Toxicity of cigarette butts, and their chemical components, to marine and freshwater fish

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ABSTRACT

Background Cigarette butts are the most common form of litter, as an estimated 4.5 trillion cigarette butts are thrown away every year worldwide. Many chemical products are used during the course of growing tobacco and manufacturing cigarettes, the residues of which may be found in cigarettes prepared for consumption. Additionally, over 4000 chemicals may also be introduced to the environment via cigarette particulate matter (tar) and mainstream smoke.

Methods Using US Environmental Protection Agency standard acute fish bioassays, cigarette butt-derived leachate was analysed for aquatic toxicity. Survival was the single endpoint and data were analysed using Comprehensive Environmental Toxicity Information System to identify the LC50 of cigarette butt leachate to fish.

Results The LC50 for leachate from smoked cigarette butts (smoked filter + tobacco) was approximately one cigarette butt/l for both the marine topsmelt (*Atherinops affinis*) and the freshwater fathead minnow (*Pimephales promelas*). Leachate from smoked cigarette filters (no tobacco), was less toxic, with LC50 values of 1.8 and 4.3 cigarette butts/l, respectively for both fish species. Unsmoked cigarette filters (no tobacco) were also found to be toxic, with LC50 values of 5.1 and 13.5 cigarette butts/l, respectively, for both fish species.

Conclusion Toxicity of cigarette butt leachate was found to increase from unsmoked cigarette filters (no tobacco) to smoked cigarette filters (no tobacco) to smoked cigarette butts (smoked filter + tobacco). This study represents the first in the literature to investigate and affirm the toxicity of cigarette butts to fish, and will assist in assessing the potential ecological risks of cigarette butts to the aquatic environment.

BACKGROUND

Cigarette butts are the most common form of litter in the world, as approximately 5.6 trillion cigarettes are smoked every year worldwide.[1] Cigarette waste constitutes an estimated 30% of the total litter (by count) on US shorelines, waterways and on land (LitterFreePlanet, 2009). In fact, cigarette butts are the most common debris item collected along waterways during the Ocean Conservancy's yearly International Coastal Cleanup. In all, 2 189 252 cigarettes were collected during the 2009 cleanup. (Ocean Conservancy, 2010) Conservatively, this quantity of cigarettes weighs approximately 821 lb and displaces a volume of 1095 litres.[2] Owing to the ubiquitous nature and magnitude of cigarette butts discharged into the environment, studies are needed to determine whether cigarette butt waste can exert ecotoxic effects when in aquatic environments.

Many chemical products are used during the course of growing tobacco and manufacturing cigarettes, the residues of which may be found in cigarettes prepared for consumption.[3 4] These include pesticides, herbicides, insecticides, fungicides and rodenticides.[5] Additionally, over 4000 chemicals may also be introduced to the environment via cigarette particulate matter (tar) and mainstream smoke.[4] These include chemicals such as carbon monoxide, hydrogen cyanide, nitrogen oxides, polycyclic aromatic hydrocarbons, ammonia, acetaldehyde, formaldehyde, benzene, phenol, argon, pyridines and acetone, over 50 of which are known to be carcinogenic to humans.[4] A study performed by Moriwaki *et al* found that arsenic, nicotine, polycyclic aromatic hydrocarbons and heavy metals are released into the environment by littered 'roadside waste' cigarette butts.[6] Moreover, previous studies have shown chemicals in cigarette butt leachate can be acutely toxic to aquatic organisms.[2 7 8] Register found leachates from smoked cigarette tobacco, smoked cigarette filters and unsmoked cigarette filters to be acutely toxic to the freshwater cladoceran *Daphnia magna* between 0.125 and 0.25, 1 and 2, and >16 cigarette butts/l (48-hour LC50 (lethal concentration the concentration that kills 50% of a sample population)), respectively.[2] Warne *et al* found leachates from smoked cigarette butts, smoked cigarette filters, and unsmoked cigarette filters to be acutely toxic to the freshwater cladoceran *Ceriodaphnia* cf *dubia* at 0.06, 0.16, and 1.7 cigarette butts/l, respectively (48-hour EC50 (immobilisation)), and to the marine bacterium *Vibrio fischeri* at 0.58, 1.25, and >970 cigarette butts/l, respectively (30-minute EC50 (bioluminescence)).[7] The EC50 is the concentration at which 50% of the test organisms exhibit a specified effect (eg, immobilisation). Lastly, Micevska *et al* found that leachate from various brands of smoked cigarette butts were toxic to *Ceriodaphnia* cf *dubia* at concentrations between 8.9 and 25.9 mg butts/l (which corresponds to 0.03–0.08 butts/l) (48-hour EC50 (immobilisation)) and to *Vibrio fischeri* at concentrations between 104 and 832 mg butts/l (which corresponds to 0.3–2.7 butts/l) (30-minute EC50 (bioluminescence)).[8] This research also found that toxicity for both test species was related to cigarette brand as well as tar content. There is no research to support that flavoured cigarettes (eg, menthol) alter toxicity or impart additional toxicity.

All previous studies used non-vertebrate species (ie, daphnids and marine bacteria) for testing the

toxicity of cigarette butts in water, while similar studies investigating the toxicity to vertebrates, specifically marine and freshwater fish, have not been performed. Fish are ecologically important organisms, and are often used as bioindicators of healthy aquatic systems. Furthermore, toxicity data for a number of aquatic species are the minimum needed to derive water quality guidelines or to conduct hazard assessments. Therefore, it is important to determine the toxicity of cigarette butt leachate to fish. In this study, we investigated the toxicity of smoked cigarette butts (smoked filter + tobacco), smoked cigarette filters (no tobacco), as well as unsmoked cigarette filters (no tobacco) to the marine fish, topsmelt (*Atherinops affinis*), and to the freshwater fathead minnow (*Pimephales promelas*) in order to better understand the impact of cigarette waste on both marine and freshwater ecosystems. The specific aims of this study were to:

1. Determine if smoked cigarette butts (SCB) (smoked filter + tobacco), smoked filters (SF) (no tobacco) and unsmoked filters (USF) (no tobacco) were acutely toxic to a representative marine and freshwater fish.
2. Determine whether most of the toxicity of a cigarette butt was in the filter or the remnant tobacco.
3. Compare the sensitivity of fish to cigarette butts with other aquatic test organisms.
4. Determine if smoking increases the toxicity of cigarette filters.

MATERIALS AND METHODS

Toxicity test methods followed US Environmental Protection Agency (EPA) acute protocols.[9] Tests on both topsmelt and fathead minnows were performed utilising three different cigarette leachates: (1) leachate from smoked cigarette butts (SCB), with 1–2 cm of remnant tobacco left intact with the filter. This test was performed twice, once with artificially smoked cigarettes and again with naturally smoked cigarettes; (2) leachate from smoked cigarette filters (SF), with all remnant tobacco removed. This test was performed three times, once with artificially smoked cigarettes and twice with naturally smoked cigarettes; (3) leachate from unsmoked cigarette filters (USF), without tobacco. This test was performed once.

Test cigarettes consisted of regular filtered cigarettes (ie, no flavoured or light cigarettes were used). Cigarettes were purchased new and artificially smoked at the University of California, San Francisco, in order to control for variability and to decrease the risk of contamination from external sources. Cigarettes were smoked according to ISO Standard 3308:2000 using a TE10z smoking machine (Teague Enterprises, 530-406-88931237 E Beamer, Suite E Woodland, CA 95776, USA). Cigarettes that self-extinguished prior to completion of a complete smoking cycle were relit with a disposable butane lighter. For comparative purposes, toxicity tests were also carried out using naturally smoked cigarettes, defined as cigarettes that were actually smoked by people, extinguished in cigarette disposal units and collected within 24 hours of deposition.

To produce the leachate stock, cigarette butts were submerged and allowed to soak in dilution water (diluted mineral water for freshwater tests and natural seawater for saltwater tests), prepared according to EPA protocol, for 24 hours.[9] Diluted mineral water consisted of eight parts nanopure deionised water for every two parts Perrier mineral water. Following overnight aeration, the diluted mineral water would yield a pH range of 7.9–8.3 and a hardness range of 80–100 mg/l $CaCO_3$. Natural seawater was obtained from Scripps Institution of Oceanography and transported to the laboratory. Seawater was held in a flow-through system with a 20 μm in-line fibre filter and chiller unit. The leachate stock for the smoked cigarette butt (SCB) (smoked filter + tobacco) test was made by adding eight cigarette butts to 2 litres of dilution water. A 0.5× dilution series was then performed to obtain subsequent lower concentrations. Concentrations for this test were 4, 2, 1, 0.5, 0.25, 0.125 cigarette butts/l. The leachate stock for the smoked cigarette filter (SF) (no tobacco) test was made by adding 16 filters to 2 litres of dilution water. Concentrations for this test were 8, 4, 2, 1, 0.5, 0.25, 0.125 cigarette butts/l. The leachate stock for the unsmoked cigarette filter (USF) (no tobacco) test was made by adding 32 filters to 2 litres of dilution water. Concentrations for this test were 16, 8, 4, 2, 1, 0.5 cigarette butts/l. All tests were run with laboratory controls comprised of clean dilution water of either natural seawater for the saltwater tests or diluted mineral water for the freshwater tests.

There were four replicates for every concentration, each replicate containing five fish, for a total of 20 fish per concentration. Topsmelt were 7–14 days old and fathead minnows were 12–14 days old. Both were fed *Artemia* (brine shrimp) prior to initiation and again after 48 hours of testing. Fish were provided by Aquatic Bio Systems in Fort Collins, Colorado. All tests received continuous light aeration, a water renewal at 48 hours, and a light cycle of 16 hours of light and 8 hours of darkness. Water quality readings (pH, conductivity, salinity, dissolved oxygen and temperature) and survival counts were performed on a daily basis until test termination at 96 hours, to ensure a controlled environment. Water was to have a dissolved oxygen content between 6 mg/l and 9 mg/l at initiation and at the 48-hour renewal, and was never allowed to fall below 4 mg/l during testing. Temperature was to remain between 20±1°C for saltwater tests and 25±1°C for freshwater tests. Water quality parameters were measured by various meters: the Orion 250A+ pH meter, the YSI 550A dissolve oxygen meter and the Orion 130 to measure temperature, conductivity and salinity. Mean survival in the laboratory controls must be 90% or greater in order for the test to be deemed acceptable.[9]

Survival was the endpoint evaluated and data were analysed to identify the median lethal effect concentration (LC50), the concentration of cigarette butt leachate resulting in 50% mortality. LC50 values were determined using the Trimmed Spearman-Karber method, as outlined in US EPA 2002, using Comprehensive Environmental Toxicity Information System v1.6.3revE, Tidepool Scientific Software.[9 10] To determine whether there were statistically significant ($p<0.05$) differences in the toxicity of cigarette butt leachates, concentration-response curves were compared with an F test using Prism version 4.02, GraphPad Software, Inc.[11]

RESULTS

Toxicity of leachate from smoked cigarette butts

Leachate from smoked cigarette butts (SCB) (smoked filter + tobacco) was found to be acutely toxic to both the saltwater topsmelt *(Atherinops affinis)* and the freshwater fathead minnow *(Pimephales promelas)*. An LC50 of approximately 1 cigarette butt/l of water was obtained for both species. The concentration-response curve for the topsmelt is shown in figure 1 and for the fathead minnow in figure 2. Survival in all laboratory controls was 90% or greater, as required by EPA protocol for test validity.[9] For comparative purposes, this test was performed twice, once with artificially smoked cigarettes and again with naturally smoked cigarettes. Both methods of smoking the cigarette yielded similar results, as concentration-response curves for this test were not found to be statistically different (as

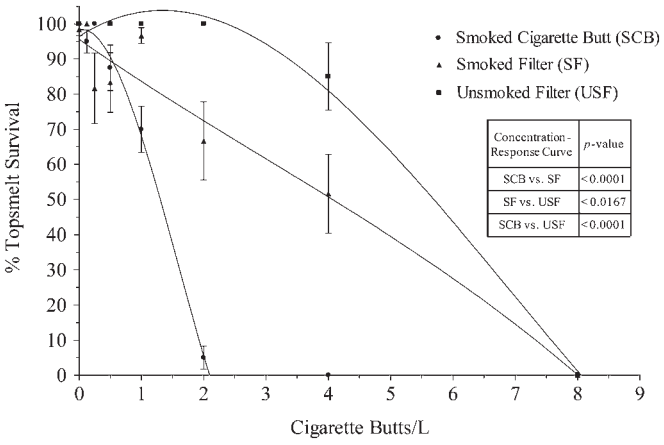

Figure 1 Concentration-response curves for topsmelt (*Atherinops affinis*). LC50 values for leachate from smoked cigarette butts (SCB) (smoked filter + tobacco), smoked cigarette filters (SF) (no tobacco) and unsmoked cigarette filters (USF) (no tobacco) were determined to be 1.1, 1.8 and 5.1 cigarette butts/l, respectively. Survival in all laboratory controls exceeded 90%. Error bars represent one SE of the mean. Dose-response curves are significantly ($p<0.05$) different.

$p>0.05$) when comparing artificially smoked versus naturally smoked cigarette leachates for either fish species.

Toxicity of leachate from smoked cigarette filters

Leachate from smoked cigarette filters (SF) (no tobacco) was also found to be acutely toxic to topsmelt at the concentration of 1.8 cigarette butts/l (figure 1), and to fathead minnows at 4.3 cigarette butts/l (figure 2). Survival in all laboratory controls was 90% or greater, as required by EPA protocol for test validity.[9] The toxicity tests for smoked filters (SF) (no tobacco) were performed three times, once with artificially smoked cigarette filters and twice with naturally smoked cigarette filters. The different methods of smoking the cigarette yielded different results. Concentration-response curves for both species were found to be statistically different ($p<0.05$) when comparing artificially smoked versus naturally smoked filter leachates. Artificially smoked filters were found to be more toxic than naturally smoked filters for both fish species. The reasons for this discrepancy are unclear.

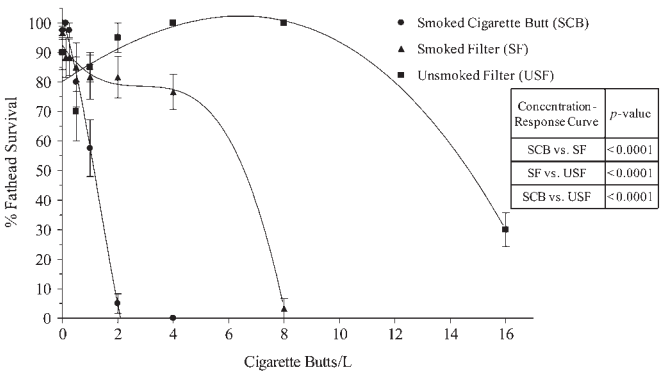

Figure 2 Concentration-response curves for the fathead minnow (*Pimephales promelas*). LC50 values for leachate from smoked cigarette butts (SCB) (smoked filter + tobacco), smoked cigarette filters (SF) (no tobacco) and unsmoked cigarette filters (USF) (no tobacco) were determined to be 0.97, 4.3, and 13.5 cigarette butts/l, respectively. Survival in all laboratory controls exceeded 90%. Error bars represent one SE of the mean. Dose-response curves are significantly ($p<0.05$) different.

Toxicity of leachate from unsmoked cigarette filters

One surprising result of our study was that leachate from unsmoked cigarette filters (USF) (no tobacco) was found to be acutely toxic to both fish species with an LC50 value of 5.1 for the topsmelt (figure 1) and 13.5 cigarette butts/l for the fathead minnow (figure 2). Survival in all laboratory controls was 90% or greater, as required by EPA protocol for test validity.[9]

DISCUSSION

Sensitivity of fish to smoked cigarette leachate compared to other species

A summary of toxicity (LC50 and EC50 values) from all research completed to date can be found in table 1. Results of a previous study found that smoked cigarette butt (SCB) leachate was acutely toxic to the daphnid, *Ceriodaphnia* cf *dubia*, at concentrations between 8.9 and 25.9 mg butts/l when remnant tobacco is left intact.[8] Given that the mean weight of a single smoked cigarette butt is approximately 310 mg, it can be calculated that smoked cigarette butt (SCB) leachate was found to be acutely toxic to daphnids between 0.03 and 0.08 cigarette butts/l (48-hour EC50 (immobilisation)). A study conducted by Warne *et al* supports this finding, as a similar EC50 (48-hour (immobilisation)) of 0.06 cigarette butts/l was identified, utilising the same test species.[7] However, a study conducted by Register found leachate from smoked cigarette tobacco (no filter) to be acutely toxic to *Daphnia magna* at slightly higher concentrations, between 0.125 and 0.25 cigarette butts/l (48-hour LC50).[2] In comparison, our current study found smoked cigarette butt (SCB) leachate to be less toxic to topsmelt and fathead minnows, than to daphnids tested in previous studies, as the LC50 (96-hour) for fish was identified as approximately 1 cigarette butt/l.

The toxicity of smoked cigarette butts (SCB) has also been evaluated using a marine bacterium (*Vibrio fischeri*). Warne *et al* found SCB leachate to be acutely toxic to *V fischeri* at 0.58 cigarette butts/l (30-minute EC50 (bioluminescence)) and Micevska *et al* supported this finding with a SCB leachate EC50 (30-minute bioluminescence) between 0.3 and 2.7 cigarette butts/l.[7 8] Since fish were found to have an LC50 of 1 cigarette butt/l for this same cigarette leachate, fish and marine bacteria may have similar sensitivities to smoked cigarette butt (SCB) leachate.

Register found leachate from smoked cigarette filters (SF) (no tobacco) to be toxic to *D magna* between 1 and 2 cigarette butts/l (48-hour LC50).[2] Warne *et al* found daphnids to be more sensitive to leachate from smoked filters (SF) (no tobacco), as leachate was found to be toxic to *C* cf *dubia* at approximately 0.16 cigarette butts/l (48-hour EC50 (immobilisation)).[7] Compared to the current study, fish were found to be less sensitive to smoked filter (SF) leachate than daphnids in previous studies, with LC50s of 1.8 and 4.3 cigarette butts/l for the topsmelt and fathead minnow, respectively.

The reason for the greater sensitivity of daphnids to cigarette butt toxicity, compared to fish, is currently unknown, but may be due to the presence of nicotine and/or pesticide residues in cigarette butt leachates, or to metabolic differences between the species. Daphnids, specifically *Daphnia magna* and *Ceriodaphnia dubia*, are largely herbivorous and detritivorous and are known to be more susceptible to nicotine than fish.[12]

Potential causes of toxicity

Pesticides, potentially remaining in unsmoked cigarettes, may contribute to the toxicity of cigarette leachate. Daphnids may be more sensitive to pesticides than are fish, which would explain the observed greater sensitivity to cigarette leachate with

Table 1 Toxicity summary

Species	Cigarette butts/l USF	SF	SCB
Topsmelt LC50	5.1 (4.6–5.7)	1.8 (1.5–2.0)	1.1 (0.95–1.3)
Fathead minnow LC50	13.5 (11.4–15.9)	4.3 (3.7–5.1)	0.97 (0.84–1.1)
Daphnid (*D magna*)* LC50	>16	1.0–2.0	0.125–0.25†
Daphnid (*C* cf *dubia)*‡ EC50 (immobilisation)	NA	NA	0.03–0.08 (0.02–0.12)
Daphnid (*C* cf *dubia*)§ EC50 (immobilisation)	1.7 (1.4–2.06)	0.16 (0.09–0.27)	0.06 (0.05–0.08)
Marine bacterium (*V fischeri*)‡ EC50 (bioluminescence)	NA	NA	0.3–2.7 (0.3–3.5)
Marine bacterium (*V fischeri*)§ EC50 (bioluminescence)	> 970	1.25 (1.21–1.33)	0.58 (0.53–0.63)

LC50 and EC50 values (with 95% CIs) for leachates from unsmoked cigarette filters (USF) (no tobacco), smoked cigarette filters (SF) (no tobacco), and smoked cigarette butts (SCB) (smoked filter + tobacco).
*Courtesy: Register.[2] No CIs reported.
†Test utilised smoked cigarette tobacco (no filter).
‡Courtesy: Micevska *et al*.[8] 95% fiducial limits reported.
§Courtesy: Warne *et al*.[7]

daphnids, compared to fish. A US Government Accountability Office report discusses the use of pesticides on cigarette tobacco crops.[13] The US EPA regulates which specific pesticides may be used on tobacco crops, as well as how they are used, but it does not regulate pesticide residues on tobacco, as is required for human foods and animal feed crops. The USDA, however, has found that some imported and domestic tobacco exceeds current residue limits considered safe for human health and environmental effects.[13] A 2006 study performed by Dane *et al* also found three previously undetected pesticides (flumetralin, pendimethalin and trifluralin) in both mainstream and sidestream cigarette smoke, which could also be retained by the cigarette filters causing toxicity to aquatic organisms as they leach out of the cigarette butts.[14]

Micevska *et al* conducted toxicity identification evaluations (TIEs) on smoked cigarette butt leachates and found that nicotine and ethylphenol may play significant roles in causing the toxicity observed in daphnids and marine bacteria.[8] Nicotine is an antiherbivore chemical derived from the tobacco plant *Nicotiana* sp and it has commonly been used as an insecticide.[15] It has also been reported that ethylphenol is commonly used in the tobacco industry as a tobacco flavouring agent and is present in cigarette smoke.[16–18] Ethylphenol has been shown to be capable of biocentration in aquatic organisms.[19]

Chemical additives are often introduced to make tobacco products more attractive to consumers. For example, sugars and humectants make smoke milder and easier to inhale, humectants can prolong shelf life, ammonia may enhance the delivery of nicotine and menthol and eugenol effectively numb the throat.[5] In fact, approximately 600 additives were in use by major American cigarette companies in 1994.[20] Many of these chemicals may be harmful to humans as a result of smoking. The major humectants used for cigarettes are glycerol, diethylene glycol and/or propylene glycol which may be carcinogenic to humans.[4] However, little is known about the fate of such additives in cigarette butt leachates.

There are several chemicals in an unsmoked cigarette filter that may contribute to aquatic toxicity. The filter of a filter-tipped cigarette is composed of cellulose acetate fibres.[21] These fibres, each approximately 20 μm in diameter, are treated with titanium dioxide (a delustrant) and over 15 000 of them are packed tightly together, using triacetin (glycerol triacetate) as a binding agent, to create a single filter.[22 23] Most cigarette filters are surrounded by two layers of paper and/or rayon wrapping, which contain chemicals, such as glues to hold the paper together, and alkali metal salts of organic acids (eg, sodium acetate) in order to maintain burning while the cigarette is being smoked.[22] It is also possible that cigarette filters attached to tobacco absorb toxicants from the adjacent unsmoked tobacco column; however, this has not been investigated in the literature.

Toxicity of leachate from smoked cigarette butts, smoked filters and unsmoked filters

Both fish species exhibited statistically different concentration-responses to the different cigarette leachates, as reported by the p values in figures 1 and 2. For both fish species, the toxicity increased significantly from unsmoked filters (USF) to smoked filters (SF) to smoked cigarette butts (SCB). These findings are consistent with findings published by Register and Warne *et al*, who also found a progressive increase in toxicity from USF to SF to SCB.[2 7] Although, it has been shown that less than 2% of the quantity of all elements in cigarette tobacco and paper adsorb onto the filter as a result of smoking, our results show that the chemicals solely in the smoked filter still exert considerable toxicity to fish.[24] However, the remnant tobacco of the cigarette butt contributed a degree of toxicity (to both topsmelt and fathead minnows) significantly ($p<0.05$) greater than that conferred by chemicals trapped and leached from the smoked filter itself. Remnant tobacco comprised unburned tobacco as well as a burnt tobacco tip and including such remnant tobacco effectively exacerbated toxicity. The chemicals in smoked cigarette butts (SCB) may be significantly greater and different from those retained within the smoked filter (SF) itself; the former may contain additional toxic products of combustion. Chemicals in smoked versus unsmoked cigarette butts may not only contribute differently to toxicity, but also may have different fates and/or potential for bioaccumulation in the environment.

Despite the gathering evidence on the toxicity of cigarette butt leachates to various organisms, it is difficult to assess the risk that cigarette waste may have on the actual aquatic environment. Pathways of cigarette waste to aquatic environments are complex and varied. In 2002, a hazard assessment concluded that, while definitive quantification is still needed, it is likely that littered cigarette butts pose a low to moderate risk to aquatic organisms.[7] However, aside from toxicity, little is known about the specific chemicals, fate and bioaccumulation potential of such cigarette butt leachates, and the actual effects they may have on aquatic life. This study represents the first in the literature to show that cigarette butt leachate is toxic to representative marine and freshwater fish species. Additional research is

What this paper adds

- Cigarette butts are the most common form of litter in the world. Thousands of chemicals are present in a cigarette, the residues of which may be found in littered cigarette butts. Previous studies have shown chemicals in cigarette butt leachate can be acutely toxic to aquatic organisms; however, all previous studies used non-vertebrate species for testing.
- This study represents the first in the literature to show that leachate from cigarette butts is acutely toxic to representative marine and freshwater fish species. Leachates from smoked cigarette butts with remnant tobacco were significantly more toxic to fish than the smoked filters alone, but even unsmoked filters exhibited a small level of toxicity.

necessary to explore the actual risks that cigarettes pose to freshwater and marine environments.

CONCLUSION

- Smoked cigarette butts (SCB) (smoked filter + tobacco), smoked cigarette filters (SF) (no tobacco) and unsmoked cigarette filters (USF) (no tobacco) were all found to be acutely toxic to representative marine and freshwater fish.
- Remnant tobacco was found to contribute a degree of toxicity above that which was conferred by the smoked filter alone.
- Fish were found to be less sensitive to cigarette butt leachate than daphnids previously tested, but to have a similar sensitivity as marine bacteria.
- Smoking was found to increase the toxicity of cigarette filters.

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