive Ion Mode and (B) Negative Ion Mode



**Figure 10. Comparison of Headspace GC–MS Chromatograms of Various Crumb Rubber Fractions and Unsieved Bulk Material Following Incubation at 100°C: (A) Full- and (B) Expanded-Scale Chromatograms**

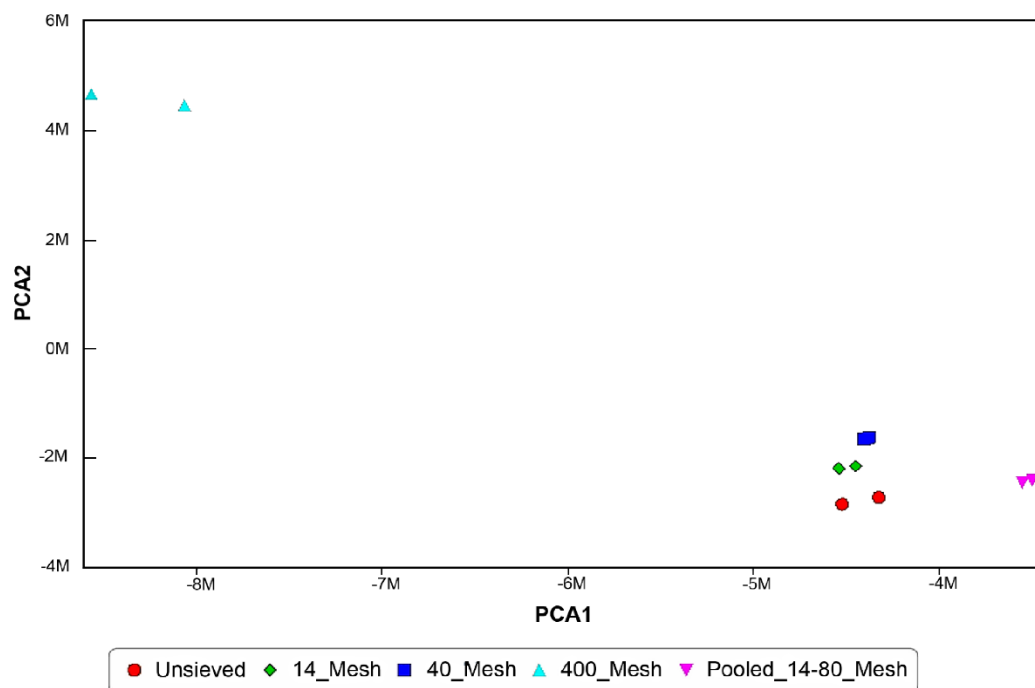




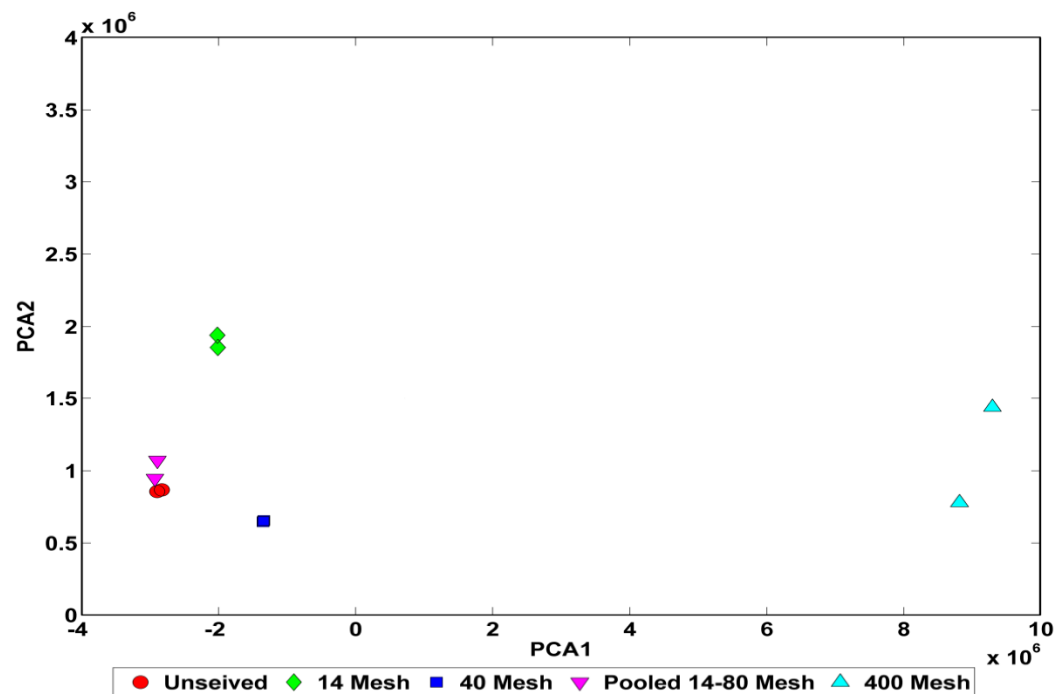
**Figure 11. Cloud Plot of GC–MS Non-Targeted Data Showing 177 Significantly Different Features Between Various Crumb Rubber Fractions**

Each peak in the chromatogram is associated with a mass-to-charge ratio, retention time, fold change between different samples, p-value, and relative intensity. A cloud plot, which is a visualization of this multidimensional data to facilitate compound identification and data interpretation, identifying 177 significantly different spectral features is shown. The cloud plot shows all chromatograms overlaid along the x-axis based on the retention time and red circles at each mass-to-charge ratio (y-axis) that met the significance criteria (with size of the circle showing the relative difference between the samples).

A

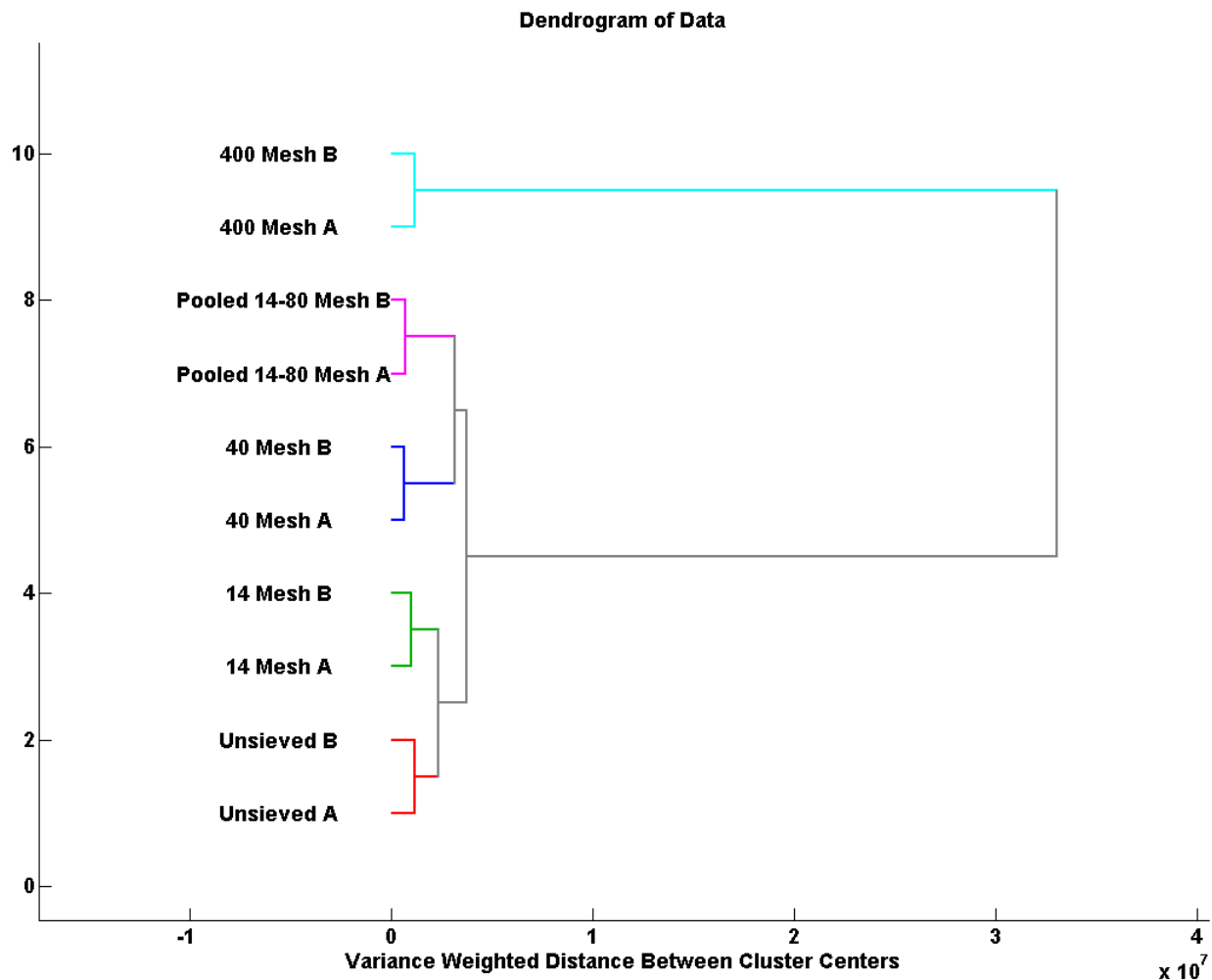


B



**Figure 12. Principal Component Analysis Scores Plots of GC–MS Non-Targeted Data Comparing Various Crumb Rubber Fractions: (A) Using Mass Fragments (177 Features) and (B) Using Individual Peaks**

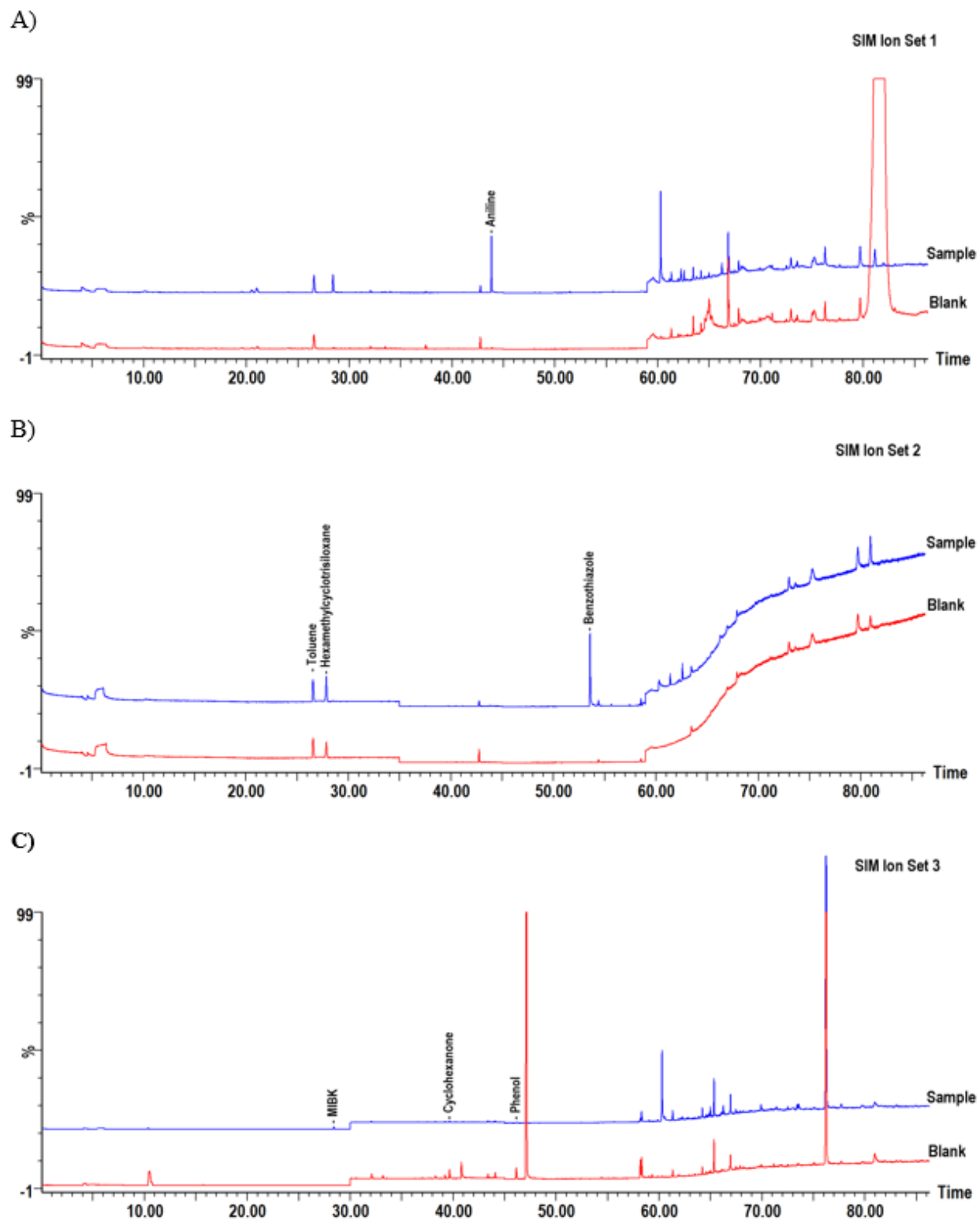
Individual data point reflects a single sample.



**Figure 13. Hierarchical Cluster Analysis of GC-MS Non-Targeted Data Comparing Various Crumb Rubber Fractions Using Individual Peaks**

Data from individual replicates are shown.

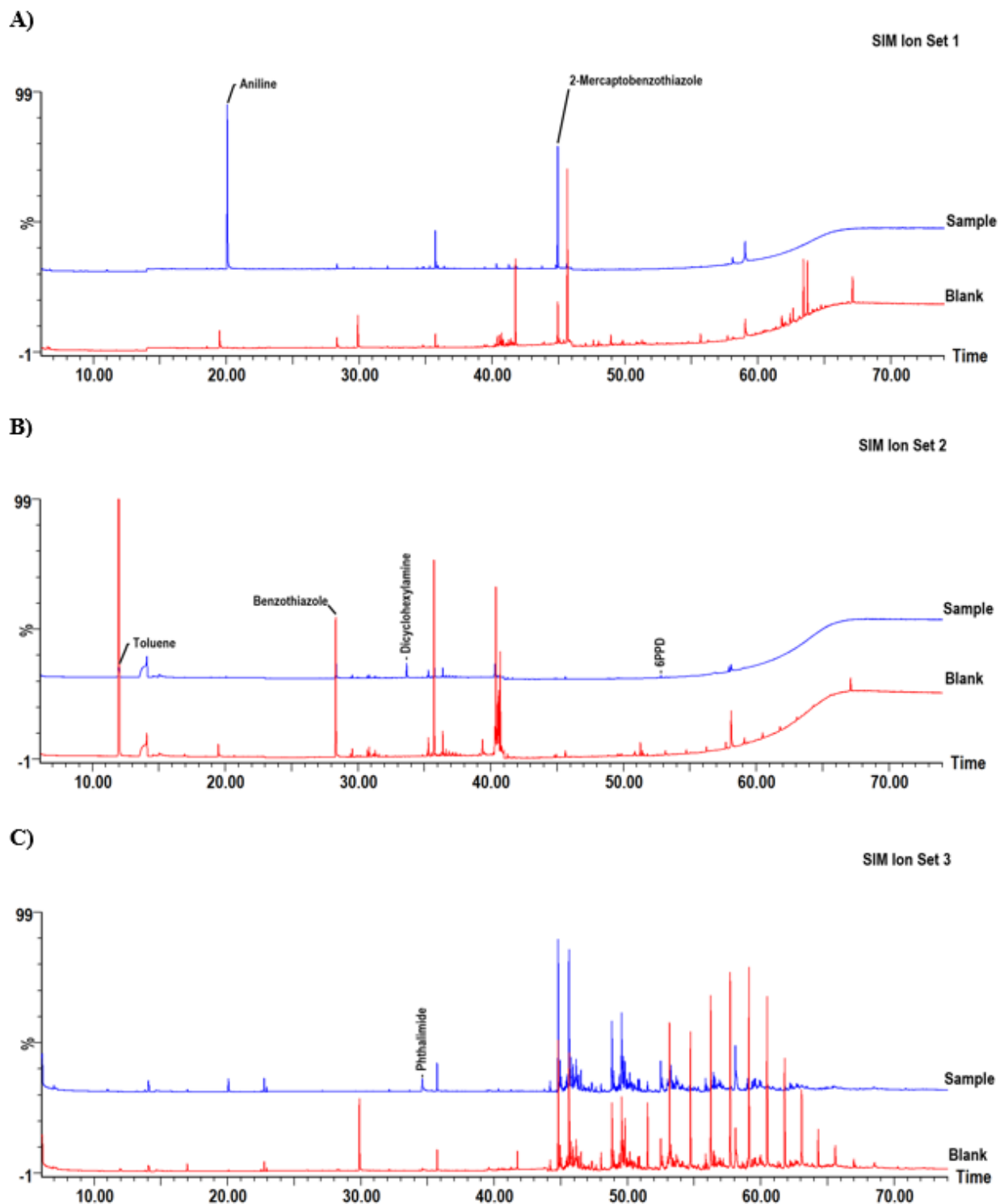
# Chemical and Physical Characterization of Recycled Tire Crumb Rubber



**Figure 14. Headspace GC–MS Chromatograms at 100°C Incubation for Crumb Rubber Extracts Using Simulated Ingestion Fluid Extract and Corresponding Method Blanks**

Due to the large number of analytes, each sample was injected three times with a subset of analytes in each run.

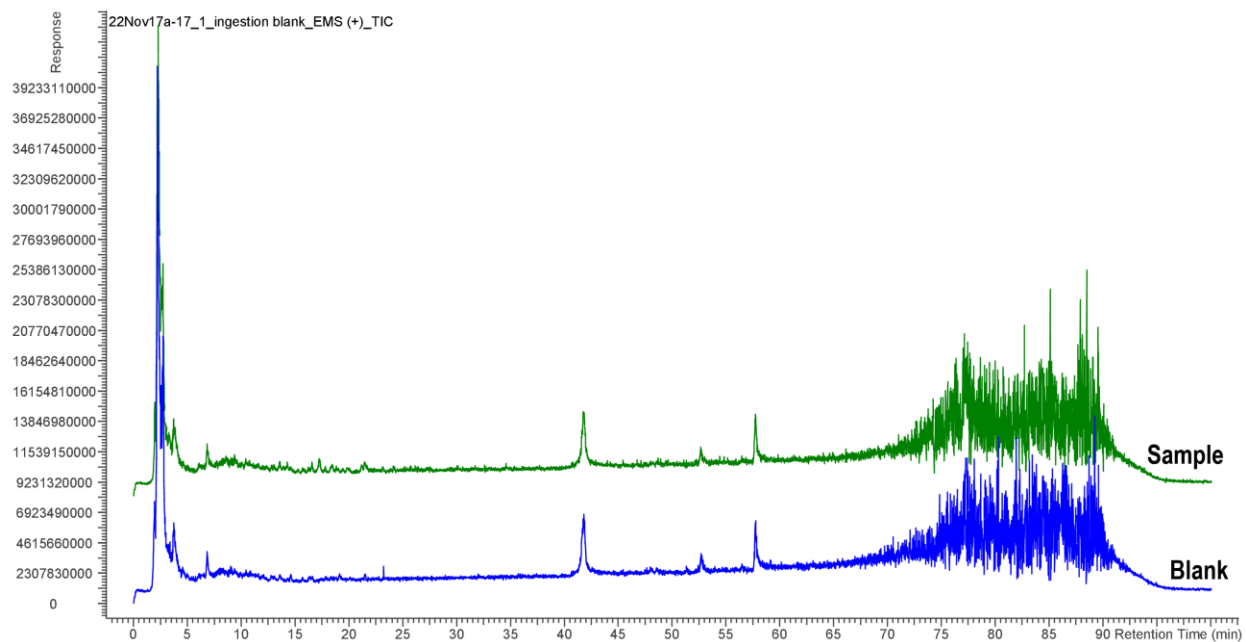
## Chemical and Physical Characterization of Recycled Tire Crumb Rubber



**Figure 15. GC–MS Liquid Injection Chromatograms for Crumb Rubber Extracts Using Simulated Ingestion Fluid Extract and Corresponding Method Blanks**

To keep the mass spectrometer cycle time short enough to generate a sufficient number of data points to improve sensitivity, the samples and blanks were injected three times, each time with a different set of SIM ions monitored (SIM ion Sets 1 to 3) as shown in the figure.

## Chemical and Physical Characterization of Recycled Tire Crumb Rubber



**Figure 16. LC-MS Chromatograms for Crumb Rubber Extracts Using Simulated Ingestion Fluid Extract and Corresponding Method Blank**

## Appendix A. Individual and Mixtures of Standards Procured to Identify Crumb Rubber Constituents

### Tables

Table A-1. Individual Standards Procured.....	A-2
Table A-2. Standards Mixes Procured.....	A-7
Table A-3. EPA VOC Mix 1 Components .....	A-8
Table A-4. EPA VOC Mix 2 Components .....	A-8
Table A-5. EPA VOC Mix 3 Mix Components.....	A-9
Table A-6. EPA VOC Mix 4 Mix Components.....	A-9
Table A-7. EPA VOC Mix 5 Mix Components.....	A-9
Table A-8. EPA VOC Mix 6 Mix Components.....	A-10
Table A-9. Phthalates in Methanol Components .....	A-10
Table A-10. C7–C30 Saturated Alkanes.....	A-11
Table A-11. Multicomponent Alcohol Mix-100.....	A-11
Table A-12. PAH Analyte Mix.....	A-12
Table A-13. EPA 610 PAH Mix.....	A-12

Chemical and Physical Characterization of Recycled Tire Crumb Rubber

**Table A-1. Individual Standards Procured<sup>a</sup>**

Compound	Used For <sup>b</sup>	Lot No. (Supplier, City, State)	CAS No.	Date Received	Amount Received	Vendor Purity (%)	Appearance	Storage Condition
1,2,4-Trimethylbenzene	4	MKBJ6229V (Sigma-Aldrich, Milwaukee, WI)	95-63-6	6/29/17	25 mL	98.8	Colorless Liquid	Ambient
1,4-Dimethylnaphthalene	1,2	SZBE010XV SZBE083XV (Sigma-Aldrich, Milwaukee, WI)	571-58-4	5/6/2016	100 mg 100 mg	95.0 97.0	Liquid	Ambient
1-Chlorooctadecane	2	XA12629 (Sigma-Aldrich, Milwaukee, WI)	3386-33-2	5/6/2016	1 mL	99.6	Solution	2°C to 8°C
1-Methoxy-2-Propanol	1	BCBL3411V (Sigma-Aldrich, Milwaukee, WI)	107-98-2	2/8/2016	5 mL	99.8	Colorless Liquid	Ambient
1-Methylnaphthalene	1,2	XA13461V (Sigma-Aldrich, Milwaukee, WI)	90-12-0	5/6/2016	2 mL	98.4	Liquid	2°C to 8°C
2-(Morpholinothio)- benzothiazole	1,2	B02308158 (Sigma-Aldrich, Milwaukee, WI)	102-77-2	2/10/2016	1 g	Not Provided	Not Provided	Ambient
2,4,4-Trimethyl-1-pentene	1,2,4	MKBV5694V (Sigma-Aldrich, Milwaukee, WI)	107-39-1	4/26/2016	100 g	99	Colorless Liquid	Ambient
2-Ethyl-naphthalene	1,2	STBD0765V( Sigma-Aldrich, Milwaukee, WI)	939-27-5	5/6/2016	5 g	99.9	Colorless Liquid	Ambient
2-Furaldehyde	1,2	SHBG2086V (Sigma-Aldrich, Milwaukee, WI)	98-01-1	5/4/2016	5 mL	99.8	Very Light Yellow Liquid	Ambient
2-Hydroxybenzothiazole	1,2,4	STBD4107 (Sigma-Aldrich, Milwaukee, WI)	934-34-9	2/8/2016	5 g	99.1	Faint Beige Powder	Ambient
2-Mercaptobenzothiazole	4	MKBZ5040V (Sigma-Aldrich, Milwaukee, WI)	149-30-4	6/29/17	10 g	99.9	Light Yellow Powder	Ambient
2-Methylnaphthalene	1,2,4	XA13611V (Sigma-Aldrich, Milwaukee, WI)	91-57-6	5/6/2016	2 mL	98.8	Liquid	2°C to 8°C
3-Buten-2-one	1	MKBP70746V (Sigma-Aldrich, Milwaukee, WI)	78-94-4	5/6/2016	5 mL	98.5	Colorless Liquid	2°C to 8°C



Chemical and Physical Characterization of Recycled Tire Crumb Rubber

Compound	Used For <sup>b</sup>	Lot No. (Supplier, City, State)	CAS No.	Date Received	Amount Received	Vendor Purity (%)	Appearance	Storage Condition
3-Ethyltoluene	1,2,4	STBD6464V (Sigma-Aldrich, Milwaukee, WI)	620-14-4	5/6/2016	10 g	99.7	Colorless Liquid	Ambient
3-Methylfuran	1	B02323174 (Sigma-Aldrich, Milwaukee, WI)	930-78-8	5/4/2016	100 mg	Not Provided	Liquid	Ambient
3-Methylhexane	1,2	MKBW2372V (Sigma-Aldrich, Milwaukee, WI)	589-34-4	4/26/2016	5 g	99.8	Colorless Liquid	Ambient
4-tert-Octylphenol	1,2,4	MKBT8005 (Sigma-Aldrich, Milwaukee, WI)	140-66-9	2/8/2016	500 mg	98.7	Not Provided	Ambient
5-Methyl-2-Hexanone	1,2,4	SHBC3669V (Sigma-Aldrich, Milwaukee, WI)	110-12-3	5/6/2016	1 L	98.75	Colorless Liquid	Ambient
Acetaldehyde	1	LC17886V (Sigma-Aldrich, Milwaukee, WI)	75-07-0	2/8/2016	1 mL	99.9	Colorless Liquid	2°C to 8°C
Aniline	1,2,3,4	SHBG6394V (Sigma-Aldrich, Milwaukee, WI)	62-53-3	4/1/2016	5 mL	100	Faint Yellow Liquid	Ambient
Benzothiazole	1,2,3,4	BCBP5624V (Sigma-Aldrich, Milwaukee, WI)	95-16-9	2/8/16 6/1/16 9/6/16	200 mg 200 mg 300 mg	99.7	Light-Brown-Yellow Liquid	Ambient
Bis-2-ethylhexyl Phthalate	4	BCBL8383V (Sigma-Aldrich, Milwaukee, WI)	117-81-7	6/29/17	100 mg	99.6	Not Provided	Ambient
Bisphenol P	3,4	BCBN1660V (Sigma-Aldrich, St. Louis, MO)	2167-51-3	6/29/17	200 mg	99.9%	White powder	Ambient
Butylated Hydroxyanisole	1,2	BCBN8023V (Sigma-Aldrich, Milwaukee, WI)	25013-16-5	2/8/2016	500 mg	99.9	White Crystals	Ambient
Butylated Hydroxytoluene	1,2,4	LRAA7701 (Sigma-Aldrich, Milwaukee, WI)	128-37-0	4/1/2016	1 g	99.9	White Powder	Ambient
Butyraldehyde	1	SHBD8136V (Sigma-Aldrich, Milwaukee, WI)	123-72-8	4/26/2016	100 mL	99.86	Colorless Liquid	Ambient
Crotonaldehyde	1,2	BCBM3428V (Sigma-Aldrich, Milwaukee, WI)	123-73-9	4/26/2016	5 mL	99.7	Colorless Liquid	2°C to 8°C

Chemical and Physical Characterization of Recycled Tire Crumb Rubber

Compound	Used For <sup>b</sup>	Lot No. (Supplier, City, State)	CAS No.	Date Received	Amount Received	Vendor Purity (%)	Appearance	Storage Condition
Cyclohexanone	1,2,4	BCBL8626V (Sigma-Aldrich, Milwaukee, WI)	108-94-1	4/1/2016	3 mL	99.98	Colorless Liquid	Ambient
Cyclohexene	1	BCBP8188V (Sigma-Aldrich, Milwaukee, WI)	110-83-8	4/26/2016	5 mL	99.7	Colorless Liquid	Ambient
Dicyclohexylamine	2,4	MKBB9424V (Sigma-Aldrich, Milwaukee, WI)	101-83-7	5/6/2016	5 g	99.4	Colorless Liquid	Ambient
Diphenylamine	2,4	MKBV3210V (Sigma-Aldrich, Milwaukee, WI)	122-39-4	4/26/2016	5 g	100	White Crystals	Ambient
Ethylene Glycol Butyl Ether (2-Butoxyethanol)	1	BCBN8459V (Sigma-Aldrich, Milwaukee, WI)	111-76-2	2/8/2016	5 mL	99.6	Colorless Liquid	Ambient
Fluorene	4	BCBS0780V (Sigma-Aldrich, Milwaukee, WI)	86-73-7	6/29/17	100 mg	98.7%	Not Provided	Ambient
Hexadecane	1,2,3	BCBQ7425V (Sigma-Aldrich, Milwaukee, WI)	544-76-3	2/8/2016	5 mL	99.9	Colorless Liquid	Ambient
Hexamethylcyclotrisiloxane	1,2,3	BCBM4918V (Sigma-Aldrich, Milwaukee, WI)	541-05-9	4/1/2016	100 mg	99.8	White Crystalline Chunks	Ambient
Hexanal	1,2,4	BCBQ1155V (Sigma-Aldrich, Milwaukee, WI)	66-25-1	2/8/2016	2 mL	98.1	Colorless Liquid	Ambient
Methyl Ethyl Ketone (MEK)	1,2	P500207 (Sigma-Aldrich, Milwaukee, WI)	78-93-3	2/8/2016	3.6 mL	99.7	Clear Liquid	Ambient
m-Xylene	4	BCBS5438V (Sigma-Aldrich, Milwaukee, WI)	108-38-3	4/25/17	5 mL	100%	Colorless liquid	Ambient
Methyl Isobutyl Ketone (MIK)	1,2,3	LRAA4472 (Sigma-Aldrich, Milwaukee, WI)	108-10-1	2/8/2016	3.6 mL	99.9	Clear Liquid	Ambient
Methylcyclohexane	1,2	BCBQ6849V (Sigma-Aldrich, Milwaukee, WI)	108-87-2	4/26/2016	2 mL	99.6	Colorless Liquid	Ambient
N-(1,3,-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD)	2,3	B02323173 (Sigma-Aldrich, Milwaukee, WI)	793-24-8	5/4/2016	500 mg	Not Provided	Black Powder	Ambient

Chemical and Physical Characterization of Recycled Tire Crumb Rubber

Compound	Used For <sup>b</sup>	Lot No. (Supplier, City, State)	CAS No.	Date Received	Amount Received	Vendor Purity (%)	Appearance	Storage Condition
Naphthalene	4	BCBR2099V (Sigma-Aldrich, Milwaukee, WI)	91-20-3	8/30/2016	1 g	99.9	White Crystals	Ambient
N-Nitrosodimethylamine	1	LC14356V (Sigma-Aldrich, Milwaukee, WI)	62-75-9	2/8/2016	1 mL	99.9	Not Provided	2°C to 8°C
N-Nitrosodiphenylamine	1	LC17695V (Sigma-Aldrich, Milwaukee, WI)	86-30-6	2/8/2016	1,000 mg	98.1	Brownish-Yellow Fine Crystals	Ambient
N-tert-Butyl-2-benzothiazolesulfenamide	1,2	10119609 (Alpha Aesar, Ward Hill, MA)	107-98-2	2/8/2016	50 g	97.4	Pale Cream Powder	Ambient
n-tert-Butylformamide	1,2	1447853V (Sigma-Aldrich, Milwaukee, WI)	2425-74-3	4/1/2016	25 g	99.9	Colorless Clear Liquid	Ambient
Phenol	1,2,4	STBG0464V (Sigma-Aldrich, Milwaukee, WI)	108-95-2	5/4/2016	100 g	>99.9	White Crystals	Ambient
Phthalamide	2	MKBB9424V (Sigma-Aldrich, Milwaukee, WI)	85-41-6	5/6/2016	1 g	99.9	Beige Flakes	Ambient
Phthalimide	4	MKBW6020V (Sigma-Aldrich, Milwaukee, WI)	85-41-6	8/22/2016	50 g	99.8	White Powder	Ambient
Propylene Glycol (1,2-Propanediol)	1	BCBQ0887V (Sigma-Aldrich, Milwaukee, WI)	57-55-6	2/8/2016	5 mL	99.99	Colorless Viscous Liquid	Ambient
Pyrene	4	BCBR5529V (Sigma-Aldrich, Milwaukee, WI)	129-00-0	6/29/17	100 mg	99.2	Not Provided	Ambient
Styrene	4	LC26706V (Sigma-Aldrich, Milwaukee, WI)	100-42-5	9/1/17	1 g	99.9	Colorless Liquid	Ambient
tert-Butylamine	1	SHBG2764V (Sigma-Aldrich, Milwaukee, WI)	75-64-9	4/1/2016	100 mL	99.99	Colorless Liquid	Ambient
Tetraethylthiuram Disulfide	2	BCBL7406V (Sigma-Aldrich, Milwaukee, WI)	97-77-8	2/8/2016	50 g	99.4	Faint Beige Powder	Ambient
Tetramethylthiuram Disulfide	2	STBF1299V (Sigma-Aldrich, Milwaukee, WI)	137-26-8	2/8/2016	5 g	>99.9	White Powder	Ambient

Chemical and Physical Characterization of Recycled Tire Crumb Rubber

Compound	Used For <sup>b</sup>	Lot No. (Supplier, City, State)	CAS No.	Date Received	Amount Received	Vendor Purity (%)	Appearance	Storage Condition
Tetramethylthiuram Monosulfide	2	SHBC3940V (Sigma-Aldrich, Milwaukee, WI)	97-74-5	2/8/2016	100 g	97	Yellow Powder	Ambient
Toluene	3,4	BCBR9283V (Sigma-Aldrich, Milwaukee, WI)	108-88-3	4/25/17	5 mL	99.91%	Colorless Liquid	Ambient
Trans-1,2-Dimethylcyclohexane	1,2	12222ASV (Sigma-Aldrich, Milwaukee, WI)	6876-23-9	4/26/2016	5 g	99.8	Colorless Liquid	Ambient
Trans-Cinnamaldehyde (3-phenyl-2propanal)	1,2	BCBP7078V (Sigma-Aldrich, Milwaukee, WI)	14371-10-9	2/8/2016	100 mg	96.1	Green-Yellow Liquid	Ambient
Tetracosane	4	MKBZ5406V (Sigma-Aldrich, St. Louis, MO)	646-31-1	6/29/17	25 g	99.8%	White Crystals	Ambient
Tetratriacontane	4	BCBV2796 (Sigma-Aldrich, St. Louis, MO)	14167-59-0	6/29/17	1 g	99.4%	White Flakes	Ambient
Tricosane	4	MKBX1642V (Sigma-Aldrich, Milwaukee, WI)	638-68-5	8/30/2016	1 g	99.6	White Crystals	Ambient

<sup>a</sup>Information from the vendors certificate of analysis.

<sup>b</sup>1 = Headspace GC/MS Characterization, 2 = Liquid Injection GC/MS Characterization, 3 = LC/MS Characterization, 4 = Bioaccessibility and Size Fractionation Analyses.

Chemical and Physical Characterization of Recycled Tire Crumb Rubber

**Table A-2. Standards Mixes Procured<sup>a, b</sup>**

Mixture	Used For <sup>c</sup>	Lot No. (Supplier, City, State)	Date Received	Amount Received (mL)	Vendor Purity (%)	Appearance	Storage Condition
EPA VOC Mix 1	1,2	LC09299V (Sigma-Aldrich, Milwaukee, WI)	2/8/16	3	N/A	Not Provided	2°C to 8°C
EPA VOC Mix 2	1,2	LC09300V (Sigma-Aldrich, Milwaukee, WI)	2/8/16	3	N/A	Not Provided	2°C to 8°C
EPA VOC Mix 3	1,2	XA18188V (Sigma-Aldrich, Milwaukee, WI)	2/8/16	3	N/A	Not Provided	2°C to 8°C
EPA VOC Mix 4	1	XA16770V (Sigma-Aldrich, Milwaukee, WI)	2/10/16	3	N/A	Not Provided	2°C to 8°C
EPA VOC Mix 5	1	LC09303V (Sigma-Aldrich, Milwaukee, WI)	2/8/16	3	N/A	Not Provided	2°C to 8°C
EPA VOC Mix 6	1	LC16135V (Sigma-Aldrich, Milwaukee, WI)	2/8/16	3	N/A	Not Provided	2°C to 8°C
Phthalates in Methanol	2	SRM 3074 (Sigma-Aldrich, Milwaukee, WI)	2/15/16	6	N/A	Not Provided	Ambient
C7–C30 Saturated Alkanes	1,2	XA17133V (Sigma-Aldrich, Milwaukee, WI)	4/26/16	2	N/A	Not Provided	2°C to 8°C
Multicomponent Alcohol Mix-100	1,2	FN03251502 (Sigma-Aldrich, Milwaukee, WI)	4/25/16	2.4	N/A	Not Provided	2°C to 8°C
PAH Analyte Mix	2	EN151214002 (SPEX Certiprep, Metuchen, NJ)	7/8/16	2	N/A	Liquid	Ambient
EPA 610 PAH Mix	4	XA21638V (Sigma-Aldrich, Milwaukee, WI)	8/15/16	6	N/A	Liquid	2°C to 8°C

<sup>a</sup>See Tables A-3 through A-13 for composition of each mixture.

<sup>b</sup>Information from the vendors certificate of analysis.

<sup>c</sup>1 = Headspace GC/MS Characterization, 2 = Liquid Injection GC/MS Characterization, 3 = LC/MS Characterization, 4 = Bioaccessibility and Size Fractionation Analyses.

## Chemical and Physical Characterization of Recycled Tire Crumb Rubber

**Table A-3. EPA VOC Mix 1 Components**

<b>Compound</b>	<b>Lot No.</b>	<b>CAS No.</b>	<b>Analytical Conc. (µg/mL)</b>
<i>sec</i> -Butylbenzene	LB77150	135-98-8	1962.7
<i>tert</i> -Butylbenzene	LB40373	98-06-6	1956.2
Chlorobenzene	LB93949	109-90-7	1977.3
2-Chlorotoluene	LB01464	95-49-8	1947.3
4-Chlorotoluene	LB02742	106-43-4	2008.6
Cumene	LB89580	98-82-8	1958.6
1,2-Dichlorobenzene	LB58923	95-50-1	1953.9
1,3-Dichlorobenzene	LB68066	541-73-1	1950.8
1,4-Dichlorobenzene	LB90630	106-46-7	1957.0
<i>n</i> -Propylbenzene	LB69017	103-65-1	2070.6
<i>o</i> -Xylene	LB92424	95-47-6	1933.4
<i>p</i> -Xylene	LB86113	106-42-3	1984.3

**Table A-4. EPA VOC Mix 2 Components**

<b>Compound</b>	<b>Lot No.</b>	<b>CAS No.</b>	<b>Analytical Conc. (µg/mL)</b>
Benzene	LC03683	71-43-2	1878.0
Bromobenzene	LB99843	108-86-1	1935.2
<i>n</i> -Butylbenzene	LB81630	104-51-8	1947.3
Ethylbenzene	LB69556	100-41-4	1960.5
<i>p</i> -Isopropyltoluene	LB40003	99-87-6	1898.7
Naphthalene	LB89929	91-20-3	1951.3
Styrene	LB86229	100-42-5	1946.9
Toluene	LB94376	108-88-3	1922.4
1,2,3-Trichlorobenzene	LA50762	87-61-6	1949.6
1,2,4-Trichlorobenzene	LB48083	120-82-1	1960.2
1,2,4-Trimethylbenzene	LB97224	95-63-6	1911.1
1,3,5-Trimethylbenzene	LB82157	108-67-8	1938.3
<i>m</i> -Xylene	LB87531	108-38-3	1941.2

Chemical and Physical Characterization of Recycled Tire Crumb Rubber

**Table A-5. EPA VOC Mix 3 Mix Components**

Compound	Lot No.	CAS No.	Analytical Conc. (µg/mL)
1,2-Dibromo-3-Chloropropane	LB06608	96-12-8	2008.7
1,2-Dibromoethane	LB42792	106-93-4	2000.0
1,2-Dichloroethane	LB74294	107-06-2	2005.7
1,2-Dichloropropane	LC14320	78-87-5	2030.8
1,3-Dichloropropane	LC10811	142-28-9	2025.3
1,1-Dichloropropene	LC11148	563-58-6	1837.7
<i>cis</i> -1,3-Dichloroprepene	LB96292	10061-01-5	2044.2
<i>trans</i> -1,3-Dichloropropene	LC11733	10061-02-6	2004.4
Hexachlorobutadiene	LB75566	87-68-3	2006.7
1,1,1,2-Tetrachloroethane	LC14810	630-20-6	2017.2
1,1,2,2-Tetrachloroethane	LB51327	79-34-5	2052.4
1,1,2-Trichloroethane	LB87978	79-00-5	1993.0
Trichloroethene	LB56674	79-01-6	1977.5
1,2,3-Trichloropropane	LB82841	96-18-4	2016.0

**Table A-6. EPA VOC Mix 4 Mix Components**

Compound	Lot No.	CAS No.	Analytical Conc. (µg/mL)
Bromochloromethane	LB39040	74-97-5	1953.3
Bromoform	LB99100	75-25-2	1990.6
Carbon Tetrachloride	LA55583	56-23-5	1949.4
Chloroform	LB97804	67-66-3	2000.0
Dibromomethane	LB81118	74-95-3	2055.2
1,1-Dichloroethane	LB68167	75-34-3	1842.0
2,2-Dichloropropane	LB90123	594-20-7	1932.9
Tetrachloroethene	LB67182	127-18-4	1977.4
1,1,1-Trichloroethane	LC06533	71-55-6	1976.8

**Table A-7. EPA VOC Mix 5 Mix Components**

Compound	Lot No.	CAS No.	Analytical Conc. (µg/mL)
Bromodichloromethane	LB78267	75-27-4	2000.0
Dibromochloromethane	LB89245	124-48-1	2016.6
1,1-Dichloroethylene	LC08188	75-34-4	1822.1
<i>cis</i> -1,2-Dichloroethylene	LB73428	156-59-2	1967.2
<i>trans</i> -1,2-Dichloroethylene	LB82639	156-60-5	1914.2
Methylene Chloride	LB87418	75-09-2	1968.9

Chemical and Physical Characterization of Recycled Tire Crumb Rubber

**Table A-8. EPA VOC Mix 6 Mix Components**

Compound	Lot No.	CAS No.	Analytical Conc. (µg/mL)
Bromomethane	LB22203	74-83-9	2030
Chloroethane	LB55667	75-00-3	2029
Chloromethane	LB64986	74-87-3	2063
Dichlorodifluoromethane	LB68837	75-71-8	1867
Trichlorofluoromethane	LA91320	75-69-4	2086
Vinyl Chloride	LB66643	75-01-4	2031

**Table A-9. Phthalates in Methanol Components**

Compound	Lot No.	CAS No.	Analytical Conc. (mg/L)
Dimethylphthalate	–	131-11-3	44
Diethylphthalate	–	84-66-2	40.7
Di-n-butylphthalate	NA <sup>a</sup>	84-74-2	40.5
Benzylbutylphthalate	–	85-68-7	41.3
Bis(2-ethylhexyl)phthalate	–	117-81-7	46.4
Di-n-octylphthalate	–	117-84-0	38.2

<sup>a</sup>Separate lot numbers for constituents not provided.



**Table A-10. C7–C30 Saturated Alkanes**

Compound	Lot No.	CAS No.	Analytical Conc. (µg/mL)
n-Decane	LC07947	124-18-5	1025.3
n-Docosane	LC11073	629-97-0	1018.5
n-Dodecane	LC08158	112-40-3	1025.1
n-Eicosane	LB97320	112-95-8	1016.7
n-Heneicosane	LB85393	629-94-7	1017.1
n-Heptacosane	LC11618	593-49-7	1015.7
n-Heptadecane	LC12302	629-78-7	1023.6
n-Heptane	LC07614	142-82-5	1028.0
n-Hexacosane	LC11398	630-01-3	1016.8
n-Hexadecane	LC02125	544-76-3	1021.0
n-Nonacosane	LC08881	630-03-5	1012.7
n-Nonadecane	LB30980	629-92-5	1018.1
n-Nonane	LC00219	111-84-2	1026.5
n-Octacosane	LB95341	630-02-4	1018.8
n-Octadecane	LB89629	593-45-3	1019.6
n-Octane	LB63797	111-65-9	1028.1
n-Pentacosane	LC15080	629-99-2	1016.8
n-Pentadecane	LC08640	629-62-9	1022.8
n-Tetracosane	LC13242	646-31-1	1018.9
n-Tetradecane	LC15053	629-59-4	1019.8
n-Triacontane	LC07823	638-68-6	1019.1
n-Tricosane	LB36889	638-67-5	1018.3
n-Tridecane	LC14314	629-50-5	1022.2
n-Undecane	LC08959	1120-21-4	1025.2

**Table A-11. Multicomponent Alcohol Mix-100**

Compound	Lot No.	CAS No.	Analytical Conc.(µg/mL)
Acetone	–	67-64-1	101.4
Ethanol	FN03251502	64-17-5	103.4
Isopropanol	–	67-63-0	102.2
Methanol	–	67-56-1	102.3

Chemical and Physical Characterization of Recycled Tire Crumb Rubber

**Table A-12. PAH Analyte Mix**

Compound	Lot No.	CAS No.	Analytical Conc. (µg/mL)
Acenaphthene	–	83-32-9	2001
Acenaphthylene	–	208-96-8	2001
Anthracene	–	120-12-7	2001
Benz(a)anthracene	–	56-55-3	1995
Benzo(a)pyrene	–	50-32-8	2002
Benzo(b)fluoranthene	–	205-99-2	2004
Benzo(g,h,i)perylene	–	191-24-2	1996
Benzo(k)fluoranthene	NA <sup>a</sup>	207-08-9	1999
Chrysene	–	218-01-9	2002
Dibenz(a,h)anthracene	–	53-70-3	1999
Fluoranthene	–	206-44-0	2003
Fluorene	–	86-73-7	2004
Indeno(1,2,3-c,d)pyrene	–	193-39-5	2000
Naphthalene	–	91-20-3	1995
Phenanthrene	–	85-01-8	2000
Pyrene	–	129-00-0	2007

<sup>a</sup>Separate lot numbers for constituents not provided.

**Table A-13. EPA 610 PAH Mix**

Compound	Lot No.	CAS No.	Analytical Conc. (µg/mL)
Acenaphthene, CRM	LC15478	83-32-9	1059.6
Acenaphthylene, CRM	LC20622	208-96-8	
Anthracene, CRM	LC20462	120-12-7	107.4
Benz(a)anthracene, CRM	LB94996	56-55-3	106.5
Benzo(a)pyrene, CRM	LC19497	50-32-8	111.5
Benzo(b)fluoranthene, CRM	LB89946	205-99-2	217.4
Benzo(ghi)perylene, CRM	LB96971	191-24-2	201.9
Benzo(k)fluoranthene, CRM	LB89276	207-08-9	107.8
Chrysene, CRM	LC20748	218-01-9	106.4
Dibenz(a,h)anthracene, CRM	LC20472	53-70-3	213.4
Fluoranthene, CRM	LC15477	206-44-0	215.9
Fluorene, CRM	LC15479	86-73-7	216.1
Indeno(1,2,3-C,D)pyrene, CRM	LC20473	193-39-5	107.0
Naphthalene, CRM	LC11683	91-20-3	1052.7
Phenanthrene, CRM	LC18318	85-01-8	106.7
Pyrene, CRM	LC15727	129-00-0	99.3

## Appendix B. Potential Crumb Rubber Constituents Identified in the Literature and Initial Screening Analyses

**Table B-1. Potential Constituents in Crumb Rubber Based on Literature<sup>a</sup> and Initial Screening**

Name	CASRN	Molecular Wt
Acenaphthene	83-32-9	154.21
Acenaphthylene	208-96-8	152.20
Acetaldehyde	75-07-0	44.05
Acetic Acid	64-19-7	60.05
Acetone	67-64-1	58.08
Acetonitrile	75-05-8	41.05
Acetophenone	98-86-2	120.15
Allyl Chloride	107-05-1	76.52
Alpha Pinene	80-56-8	136.24
<i>trans</i> -Anethole	50770-19-9	148.21
Aniline	62-53-3	93.13
Anthanthrene	191-26-4	276.34
Anthracene	120-12-7	178.23
Anthracene, 2-methyl-	613-12-7	192.26
Anthracene, 9-methyl-	779-02-2	192.26
Azobenzene	103-33-3	182.23
Benz(a)anthracene	56-55-3	228.29
Benzamide, <i>N,N</i> -diethyl-3-methyl- (DEET)	134-62-3	191.27
Benzenamine, 4-octyl- <i>N</i> -(4-octylphenyl)-	101-67-7	393.66
Benzene	71-43-2	78.14
1,4-Benzenediamine, <i>N</i> -(1,3-dimethylbutyl)- <i>N'</i> -phenyl- (6PPD)	793-24-8	268.40
1,4-Benzenediamine, <i>N</i> -(1-methylethyl)- <i>N'</i> -phenyl- (IPPD)	101-72-4	226.32
Benzene, 1-ethyl-2-methyl-(2-ET)	611-14-3	120.20
Benzenemethanol, ar-ethenyl-	53957-34-9	136.19
Benzenesulfonanilide	1678-25-7	233.29

Chemical and Physical Characterization of Recycled Tire Crumb Rubber

Name	CASRN	Molecular Wt
Benzidine	92-87-5	184.24
Benzo(a)fluoranthene	203-33-8	252.32
Benzo(a)pyrene	50-32-8	252.32
Benzo(b)fluoranthene	205-99-2	252.31
Benzo(b)fluorene	243-17-4	216.28
Benzo(e)pyrene	192-97-2	252.32
Benzo(g,h,i)perylene	191-24-2	276.33
Benzo(ghi)fluoranthene	203-12-3	226.28
Benzo[k]fluoranthene	207-08-9	252.32
Benzoic Acid	65-85-0	122.12
Benzothiazole	95-16-9	135.18
Benzothiazole, 2-(methylthio)-	615-22-5	181.27
Benzothiazole, 2-(morpholiniothio)-	102-77-2	252.35
<i>1H</i> -Benzotriazol-5-amine, 1-methyl-	27799-83-3	148.17
Benzyl Alcohol	100-51-6	108.14
Biphenyl	92-52-4	154.21
Bis(2-chloroethoxy)methane	111-91-1	173.04
Bis(2-chloroethyl)ether	111-44-4	143.01
<i>N,N'</i> -Bis(2'-dimethylphosphinothioylethyl)- <i>N,N'</i> -Diethylpropylenediamine	Unknown	Unknown
Bis(2-ethylhexyl) Phthalate	117-81-7	390.56
Bisphenol P	2167-51-3	346.47
Bromodichloromethane	75-27-4	163.82
Bromoform	75-25-2	252.73
Bromomethane	74-83-9	94.94
4-Bromophenyl Phenyl Ether	101-55-3	249.11
1,3-Butadiene, 2-methyl-	78-79-5	68.12
Butane	106-97-8	58.12
Butane, 2-iodo-2-methyl-	594-38-7	198.05
Butane, 2-methyl-	78-78-4	72.15

Chemical and Physical Characterization of Recycled Tire Crumb Rubber

Name	CASRN	Molecular Wt
1-Butanol, 4-methoxy-	111-32-0	104.15
2-Butanone	78-93-3	72.11
2-Butene, (E)-	624-64-6	56.11
2-Butoxyethanol	111-76-2	118.18
2-(2-Butoxyethoxy)ethanol	112-34-5	162.23
2-(2-Butoxyethoxy)ethyl acetate	124-17-4	204.27
Butylated Hydroxyanisole	25013-16-5	180.25
Butylated Hydroxytoluene	128-37-0	220.36
<i>sec</i> -Butylbenzene	135-98-8	134.22
Butyl Butanoate	109-21-7	144.21
<i>n-tert</i> -Butylbenzothiazolesulfenamide	95-31-8	238.37
Butyl Benzyl Phthalate	85-68-7	312.36
<i>p-tert</i> -Butyl Phenol	98-54-4	150.22
2-Butyltetrahydrothiopenene	Unknown	Unknown
Butyraldehyde	123-72-8	72.11
Carbazole	86-74-8	167.21
Carbon Disulfide	75-15-0	76.13
Carbon Tetrachloride	56-23-5	153.81
3-Carene	13466-78-9	136.24
4-Chloroaniline	106-47-8	127.57
Chlorobenzene	108-90-7	112.56
Chloroethane	75-00-3	64.51
1-Chloro-2-ethenyl-1-methylcyclopropane	62337-93-3	116.59
2-Chloroethyl Vinyl Ether	110-75-8	106.55
Chloroform	67-66-3	119.37
Chloromethane	74-87-3	50.49
4-Chloro-3-methylphenol	59-50-7	142.58
2-Chloronaphthalene	91-58-7	162.62
2-Chlorophenol	95-57-8	128.56

Chemical and Physical Characterization of Recycled Tire Crumb Rubber

Name	CASRN	Molecular Wt
4-Chlorophenyl Phenyl Ether	7005-72-3	204.65
4-Chlorotoluene	106-43-4	126.58
Chrysene	218-01-9	228.29
Cinnamaldehyde	14371-10-9	132.16
Cycloheptane	291-64-5	98.19
Cyclohexane	110-82-7	84.16
Cyclohexane, 1,3-dimethyl-, <i>cis</i> -	638-04-0	112.22
Cyclohexane, 1,4-dimethyl-	589-90-2	112.22
Cyclohexane, ethyl-	1678-91-7	112.22
Cyclohexanethiol	1569-69-3	116.22
Cyclohexane, 1,1,3-trimethyl-	3073-66-3	126.24
Cyclohexanol	108-93-0	100.16
Cyclohexanone	108-94-1	98.15
Cyclohexyl isothiocyanate	1122-82-3	141.23
Cyclopentane	287-92-3	70.14
Cyclopentanone, 2-methyl-	1120-72-5	98.15
Cyclopropyl Methyl Ketone	765-43-5	84.12
<i>m</i> -Cymene	535-77-3	134.22
<i>o</i> -Cymene	527-84-4	134.22
<i>p</i> -Cymene	99-87-6	134.22
Decamethyl Cyclopentasiloxane	541-02-6	370.77
Decanal	112-31-2	156.27
Decane	124-18-5	142.29
Decane, 2,3,8-trimethyl-	62238-14-6	184.37
Decane, 2,5,6-trimethyl-	62108-23-0	184.36
Decane, 5-methyl-	13151-35-4	156.31
Decane, 6-ethyl-2-methyl-	62108-21-8	184.37
Dehydroabiatic Acid	1740-19-8	300.44
2,4'-Diaminotriphenylmethane	Unknown	274.36

Chemical and Physical Characterization of Recycled Tire Crumb Rubber

Name	CASRN	Molecular Wt
Dibenz(a,h)anthracene	53-70-3	278.35
Dibenzofuran	132-64-9	168.19
2-Dibenzofuranamine	3693-22-9	183.21
3-Dibenzofuranamine	4106-66-5	183.21
4-Dibenzofuranamine	50548-43-1	183.21
Dibenzothiophene	132-65-0	184.26
Dibromochloromethane	124-48-1	208.28
Di-n-butyl Phthalate	84-74-2	278.34
Di-n-octyl Phthalate	117-84-0	390.56
2,6-Di( <i>t</i> -butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one (BHT-OH)	10396-80-2	236.36
3,5-Di- <i>tert</i> -butyl-4-hydroxybenzaldehyde	1620-98-0	234.34
1,2-Dichlorobenzene	95-50-1	147.00
1,3-Dichlorobenzene	541-73-1	147.00
1,4-Dichlorobenzene	106-46-7	147.00
3,3'-Dichlorobenzidine	91-94-1	253.13
Dichlorodifluoromethane	75-71-8	120.91
1,1-Dichloroethane	75-34-3	98.95
1,2-Dichloroethane	107-06-2	98.95
1,1-Dichloroethene	75-35-4	96.94
2,4-Dichlorophenol	120-83-2	163.00
1,2-Dichloropropane	78-87-5	112.98
<i>trans</i> -1,3-Dichloropropene	542-75-6	110.97
Dicyclohexylamine	101-83-7	181.32
Diethyl Phthalate	84-66-2	222.24
2,3-Dihydro-1,1,3-trimethyl-3-phenyl- <i>IH</i> -indene	3910-35-8	236.36
1,2-Dihydro-2,2,4-trimethylquinoline	147-47-7	173.26
Dimethyl Adipate	627-93-0	174.20
9,10-Dimethylantracene	781-43-1	206.28
2,6-Dimethylnaphthalene	581-42-0	156.23

Chemical and Physical Characterization of Recycled Tire Crumb Rubber

Name	CASRN	Molecular Wt
Dimethyl Pentanedioate	1119-40-0	160.17
2,7-Dimethylphenanthrene	1576-69-8	206.29
2,4-Dimethylphenol	105-67-9	122.16
Dimethyl Phthalate	131-11-3	194.18
Dimethyl Succinate	106-65-0	146.14
4,6-Dinitro-2-methylphenol	534-52-1	198.13
2,4-Dinitrophenol	51-28-5	184.11
2,4-Dinitrotoluene	121-14-2	182.13
2,6-Dinitrotoluene	606-20-2	182.13
1,3-Divinylbenzene	108-57-6	130.19
Docosane	629-97-0	310.61
Dodecanal	112-54-9	184.32
Dodecane	112-40-3	170.34
Dodecane, 2,6,11-trimethyl-	31295-56-4	212.42
Dodecane, 2,7,10-trimethyl-	74645-98-0	212.42
2-Dodecen-1-yl Succinic Anhydride	19780-11-1	266.38
Dotriacontane	544-85-4	450.88
Ethanol	64-17-5	46.07
Ethyl Acetate	141-78-6	88.11
Ethylbenzaldehyde	4748-78-1	134.18
Ethylbenzene	100-41-4	106.17
Ethyl Benzoate	93-89-0	150.18
2-Ethyl Hexanoic Acid	149-57-5	144.21
2-Ethyl-1-hexanol	104-76-7	130.23
2-Ethyltetrahydrothiopen	Unknown	Unknown
4-Ethyltoluene	622-96-8	120.19
Fluoranthene	206-44-0	202.26
Fluorene	86-73-7	166.22
Formaldehyde	50-00-0	30.03



Chemical and Physical Characterization of Recycled Tire Crumb Rubber

Name	CASRN	Molecular Wt
Freon 11	75-69-4	137.36
Freon 113	76-13-1	187.37
2(3 <i>H</i> )-Furanone, dihydro-4-hydroxy-	5469-16-9	102.09
Heneicosane	629-94-7	296.58
Hentriacontane	630-04-6	436.85
Heptacosane	593-49-7	380.74
Heptadecane	629-78-7	240.47
Heptane	142-82-5	100.21
Heptane, 2,2-dimethyl-	1071-26-7	128.26
Heptane, 2,3,4-trimethyl-	52896-95-4	142.29
Heptane, 2,4-dimethyl-	2213-23-2	128.26
Heptane, 2,5-dimethyl-	2216-30-0	128.26
Heptane, 2,6-dimethyl-	1072-05-5	128.26
Heptane, 2-methyl-	592-27-8	114.23
Heptane, 3-methyl-	589-81-1	114.23
Heptane, 4-(1-methylethyl)-	52896-87-4	142.29
1-Heptene	592-76-7	98.19
2-Hexanone (MBK)	591-78-6	100.16
Hexachlorobenzene	118-74-1	284.78
Hexachlorobutadiene	87-68-3	260.76
Hexachlorocyclopentadiene	77-47-4	272.77
Hexachloroethane	67-72-1	236.74
Hexacosane	630-01-3	366.71
Hexadecane	544-76-3	226.45
Hexadecanoic Acid, Butyl Ester	111-06-8	312.53
Hexadecanoic Acid, Methyl Ester	112-39-0	270.46
Hexanal	66-25-1	100.16
Hexane	110-54-3	86.18
Hexane, 2,2,4-trimethyl-	16747-26-5	128.26

Chemical and Physical Characterization of Recycled Tire Crumb Rubber

Name	CASRN	Molecular Wt
Hexane, 2,2,5-trimethyl-	3522-94-9	128.26
Hexane, 3,3-dimethyl-	563-16-6	114.23
Hexane, 3-methyl-	589-34-4	100.21
Hexatriacontane	630-06-8	506.98
1-Hexene, 4,5-dimethyl-	16106-59-5	112.22
1-Hexene, 3,5,5-trimethyl-	4316-65-8	126.24
2-Hexen-1-ol, (Z)-	928-94-9	100.16
5-Hexen-2-ol, (+/-)-	54774-27-5	100.16
3-[(E)-2-(4-Hexoxyphenyl)ethenyl]phenol	Unknown	294.00
2-Hydroxybenzothiazole	934-34-9	151.19
Hydroxylamine, O-decyl-	29812-79-1	173.30
Icosane	112-95-8	282.55
3H-Indazol-3-one, 1,2-dihydro-2-methyl-	1848-40-4	148.17
Indeno(1,2,3-cd)pyrene	193-39-5	276.33
1-Iodo-2-methylundecane	73105-67-6	296.24
Isobutene	115-11-7	56.11
Isobutyl Alcohol	78-83-1	74.12
Isophorone	78-59-1	138.21
Isopropylbenzene	98-82-8	120.20
4-Isopropylphenol	99-89-8	136.19
Isopropyltoluene (Cymenes)	25155-15-1	134.22
Limonene	5989-27-5	136.24
Menthol	1490-04-6	156.27
2-Mercaptobenzothiazole	149-30-4	167.24
Methanimidamide, N,N-dimethyl-N'-phenyl-	1783-25-1	148.21
1-Methoxy-2-propanol	107-98-2	90.12
Methyl $\alpha$ -D-xylofuranoside	1824-96-0	164.16
6-Methylbenzo[b]thiophene	16587-47-6	148.22
2-Methylbenzothiazole	120-75-2	149.21

Chemical and Physical Characterization of Recycled Tire Crumb Rubber

Name	CASRN	Molecular Wt
Methylcyclohexane	108-87-2	98.19
Methyl Elaidate	1937-62-8	296.50
Methylene Chloride	75-09-2	84.93
4-Methyl-4-heptanol	598-01-6	130.23
2-Methylhexane	591-76-4	100.21
2.α.-Methyl-17.β.-hopane	Unknown	Unknown
1-Methylnaphthalene	90-12-0	142.20
2-Methylnaphthalene	91-57-6	142.20
4-Methyl-2-Pentanone (MIBK)	108-10-1	100.16
2-Methylphenanthrene	2531-84-2	192.26
3-Methylphenanthrene	832-71-3	192.26
2-Methylphenol (o-Cresol)	95-48-7	108.14
4-Methylphenol (p-Cresol)	106-44-5	108.14
1-Methyl-2-pyrrolidinone	872-50-4	99.13
Methyl Salicylate	119-36-8	152.15
Methyl Stearate	112-61-8	298.50
Naphthalene	91-20-3	128.17
Neodecanoic Acid	26896-20-8	172.27
2-Nitroaniline	88-74-4	138.12
3-Nitroaniline	99-09-2	138.12
4-Nitroaniline	100-01-6	138.12
Nitrobenzene	98-95-3	123.11
Nitromethane	75-52-5	61.04
2-Nitrophenol	88-75-5	139.11
4-Nitrophenol	100-02-7	139.11
<i>N</i> -Nitrosodimethylamine	62-75-9	74.08
<i>N</i> -Nitroso-di- <i>n</i> -propylamine	621-64-7	130.19
<i>N</i> -Nitrosodiphenylamine	86-30-6	198.22
Nonacosane	630-03-5	408.80

Chemical and Physical Characterization of Recycled Tire Crumb Rubber

Name	CASRN	Molecular Wt
Nonadecane	629-92-5	268.53
Nonanal	124-19-6	142.24
Nonanamide	1120-07-6	157.26
Nonane	111-84-2	128.26
Nonane, 3-methyl-5-propyl-	31081-18-2	184.37
30-Norhopane	36728-72-0	398.72
Octacosane	630-02-4	394.77
Octadecane	593-45-3	254.50
Octadecanoic Acid/Stearic Acid	57-11-4	284.48
Octane	111-65-9	114.23
Octane, 2-methyl-	3221-61-2	128.26
Octane, 3-methyl-	2216-33-3	128.26
2-Octen-1-ol	18409-17-1	128.22
4- <i>tert</i> -Octylphenol	140-66-9	206.33
Oleic Acid	112-80-1	282.46
2,2'-Oxybis(1-chloropropane)	108-60-1	171.06
Pentachlorophenol (PCP)	87-86-5	266.32
Pentacosane	629-99-2	352.69
Pentadecane	629-62-9	212.42
1,3-Pentadiene	504-60-9	68.12
1,4-Pentadiene	591-93-5	68.12
2,2,4,6,6-Pentamethylheptane	13475-82-6	170.34
Pentanamide, 4-methyl-	1119-29-5	115.18
Pentane	109-66-0	72.15
Pentane, 2,2,3,4-tetramethyl-	1186-53-4	128.26
Pentane, 2-methyl-	107-83-5	86.18
Pentane, 3,3-dimethyl-	562-49-2	100.21
Pentatriacontane	630-07-9	492.96
Pentenylbenzene	1075-74-7	146.23

Chemical and Physical Characterization of Recycled Tire Crumb Rubber

Name	CASRN	Molecular Wt
Perylene	198-55-0	252.32
Phenanthrene	65996-93-2	178.23
Phenol	108-95-2	94.11
Phenol, 2,2'-methylenebis(6-(1,1-dimethylethyl)-4-ethyl-	88-24-4	368.55
Phthalimide	85-41-6	147.13
2-Phenyl Benzothiazole	883-93-2	211.28
1-Phenyl-1-butene	1005-64-7	132.21
1,2-Propanediol	57-55-6	76.10
Propane, 2-methyl-	75-28-5	58.12
2-Propanol	67-63-0	60.10
n-Propylbenzene	103-65-1	120.19
2-Propyl-methyl pentanoic acid	31113-56-1	158.24
1-Propynylbenzene	673-32-5	116.16
Pyrene	129-00-0	202.25
Pyrene, 1-methyl-	2381-21-7	216.28
Stigmasta-5,24(28)-dien-3-ol	17605-67-3	412.70
Styrene	100-42-5	104.15
1,1,2,2-Tetrachloroethane	79-34-5	167.84
Tetrachloroethene	127-18-4	165.82
Tetracosane	646-31-1	338.66
Tetradecane	629-59-4	198.39
Tetradecane, 1-chloro-	2425-54-9	232.84
Tetradecanoic Acid	544-63-8	228.37
Tetrahydrofuran	109-99-9	72.11
Tetratriacontane	14167-59-0	478.93
Toluene	108-88-3	92.14
Triacontane	638-68-6	422.82
1,2,4-Trichlorobenzene	120-82-1	181.44
1,1,1-Trichloroethane	71-55-6	133.40

Chemical and Physical Characterization of Recycled Tire Crumb Rubber

Name	CASRN	Molecular Wt
1,1,2-Trichloroethane	79-00-5	133.40
Trichloroethylene	79-01-6	131.38
2,4,5-Trichlorophenol	95-95-4	197.44
2,4,6-Trichlorophenol	88-06-2	197.44
Trichlorotrifluoroethane	76-13-1	187.37
Tricosane	638-67-5	324.63
Tridecane	629-50-5	184.37
2,3,6-Trimethylanisole	21573-36-4	150.22
1,2,4-Trimethylbenzene	95-63-6	120.19
1,3,5-Trimethylbenzene	108-67-8	120.19
2,2,7-Trimethyl-3-octyne	55402-13-6	152.28
2,4,4-Trimethylpentene	107-40-4	112.22
Tritriacontane	630-05-7	464.90
TXIB	6846-50-0	286.41
Undecane	1120-21-4	156.31
Undecane, 2,6-dimethyl-	17301-23-4	184.37
Undecane, 4,6-dimethyl-	17312-82-2	184.37
Undecane, 5,6-dimethyl-	17615-91-7	184.37
Undecane, 3-methyl-	1002-43-3	170.34
Undecane, 4-methyl-	2980-69-0	170.34
Vinyl Acetate	108-05-4	86.09
Vinyl Chloride	75-01-4	62.50
<i>m</i> -Xylene	108-38-3	106.17
<i>o</i> -Xylene	95-47-6	106.17
<i>p</i> -Xylene	106-42-3	106.17

<sup>a</sup>For references used please refer to Table 1 in Perkins et al.<sup>1</sup>.

## Appendix C. Additional Constituents of Crumb Rubber Identified by Various Techniques

**Table C-1. Constituents of Crumb Rubber in Methylene Chloride Extract Identified by GC-MS with Low Confidence<sup>a</sup>**

Name	CAS	ID by Match with Standard <sup>a</sup>	ID by Match with Reference <sup>b</sup>
Acenaphthylene	208-96-8	Yes	Yes
Acetophenone	98-86-2	No	Yes
Alpha Pinene	80-56-8	No	Yes
Aniline	62-53-3	No	Yes
Anthracene	120-12-7	Yes	Yes
Anthracene, 2-methyl-	613-12-7	No	Yes
Anthracene, 9-methyl-	779-02-2	No	Yes
Benz(a)anthracene	56-55-3	Yes	Yes
Benzenesulfonamide	1678-25-7	No	Yes
Benzo(a)fluoranthene	203-33-8	No	Yes
Benzo(a)pyrene	50-32-8	Yes	Yes
Benzo(b)fluorene	243-17-4	No	Yes
Benzo(ghi)fluoranthene	203-12-3	No	Yes
Benzothiazole, 2-(methylthio)-	615-22-5	No	Yes
<i>N,N'</i> -Bis(2'-dimethylphosphinothioylethyl)- <i>N,N'</i> -diethylpropylenediamine	Unknown	No	Yes
Bisphenol P	2167-51-3	No	Yes
<i>p-tert</i> -butyl Phenol	98-54-4	No	Yes
3-Carene	13466-78-9	No	Yes
Chrysene	218-01-9	Yes	Yes
Cyclohexane, 1,1,3-trimethyl-	3073-66-3	No	Yes
Cyclohexanone	108-94-1	No	Yes
<i>m</i> -Cymene	535-77-3	No	Yes
<i>o</i> -Cymene	527-84-4	No	Yes
<i>p</i> -Cymene	99-87-6	No	Yes
2,4'-Diaminotriphenylmethane	Unknown	No	Yes
Dicyclohexylamine	101-83-7	No	Yes
2,3-Dihydro-1,1,3-trimethyl-3-phenyl-1H-indene	3910-35-8	No	Yes
9,10-Dimethylanthracene	781-43-1	No	Yes
2,6-Dimethylnaphthalene	581-42-0	No	Yes
2,7-Dimethylphenanthrene	1576-69-8	No	Yes

## Chemical and Physical Characterization of Recycled Tire Crumb Rubber

Name	CAS	ID by Match with Standard <sup>a</sup>	ID by Match with Reference <sup>b</sup>
2,6-Di( <i>t</i> -butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one (BHT-OH)	10396-80-2	No	Yes
Ethylbenzene	100-41-4	No	Yes
2-Ethyl Hexanoic Acid	149-57-5	No	Yes
Fluorene	86-73-7	Yes	Yes
Hexadecanoic Acid, Methyl Ester	112-39-0	No	Yes
Hexatriacontane	630-06-8	No	Yes
1-Iodo-2-methylundecane	73105-67-6	No	Yes
Limonene	5989-27-5	No	Yes
2-Mercaptobenzothiazole	149-30-4	No	Yes
Methylcyclohexane	108-87-2	No	Yes
Methyl Elaidate	1937-62-8	No	Yes
2- $\alpha$ -Methyl-17- $\beta$ -hopane	Unknown	No	Yes
2-Methylphenanthrene	2531-84-2	No	Yes
3-Methylphenanthrene	832-71-3	No	Yes
Neodecanoic Acid	26896-20-8	No	Yes
30-Norhopane	36728-72-0	No	Yes
Perylene	198-55-0	No	Yes
Phenol	108-95-2	No	Yes
1-Phenyl-1-butene	1005-64-7	No	Yes
1-Propynylbenzene	673-32-5	No	Yes
2-Phenyl Benzothiazole	883-93-2	No	Yes
Pyrene, 1-methyl-	2381-21-7	No	Yes
Stigmasta-5,24(28)-dien-3-ol	17605-67-3	No	Yes
Styrene	100-42-5	No	Yes
Tetratriacontane	14167-59-0	No	Yes
Trichloroethylene	79-01-6	No	Yes
2,4,4-Trimethylpentene	107-40-4	No	Yes
1,2,4-Trimethylbenzene	95-63-6	No	Yes
1,3,5-Trimethylbenzene	108-67-8	No	Yes
<i>m</i> -Xylene	108-38-3	No	Yes
<i>o</i> -Xylene	95-47-6	No	Yes
<i>p</i> -Xylene	106-42-3	No	Yes

<sup>a</sup>Identification of constituents was done with retention time and a partial mass spectral match with a standard, a 30–84% match with a library reference spectrum, or both.

<sup>b</sup>Indicates a 30–84% match with a library reference spectrum.



**Table C-2. Constituents Not Found in Methylene Chloride Extracts of Crumb Rubber (No Peak for the Molecular Ion or with a Library Match Exceeding 30%)**

Name	CAS
Acetic Acid	64-19-7
<i>trans</i> -Anethole	50770-19-9
Anthanthrene	191-26-4
Azobenzene	103-33-3
Benzamide, N-N-diethyl-3-methyl- (DEET)	134-62-3
Benzenamine, 4-octyl-N-(4-octylphenyl)-	101-67-7
Benzene	71-43-2
Benzene, 1-ethyl-2-methyl- (2-ET)	611-14-3
Benzenemethanol, ar-ethenyl-	53957-34-9
Benzidine	92-87-5
Benzo(b)fluoranthene	205-99-2
Benzo(g,h,i)perylene	191-24-2
Benzo[k]fluoranthene	207-08-9
Benzoic Acid	65-85-0
Benzothiazole, 2-(morpholiniothio)-	102-77-2
<i>1H</i> -Benzotriazol-5-amine, 1-methyl-	27799-83-3
Benzyl Alcohol	100-51-6
Biphenyl	92-52-4
Bis(2-chloroethoxy)methane	111-91-1
Bis(2-chloroethyl)ether	111-44-4
Bromodichloromethane	75-27-4
Bromoform	75-25-2
4-Bromophenyl Phenyl Ether	101-55-3
Butane, 2-iodo-2-methyl-	594-38-7
1-Butanol, 4-methoxy-	111-32-0
2-Butanone	78-93-3
2-Butoxyethanol	111-76-2
2-(2-Butoxyethoxy)ethanol	112-34-5
2-(2-Butoxyethoxy)ethyl acetate	124-17-4
<i>sec</i> -Butylbenzene	135-98-8
Butyl Butanoate	109-21-7
Butyl Benzyl Phthalate	85-68-7
<i>n-tert</i> -Butylbenzothiazolesulfenamide	95-31-8
Carbon Tetrachloride	56-23-5

## Chemical and Physical Characterization of Recycled Tire Crumb Rubber

Name	CAS
4-Chloroaniline	106-47-8
Chlorobenzene	108-90-7
Chloroethane	75-00-3
2-Chloroethyl Vinyl Ether	110-75-8
1-Chloro-2-ethenyl-1-methylcyclopropane	62337-93-3
4-Chloro-3-methylphenol	59-50-7
2-Chloronaphthalene	91-58-7
2-Chlorophenol	95-57-8
4-Chlorophenyl Phenyl Ether	7005-72-3
4-Chlorotoluene	106-43-4
Cinnamaldehyde	14371-10-9
Cycloheptane	291-64-5
Cyclohexane	110-82-7
Cyclohexane, 1,3-dimethyl-, cis-	638-04-0
Cyclohexane, 1,4-dimethyl-	589-90-2
Cyclohexane, ethyl-	1678-91-7
Cyclohexanol	108-93-0
Cyclohexyl Isothiocyanate	1122-82-3
Decamethyl Cyclopentasiloxane	541-02-6
Decanal	112-31-2
Decane	124-18-5
Decane, 5-methyl-	13151-35-4
Decane, 6-ethyl-2-methyl-	62108-21-8
Decane, 2,3,8-trimethyl-	62238-14-6
Decane, 2,5,6-trimethyl-	62108-23-0
Dehydroabietic Acid	1740-19-8
Dibenz(a,h)anthracene	53-70-3
Dibenzofuran	132-64-9
3-Dibenzofuranamine	4106-66-5
4-Dibenzofuranamine	50548-43-1
2-Dibenzofuranamine	3693-22-9
Dibenzothiophene	132-65-0
Dibromochloromethane	124-48-1
Di-n-butyl Phthalate	84-74-2
1,2-Dichlorobenzene	95-50-1
1,3-Dichlorobenzene	541-73-1

Chemical and Physical Characterization of Recycled Tire Crumb Rubber

Name	CAS
1,4-Dichlorobenzene	106-46-7
3,3'-Dichlorobenzidine	91-94-1
1,1-Dichloroethane	75-34-3
1,2-Dichloroethane	107-06-2
<i>trans</i> -1,2-Dichloroethene	951-86-0
2,4-Dichlorophenol	120-83-2
Diethyl Phthalate	84-66-2
Dimethyl Adipate	627-93-0
Dimethyl Pentanedioate	1119-40-0
2,4-Dimethylphenol	105-67-9
Dimethyl Phthalate	131-11-3
Dimethyl Succinate	106-65-0
4,6-Dinitro-2-methylphenol	534-52-1
2,4-Dinitrophenol	51-28-5
2,4-Dinitrotoluene	121-14-2
2,6-Dinitrotoluene	606-20-2
Di-n-octyl Phthalate	117-84-0
1,3-Divinylbenzene	108-57-6
Dodecanal	112-54-9
Dodecane	112-40-3
Dodecane, 2,6,11-trimethyl-	31295-56-4
Dodecane, 2,7,10-trimethyl-	74645-98-0
2-Dodecen-1-yl Succinic Anhydride	19780-11-1
Ethyl Benzoate	93-89-0
Ethylbenzaldehyde	4748-78-1
2-Ethyl-1-hexanol	104-76-7
4-Ethyltoluene (4-ET)	622-96-8
2(3 <i>H</i> )-Furanone, dihydro-4-hydroxy-	5469-16-9
Heptane	142-82-5
Heptane, 2,2-dimethyl-	1071-26-7
Heptane, 2,3,4-trimethyl-	52896-95-4
Heptane, 2,4-dimethyl-	2213-23-2
Heptane, 2,5-dimethyl-	2216-30-0
Heptane, 2,6-dimethyl-	1072-05-5
Heptane, 2-methyl-	592-27-8
Heptane, 3-methyl-	589-81-1

Chemical and Physical Characterization of Recycled Tire Crumb Rubber

Name	CAS
Heptane, 4-(1-methylethyl)-	52896-87-4
1-Heptene	592-76-7
Hexachlorobenzene	118-74-1
Hexachlorobutadiene	87-68-3
Hexachlorocyclopentadiene	77-47-4
Hexachloroethane	67-72-1
Hexadecane	544-76-3
Hexadecanoic Acid, Butyl Ester	111-06-8
Hexanal	66-25-1
Hexane, 2,2,4-trimethyl-	16747-26-5
Hexane, 2,2,5-trimethyl-	111-84-2
Hexane, 3,3-dimethyl-	563-16-6
Hexane, 3-methyl-	589-34-4
2-Hexanone (MBK)	591-78-6
1-Hexene, 4,5-dimethyl-	16106-59-5
2-Hexen-1-ol, (Z)-	928-94-9
2-Hexen-1-ol, (Z)-	928-94-9
5-Hexen-2-ol, (+/-)-	54774-27-5
Hydroxylamine, O-decyl-	29812-79-1
2-Hydroxybenzothiazole	934-34-9
3 <i>H</i> -Indazol-3-one, 1,2-dihydro-2-methyl-	1848-40-4
4-Isopropylphenol	99-89-8
Indeno(1,2,3- <i>cd</i> )pyrene	193-39-5
Isobutyl Alcohol	78-83-1
Isophorone	78-59-1
Isopropylbenzene	98-82-8
Menthol	1490-04-6
Methanimidamide, <i>N,N</i> -dimethyl- <i>N'</i> -phenyl-	1783-25-1
1-Methoxy-2-propanol	107-98-2
6-Methylbenzo[ <i>b</i> ]thiophene	16587-47-6
2-Methylbenzothiazole	120-75-2
4-Methyl-4-heptanol	598-01-6
2-Methylhexane	591-76-4
2-Methylphenol ( <i>o</i> -Cresol)	95-48-7
4-Methylphenol ( <i>p</i> -Cresol)	106-44-5
1-Methyl-2-pyrrolidinone	872-50-4

Chemical and Physical Characterization of Recycled Tire Crumb Rubber

Name	CAS
Methyl $\alpha$ -D-xylofuranoside	1824-96-0
Methyl Salicylate	119-36-8
2-Nitroaniline	88-74-4
3-Nitroaniline	99-09-2
4-Nitroaniline	100-01-6
Nitrobenzene	98-95-3
Nitromethane	75-52-5
2-Nitrophenol	88-75-5
4-Nitrophenol	100-02-7
<i>N</i> -Nitrosodimethylamine	62-75-9
<i>N</i> -Nitroso-di- <i>n</i> -propylamine	621-64-7
<i>N</i> -Nitrosodiphenylamine	86-30-6
Nonanal	124-19-6
Nonanamide	1120-07-6
Nonane	111-84-2
Nonane, 3-methyl-5-propyl-	31081-18-2
Octadecanoic Acid/Stearic Acid	57-11-4
Octane	111-65-9
Octane, 2-methyl-	3221-61-2
Octane, 3-methyl-	2216-33-3
2-Octen-1-ol	18409-17-1
Oleic Acid	112-80-1
2,2'-Oxybis(1-chloropropane)	108-60-1
Pentachlorophenol (PCP)	87-86-5
Pentadecane	629-62-9
2,2,4,6,6-Pentamethylheptane	13475-82-6
Pentanamide, 4-methyl-	1119-29-5
Pentane, 2,2,3,4-tetramethyl-	1186-53-4
Pentane, 3,3-dimethyl-	562-49-2
Pentenylbenzene	826-18-6
Phenol, 2,2'-methylenebis(6-(1,1-dimethylethyl)-4-ethyl-	88-24-4
1,2-Propanediol	57-55-6
2-Propanol	67-63-0
<i>n</i> -Propylbenzene	103-65-1
2-Propyl-methyl pentanoic Acid	31113-56-1
1,1,2,2-Tetrachloroethane	

## Chemical and Physical Characterization of Recycled Tire Crumb Rubber

Name	CAS
Tetrachloroethene	127-18-4
Tetradecane	629-59-4
Tetradecane, 1-chloro-	2425-54-9
Tetradecanoic Acid	544-63-8
Tetrahydrofuran	109-99-9
1,2,4-Trichlorobenzene	120-82-1
2,4,5-Trichlorophenol	95-95-4
2,4,6-Trichlorophenol	88-06-2
Tridecane	629-50-5
2,3,6-Trimethylanisole	21573-36-4
2,2,7-Trimethyl-3-octyne	55402-13-6
TXIB	6846-50-0
Undecane	1120-21-4
Undecane, 2,6-dimethyl-	17301-23-4
Undecane, 3-methyl-	1002-43-3
Undecane, 4,6-dimethyl-	17312-82-2
Undecane, 4-methyl-	2980-69-0
Undecane, 5,6-dimethyl-	17615-91-7
Vinyl Acetate	108-05-4

<sup>a</sup>No peak for the molecular ion or with a library match exceeding 30%.

## Appendix D. Settings Used in XCMS Software

The XCMS software can be found online here: <https://xcmsonline.scripps.edu>.

```
-----
XCMSOnline version 2.3.0
XCMS        version 1.47.3
CAMERA      version 1.26.0
-----
```

### General parameters

```
Polarity      positive
Retention time format      minutes
```

### 2. Feature detection

```
method : matchedFilter
fwhm    15
snthresh 3
max     100
step    0.5
steps   2
mzdiff  0.5
```

#### Feature detection results :

```
26sep17-04.cdf [Unsieved] --> 176 Features.
26sep17-03.cdf [Unsieved] --> 204 Features.
26sep17-05.cdf [14_Mesh]  --> 176 Features.
26sep17-06.cdf [14_Mesh]  --> 177 Features.
26sep17-08.cdf [40_Mesh]  --> 176 Features.
26sep17-07.cdf [40_Mesh]  --> 186 Features.
26sep17-11.cdf [400_Mesh] --> 169 Features.
26sep17-12.cdf [400_Mesh] --> 163 Features.
26sep17-10.cdf [Pooled_14-80_Mesh] --> 155 Features.
26sep17-09.cdf [Pooled_14-80_Mesh] --> 142 Features.
```

### 3. Retention time correction

```
method : peakgroups
Initial grouping : ignore sample class TRUE
Initial grouping : bw          3
Initial grouping : mzwid       0.5
Initial grouping : minfrac     1
Initial grouping : minsamp     1
missing          1
extra            1
smooth          loess
family          gaussian
```

### 4. Grouping

```
method : density
bw     10
mzwid  0.5
minfrac 0.5
minsamp 1
```

### 5. FillPeaks

### 6. Diffreport

```
classes Unsieved
classes 14_Mesh
classes 40_Mesh
```

## Chemical and Physical Characterization of Recycled Tire Crumb Rubber

```
classes      400_Mesh
classes      Pooled_14-80_Mesh
statistical test      ANOVA parametric
statistics.threshold.pvalue      0.05
statistics.diffReport.value      into
Finished Running Statistical tests
7. Additional Plots & Statistics
  Running mummichog
  Printing MDS plot
  Printing static PCA and Select Scaling plot
8. Annotation (isotopes & adducts)
  featureAnnotation.CAMERA.annotate      isotopes
  featureAnnotation.CAMERA.mzabs      0.25
  featureAnnotation.CAMERA.ppm      100
  featureAnnotation.CAMERA.sigma      6
  featureAnnotation.CAMERA.perfwhm      0.6
  featureAnnotation.CAMERA.maxcharge      3
  featureAnnotation.CAMERA.maxiso      4
  featureAnnotation.CAMERA.intensity      into
9. Putative ID's (METLIN)
  identification.METLIN.ppm      100
  identification.METLIN.adducts      M+H
```



## Appendix E. Bioaccessibility Studies

**Table E-1. Composition of Media Used in Bioaccessibility Studies**

<b>Simulated Biofluid</b>	<b>Component</b>	<b>Concentration</b>
<b>Sweat</b>	Sodium chloride	340 mM
	Ammonium chloride	330 mM
	Urea	83 mM
	Lactic acid	170 mM
	Acetic acid	42 mM
<b>Lung Fluid</b>	Sodium chloride	150 mM
	Potassium chloride	4 mM
	Magnesium chloride	10 mM
	Sodium phosphate dibasic	1 mM
	Sodium sulfate	5 mM
	Calcium chloride	25 mM
	Sodium acetate	7 mM
	Sodium bicarbonate	24 mM
	Sodium citrate	3 mM
	Dipalmitoyl lecithin	0.2%
<b>Saliva<sup>a</sup></b>	Sodium chloride	7 mM
	Calcium chloride	4 mM
	Potassium chloride	5 mM
	Sodium phosphate dibasic	4 mM
	Urea	17 mM
	Mucin	0.4%
<b>Gastric Fluid<sup>a</sup></b>	Sodium chloride	30 mM
	Pepsin	200 mM
	Hydrochloric acid	84 mM
<b>Intestinal Fluid<sup>a</sup></b>	Sodium bicarbonate	200 mM

<sup>a</sup>Gastric only ingestion phase consisted of extraction with simulated saliva followed by simulated gastric fluid. Total ingestion consisted of simulated saliva followed by simulated gastric fluid and then simulated intestinal fluid.



# National Toxicology Program

NTP Central Data Management, MD K2-05

National Institute of Environmental Health Sciences

P.O. Box 12233

Research Triangle Park, NC 27709

<http://ntp.niehs.nih.gov>

ISSN 2473-4756