Environmental Fate, Chemical Profile, and Cytotoxicity of Leachate from Cigarette Filters

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What are we studying?

Cigarette Filters

Cigarette Filters (CF) are the most common form of litter and an emerging threat due to their chemical profile. CF are comprised of cellulose acetate (CA), a non biodegradable plastic that fragments into microfibers. **CF consist of >15,000 microfibers that can release into the environment when exposed to environmental conditions.** CF are thought to be one of the most persistent forms of microplastics due to their ubiquity and potential to end up in waterways.

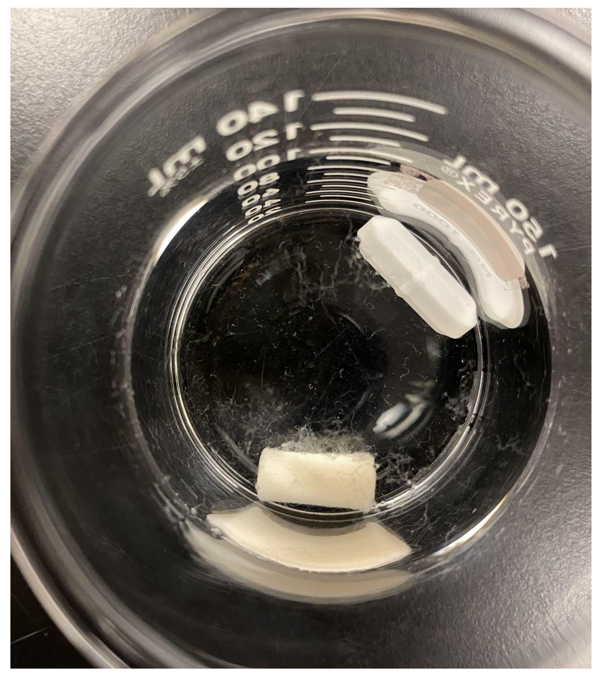

Figure 1: Cigarette filter in simulated freshwater with a magnetic stirrer.

What is the problem with cigarette filters?

CF are manufactured with various chemicals, including plasticizers, enhancers, and adhesives. Once in the environment they can leach toxic chemicals into the soil, air, or water. Microfibers are the most abundant type of microplastics in stormwater and an emerging threat to ecosystems due to bioavailability and potential toxicity.

Figure 2: Graphic displaying the impacts of CF once in an aquatic environment. Photo credit to, Smoked Cigarettes: Unignorable Source for Environmental Microplastic Fibers.

Do we know what chemicals are in cigarette filters?

Past studies have identified numerous chemicals in cigarette filters, including tobacco alkaloid products such as nicotine, nicotyrine, myosmine, and 2,2'-bipyridine. Non-targeted analysis (NTA) studies have revealed additional, previously unidentified compounds. This study investigates chemicals in aqueous leachate and examines whether exposure to sunlight leads to the formation of new or different chemical compounds

Why is non-targeted analysis useful for determining the chemicals in cigarette filters?

NTA can be used to detect thousands of compounds simultaneously in a sample, making it a powerful tool for determining a comprehensive chemical profile for cigarette filters.

Objectives

1. Determine if smoked cigarette filters will generate more chemicals in the leachate compared to unsmoked and pure cellulose acetate polymer (control).
2. Determine if cigarette filters exposed to sunlight will generate more chemicals in aqueous leachate compared to dark exposed.
3. Asses biological hazard of leachate measuring cytotoxicity of HepG2 cells.

Methods

Leaching experiments

- Leach under appropriate condition (light or dark) for 6 hours to simulate the full solar spectrum at the elevation and latitude/longitude of San Diego, CA.
- Once the timepoint is reached, filter the leachate to separate out microfibers.

NTA experiments

- Run through solid phase extraction, elute samples at rate of 1-2 drops per second until cartridge is dry.
- This separates compounds of interest from a complex sample matrix by selectively absorbing them into a solid phase.

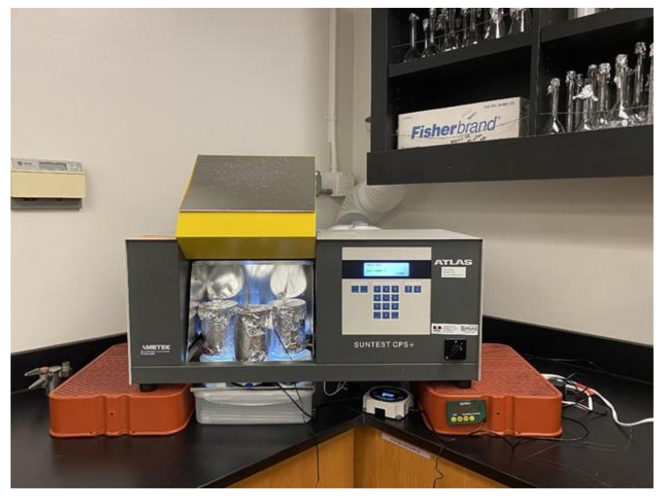

Figure 3: Leachate setup in the Atlas Suntest CPS+ photoirradiation machine.

Chemical Analysis

- Use non-targeted chemical analysis with GCxGC/TOF-MS to determine chemical profiles of the leachates
- Use "Statistical Compare" to automatically align peaks across samples.

Heatmap Analysis

- **383 "tentatively identifiable" compounds were detected.**

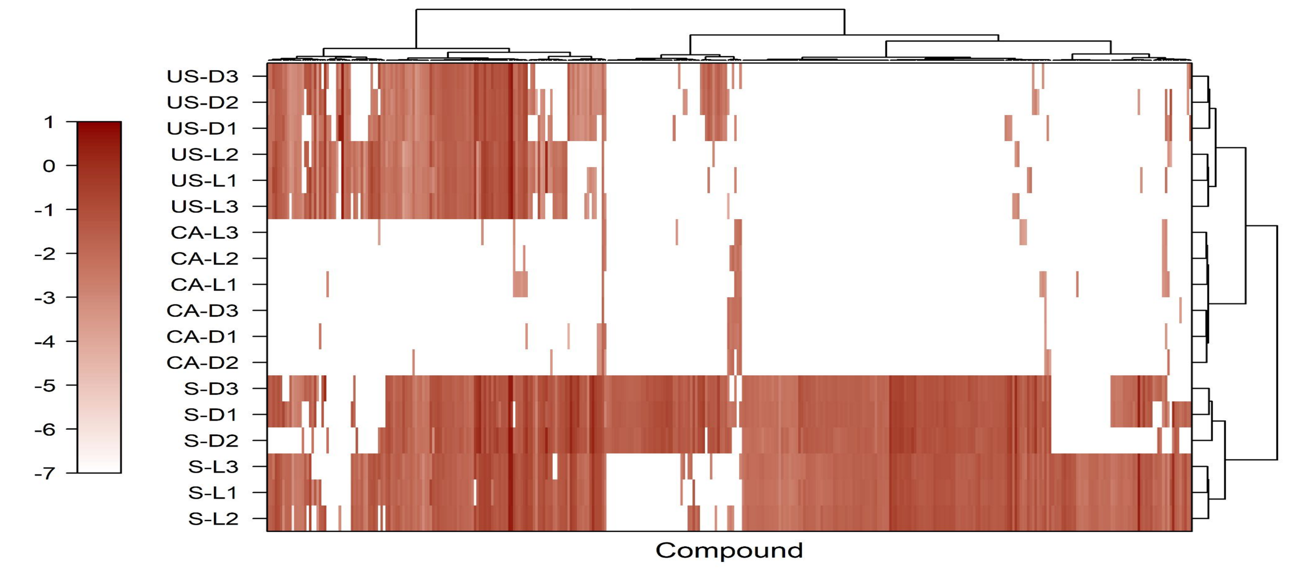

Figure 4: Heatmap produced via hierarchical clustering showing the relative abundance of detected compounds. US: unsmoked, CA: cellulose acetate, S: smoked, D: dark, L: light.

- 38 compounds (10%) found in the cellulose acetate samples both exposure conditions.
- 171 compounds (45%) found in the unsmoked samples under both exposure conditions.
- 375 (98%) compounds found in the smoked samples under both exposure conditions.
- The heatmap shows clear differences in chemical composition among sample types. Distinct clustering patterns indicate that each group (US, CA, and S) has a characteristic chemical profile. The most pronounced differences were observed in smoked cigarette filters, which display unique compounds compared to other samples.

Mass Spectrometry

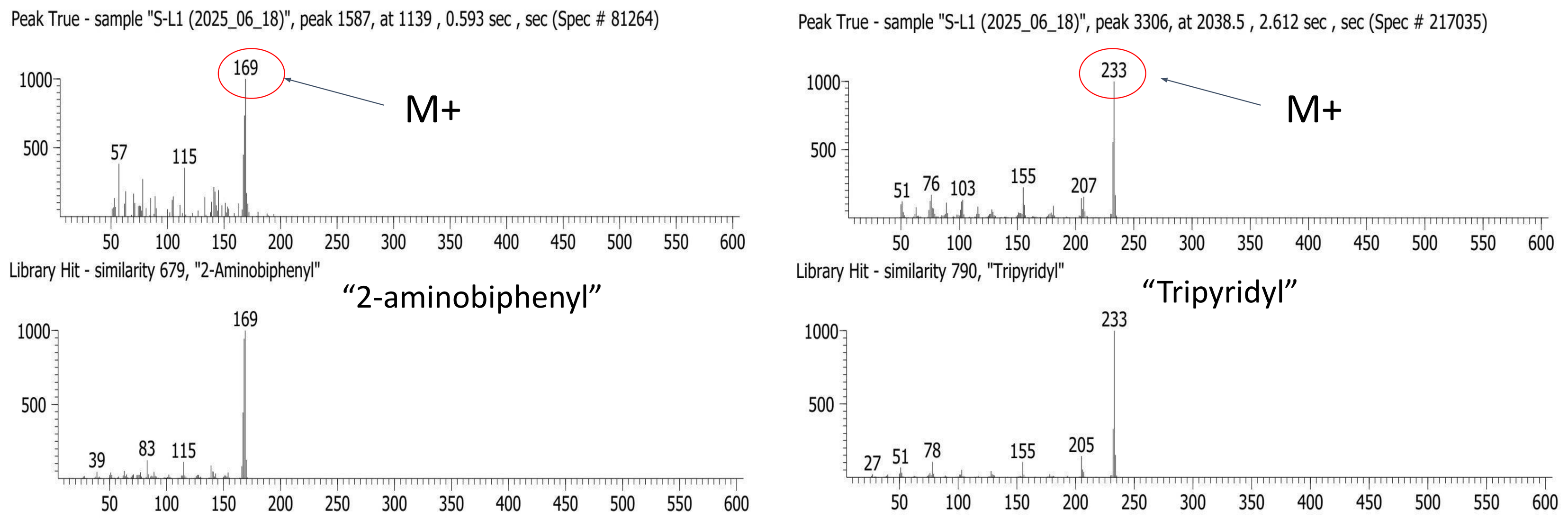

Figure 5: Mass Spectrometry data from ChromaTOF software.

- Portrays relative abundance against mass to charge ratio (m/z).
- Find the molecular ion (M+) to determine the molecular weight of the compound.
- Able to match compounds based off molecular peaks and base peaks by comparing peak true graph with the library hit from National Institute of Standards and Technology (NIST) library.
- Similarity scores <600 were eliminated, noisy mass spectra were eliminated, after manual review peak true graphs with no M+ or similar hits were eliminated.

Cytotoxicity and Hazard Data

1. Hazard ranking all of the detected compounds using the Environmental Protection Agency (EPA) CompTox Chemicals Dashboard.
2. Significant differences observed between light vs dark cellulose acetate, with increased cell death in the dark cellulose acetate condition (p=0.01).
3. Significant differences in irradiation status are also observed in death, with increased activity in dark conditions compared to light, supporting the viability seen as well (p=0.02).

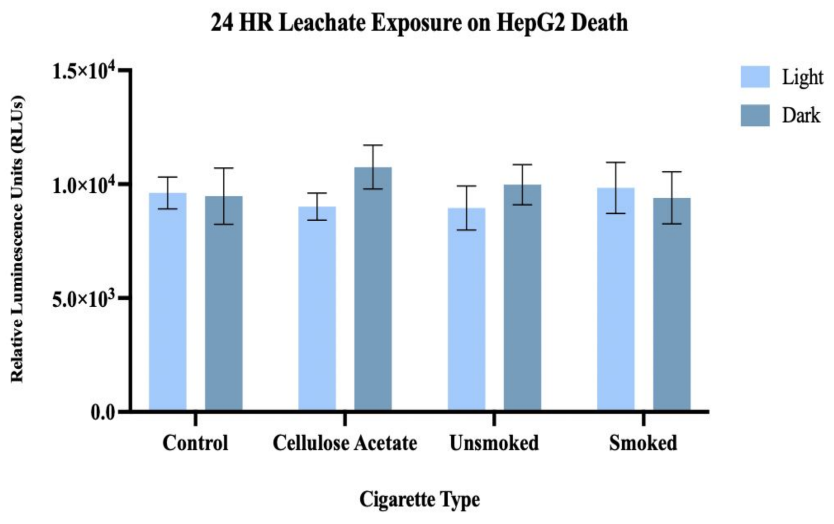

Figure 6: Effect of 24-hour cigarette filter leachate exposure on HepG2 cell death under light and dark conditions. Cell death was measured by relative luminescence units (RLUs) .

Compound	CAS #	Quality Adjusted Hazard Score	Completeness Group	Use
Human Health Effects: Chronic Exposure (7 end points)				
2-Methylquinoline	94-28-0	8.00	Low	Flavor/Fragrance
2,6-Dimethylpyrazine	108-50-9	8.00	Low	Flavor/Fragrance
3-Acetylpyridine	350-03-8	8.00	Low	Flavor
Quinoxaline	91-19-0	8.00	Low	Flavor
1,2-Diacetylglycerol	102-62-5	8.00	Low	Pharmaceutical
Human Health Effects: Short-Term Exposure (8 end points)				
(-)-Nicotine	54-11-5	8.55	Medium High	Insecticide/Formulation
Phenol	108-95-2	8.53	High	Biocide/Adhesive
p-Cresol	106-44-5	7.14	High	Biocide
m-Cresol	108-39-4	6.86	High	Biocide
3-Acetylpyridine	350-03-8	6.00	Medium Low	Flavor
Ecological End Points (4 end points)				
1,2,3-Trichlorobenzene	87-61-6	8.75	High	Not specified
Scopoletin	92-61-5	8.00	Medium Low	Pharmaceutical
1-Cyclohexylpropan-1-one	1123-86-0	8.00	Medium Low	Not specified
Isoquinoline, 3,4-dihydro-	3230-65-7	8.00	Medium Low	Not specified
4-Hydroxy-2-methylacetophenone	875-59-2	8.00	Medium Low	Not specified

Figure 7: Top 5 compounds for each exposure group based on quality adjusted hazard score.
**note: scores are calculated using methods previously published in Newmeyer et al., 2024.*

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