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Journal

Chemical Research in Toxicology, 36(11)

ISSN

0893-228X

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Publication Date

2023-11-20

DOI

10.1021/acs.chemrestox.3c00167

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Leached Compounds from Smoked Cigarettes and Their Potential for Bioaccumulation in Rainbow Trout (*Oncorhynchus mykiss*)

Published as part of the Chemical Research in Toxicology virtual special issue "Mass Spectrometry Advances for Environmental and Human Health".

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Cite This: *Chem. Res. Toxicol.* 2023, 36, 1703−1710

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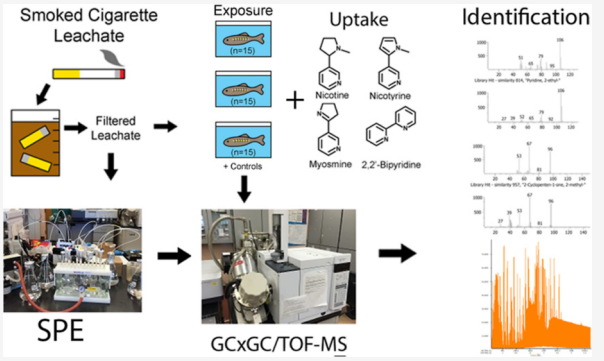
ABSTRACT: Cigarette butts are one of the most prevalent forms of litter worldwide and may leach toxic compounds when deposited in aquatic environments. Previous studies demonstrated that smoked cigarette leachate is toxic toward aquatic organisms. However, the specific bioavailable chemicals from the leachate and the potential for human and wildlife exposure through the food chain were unknown. Using a nontargeted analytical approach based on GC×GC/TOF-MS, 43 compounds were confirmed to leach from smoked cigarettes when exposed to a water source. Additionally, the bioaccumulation potential of organic contaminants in an edible fish, rainbow trout (*Oncorhynchus mykiss*), was assessed through direct exposure to the leachate of smoked cigarettes at 0.5 CB/L for 28 days. There was a significant reduction in fish mass among the exposed rainbow trout vs the control group (χ^2 (1) = 5.3, p = 0.021). Both nontargeted and targeted chemical analysis of representative fish tissue identified four tobacco alkaloids, nicotine, nicotyrine, myosmine, and 2,2′-bipyridine. Their average tissue concentrations were 466, 55.4, 94.1, and 70.8 ng/g, respectively. This study identifies leached compounds from smoked cigarettes and demonstrates the uptake of specific chemicals in rainbow trout, thus suggesting a potential for accumulation in food webs, resulting in human and wildlife exposure.



INTRODUCTION

Discarded cigarette butts are one of the most prevalent forms of marine litter worldwide. They are consistently the most collected item during the Ocean Conservancy's annual International Coastal Cleanup day.[1] Cigarette butts are also highly persistent as their cellulose acetate filters have been shown to take up to 10 years to degrade under various environmental conditions.[2] Tobacco smoke contains over 7000 chemical constituents, including polycyclic aromatic hydrocarbons, benzene, formaldehyde, aromatic amines, and metals.[3,4] Many of these compounds become trapped in the cellulose acetate filter as the cigarette is smoked, and when exposed to water, they create a toxic leachate.[3,5,6]

To date, little research has focused on the identification of leachable compounds found in discarded cigarette butts. Many studies attempting to do so have focused on the leaching of metals and have successfully identified As, Pb, Cd, Cu, Ni, Cr, Co, Al, Mn, Zn, Hg, and Fe as environmental contaminants found in smoked cigarette leachate.[7−11] Additionally, nicotine and cotinine have been measured in the influent waters of wastewater treatment plants, as well as in surface waters of rivers and lakes.[12−14] However, considering the vast number of chemicals present in tobacco and tobacco smoke, it is reasonable to assume that many more compounds leach from discarded cigarette butts than what has been previously identified.

Identification of leachable compounds is particularly important as prior research has demonstrated toxic responses to smoked cigarette leachate in aquatic life. Slaughter et al., 2011, determined an LC50 of 1 cigarette butt/L water (CB/L) for freshwater fathead minnows and saltwater topsmelt.[5] Other studies observed a 48 h EC50 for immobilization of 0.06 CB/L in *Ceriodaphnia dubia* and 1−2 CB/L in *Daphnia magna*.[15,16] A concentration of 5 CB/L was found to have a 100% mortality rate in three species of tide pool snails after 8 days of exposure.[17] While less is known regarding the effects of smoked cigarette leachate exposure on humans, Xu et al., 2019,

Received: June 8, 2023
Published: October 12, 2023

ACS Publications

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observed activation of the aryl hydrocarbon receptor, estrogen receptor, and p53 response pathways in vitro.[18] Little research, however, has addressed the bioaccumulative potential of compounds found in smoked cigarette leachate. Wright et al., 2015, examined the potential for bioaccumulation of nicotine in the marine invertebrate *Hediste diversicolor* (ragworm). After 96 h of exposure to 8 CB/L smoked cigarette leachate, a nicotine concentration of 119,654 ng/g tissue was observed.[19] Recently, Santos-Echeandía et al., 2021, observed the uptake of metals in oysters after 7 days of exposure to smoked cigarette leachate. However, metal concentrations in the oyster tissue significantly decreased after a 7 day decontamination period.[20]

The identification of previously unknown contaminants is key to accurately assessing risk and guiding future research. To determine the major compounds from smoked cigarette leachate, we used a nontargeted analytical technique based on comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC/TOF-MS). GC×GC/TOF-MS-based nontargeted analysis has been successfully implemented in order to identify known and unknown contaminants in a wide variety of sample matrixes such as human breastmilk,[21] waste and stormwater, and wildlife.[22−29] Additionally, nontargeted analysis has been implemented in conjunction with toxicological studies in order to identify potentially toxic compounds.[30] Furthermore, the United States Environmental Protection Agency (EPA) nontargeted analysis collaborative trial (ENTACT) demonstrated the power of GC-based nontargeted analysis, where GC-based methods correctly identified 809 substances and LC-based methods correctly identified 801 (539 by ESI+ and 262 by ESI−).[31]

The specific aims of this study were to (1) identify leachable organic compounds in freshwater leachate of smoked cigarettes and (2) assess whether these organic compounds have the potential to bioaccumulate in rainbow trout. We selected the fish for relevance to not only the aquatic food chain but also potential human exposure via direct consumption.

MATERIALS AND METHODS

Smoked Cigarette Leachate Preparation. Marlboro Red cigarettes (Philip Morris, Richmond, VA, USA) were machine-smoked at the University of California, San Francisco, using a TE-10z Smoking Machine (Teague Enterprises, Woodland, CA, USA) and following ISO Standard 3308:2000. The procedures of making the smoked cigarette leachate is published elsewhere[18] and are described in the Supporting Information and Figure S1. Marlboro Red cigarettes were selected due to Marlboro being the most popular cigarette brand in the United States, accounting for 40% of the total market share.[32]

Smoked Cigarette Leachate Extraction. Solid phase extraction (SPE) using OASIS HLB cartridges (Waters Corporation, Milford. MA, USA) were used for sample preparation. Cartridges were cleaned with 5 mL of dichloromethane and 5 mL of acetone prior to conditioning the cartridges. Next, cartridges were conditioned with 5 mL of methanol and 15 mL of LC/MS grade water. Then, 10 mL of leachate at 10 CB/L was loaded into the cartridges and vacuumed slowly at 1 to 2 drops per second. Cartridges were subsequently washed with 5 mL of LC/MS grade water and vacuumed until dry. The droplets were discarded as waste. Next, cartridges were eluted with 5 mL of acetone and 5 mL of dichloromethane. The extract was then treated with 5 g of sodium sulfate and ran through additional UCT Enviro-Clean (United Chemical Technologies, Bristol, PA) glass cartridges containing 1500 mg of sodium sulfate to remove any residual water. Finally, the extract was concentrated to 1 mL by a TurboVap (Zymark Corporation, Hopkinton, MA, USA) nitrogen gas concentrator in a warm water bath at 40 °C, resulting in a final extract concentration of 100 CB/L. Samples were prepared in triplicate. Additionally, triplicate blanks were prepared using the same source water that had not been exposed to cigarette butt leachate.

Assay for Fish Exposure to Cigarette Butt Leachate. Juvenile rainbow trout (*Oncorhynchus mykiss*; Thomas Fish Company, Anderson, California) aged between 30 and 60 days were randomly selected and weighed approximately 0.5 g each. Control and dilution water were 1:1 (v/v) dechlorinated tap water/deionized water. Full-scale leachate exposures were conducted with concentrations determined by the range finding study described below. The method followed the guidelines in the Organization for Economic Co-operation and Development (OECD) test no. 305: Bioaccumulation in Fish: Aqueous and Dietary Exposure,[33] with the exception of sampling for the analyte concentration during uptake because this study focused on identification of compounds accumulated in the fish.

The following specifications were common to both the rangefinder and definitive tests. Trout chow, 1−2% of body weight, was fed daily, and excess food was removed if present 1 h after feeding. Water quality measurements were taken daily to maintain pH (6.5−8.0), dissolved O_2 (8.5−10 mg/L), conductivity (350−400 μmhos/cm), and temperature (15 °C). Water hardness (80−100 mg/L $CaCO_3$), alkalinity, and total ammonia measurements (0.5−2.4 mg/L) were taken at test initiation and termination. Total ammonia was also measured at day 7, 14, and 21. 80% water renewals were conducted every other day, and continuous, light aeration was applied to all test chambers (1−2 bubbles per second).

Rangefinder Test. A 28 day exposure rangefinder test was conducted to determine the maximum aqueous cigarette butt concentration that did not induce significant mortality or behavioral changes. Testing concentrations of 0.25, 0.5, and 1.0 and 2.0 CB/L were made by serial dilution of the leachate stock, and the control was 0 CB/L freshwater. Exposures were performed in triplicate glass aquaria, each aquarium containing two rainbow trout in 800 mL of the respective freshwater leachate testing concentration. The rangefinder test, with the data summarized in Table S1, established a maximum concentration of 0.5 CB/L.

Definitive Test. Test concentrations of 0 CB/L (control) and 0.5 CB/L were used for the 28 day definitive exposure tests. At day zero (D0), 10 individual juvenile fish were randomly selected, sacrificed using tricaine methanesulfonate (MS-222, Sigma-Aldrich, St. Louis, MO, USA), and the pre-exposure wet weight was measured (Table S2). Both control and exposure groups were run in triplicate glass aquaria, with each aquarium containing 15 rainbow trout in 8 L of the water. In order to ensure measurement of only what has accumulated in the tissue and not the residual material in the gut, a depuration period was implemented. Following the exposure period, the rainbow trout were placed in sanitized test chambers containing control freshwater for 48 h and were not fed. The fish were then removed, paper towel-blotted, weighed, individually packaged in glass jars, and stored at −20 °C until analysis.

Biological Response. A linear mixed effect analysis of the association between measured individual rainbow trout weights (g) and exposure level from the 28 day definitive exposure test was performed using R and package *lme4*.[34,35] The exposure level (either control or 0.5 CB/L) was treated as a fixed effect, and the test chamber (n = 3 per exposure level) was treated as a random effect (random slope and intercept). The statistical significance of the effect of exposure (p-value) was obtained using a likelihood ratio test of the model including the exposure effect against the model without the exposure effect.[36] Visual inspections of the plot of fitted values vs residuals and the Q−Q plot of the residuals did not reveal consequential deviations from homoscedasticity or normality, respectively.

Chemical Analysis Materials. Prior to use, all glassware was baked at 450 °C for 6 h. All solvents were of GC pesticide residue analysis grade or higher (Fisher Scientific, Fair Lawn, NJ, USA). Sources of the internal standards, recovery standards, and analysts are described in the Supporting Information.

https://doi.org/10.1021/acs.chemrestox.3c00167
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Fish Sample Preparation. Following the definitive test, approximately 6−8 fish were randomly selected from each of three exposure aquaria and three control aquaria and were homogenized with a mortar and pestle. Approximately 5 g of the sample was combined with anhydrous sodium sulfate, placed in a 50 mL glass centrifuge tube, spiked with 400 ng of each internal standard, and equilibrated at 5 °C for 30 min. Next, 12 mL of acetone and 12 mL of hexane were added, samples were sonicated for 40 min at 40 °C, then centrifuged for 5 min at 3000 rpm. 10% of the resulting extract was transferred to aluminum pans, dried, and the percent lipid was determined. The remaining extract was concentrated to 1 mL by nitrogen blowdown (TurboVap, Zymark Corporation, Hopkinton, MA, USA), and lipids were removed by the automatic gel permeation chromatography (GPC) system (J2 Scientific, Columbia, MO, USA). The extract was spiked with 400 ng of each recovery standard, and the final extract volume was brought to 400 μL.

Chemical Analysis. Samples were analyzed by Pegasus 4D GC×GC/TOF-MS (LECO, St. Joseph, MI, USA), with the instrumental conditions provided in Table S3. Data were processed by LECO ChromaTOF software (version 4.50.8.0) using a signal-to-noise ratio (S/N) of 100. Isolation of compounds on interest was performed in the same manner for both smoked cigarette leachate and fish tissue samples. To determine the compounds unique to the sample group, first LECO's ChromaTOF add-in software, "statistical compare" was used to align chromatographic peaks across sample groups based retention time and mass spectral similarity. As described in Figure S2, compounds were included if they met the following criteria: (1) present in all three sample replicates and (2) either absent from the control group, or if present, the chromatographic peak abundance in the sample group was ≥3 times the abundance in the control group. Following the criteria described in studies by Xu et al., 2019, and Chang et al., 2021, compounds were tentatively identified using the 2014 National Institute of Standards and Technology's (NIST) Mass Spectral Library and were confirmed using corresponding authentic standards.[18,26] This criteria was used for both the cigarette leachate and fish tissue nontargeted analysis. Additionally, concentrations of the confirmed compounds present in fish tissue were determined using 4-point calibration curves constructed from the unlabeled target compounds and corresponding internal standards (nicotine with nicotine-d4 and the others with cotinine-d3).

The confirmed compounds present in fish tissue were also quantified in smoked cigarette leachate. For this, samples were prepared in a similar fashion to the fish tissue extracts. 3 mL of 10 CB/L leachate was placed in a 50 mL glass centrifuge tube and 3 g of sodium sulfate, 2 mL of 1:1 of dichloromethane/hexane, and 400 ng of each internal standard was added. Three replicates of the leachate mixture were analyzed, along with a 3 mL LC/MS-grade water laboratory blank. The samples were shaken for 5 min, vortexed for 1 min, and centrifuged at 3000 rpm for 15 min. The organic phase was removed and stored at −20 °C until quantified using the procedure described for the tissue extracts.

RESULTS AND DISCUSSION

Leachate Analysis. Analysis of smoked cigarette leachate yielded a total of 722 unique compounds. Reference standards were purchased for 58 compounds, in which 43 compounds were confirmed while 15 did not match the suspected compound due to differences in GC retention time. A list of confirmed compounds can be found in Table 1. Of the 43 confirmed compounds, all but phenyl carbamate have been previously identified in tobacco or tobacco smoke.[37] To the best of our knowledge, the presence of phenyl carbamate as it relates to tobacco is unclear. However, phenyl carbamate-derived pesticides, such as isopropyl-*N*-phenyl carbamate (propham) and isopropyl (3-chlorophenyl) carbamate (chlorpham), are used during the cultivation of tobacco plants.[38,39] Recently, King et al., 2021 confirmed the presence of nicotine, cotinine, nornicotyrine, and myosmine in marine sediment exposed to smoked cigarette leachate. Additionally, King et al. 2021 identified 2,3′-dipyridyl via mass spectral matching with

Table 1. 43 Compounds Confirmed to be Present in Smoked Cigarette Leachate[a]

name	CASRN	peak area percent	area rank (1−722)
nicotine	54−11−5	25.18	1
diacetin	25,395-31-7	12.95	2
triacetin	102-76-1	1.88	3
anatabine	2743-90-0	1.34	5
cotinine	486-56-6	0.64	8
myosmine	532-12-7	0.41	15
anabasine	2743-90-0	0.37	17
2-cyclopenten-1-one, 2,3-dimethyl-	1121-05-7	0.32	21
2,6-dimethylpyrazine	108-50-9	0.29	22
2-cyclopenten-1-one, 2-methyl-	1120-73-6	0.28	24
2-furanmethanol	98-00-0	0.27	28
phenol	108-95-2	0.27	30
2-methylpyrazine	109-08-0	0.26	31
*phenyl carbamate**	622-46-8	0.24	32
benzenepropanenitrile	645-59-0	0.24	33
m-cresol	108-39-4	0.23	35
2-cyclopenten-1-one, 3-methyl-	2758-18-1	0.19	36
nicotyrine	487-19-4	0.18	37
2,3′-dipyridyl	581-50-0	0.18	38
2-cyclopenten-1-one, 2-hydroxy-3-methyl-	80-71-7	0.15	41
pantolactone	599-04-2	0.12	43
2-methylindole	95-20-5	0.12	46
2-cyclohexenone	930-68-7	0.09	48
benzonitrile	100-47-0	0.09	53
2-methylpyridine	109-06-8	0.09	66
ethosuximide	77-67-8	0.09	68
2,3,5-trimethylpyrazine	14,667-55-1	0.08	69
4-oxoisophorone	1125-21-9	0.08	75
acetophenone	98-86-2	0.08	78
2(5H)-furanone, 3-methyl-	22,122-36-7	0.06	90
isoquinoline	119-65-3	0.06	92
2,3-dimethylpyrazine	5910-89-4	0.06	96
4-ethylphenol	123-07-9	0.06	98
2-ethylpyrazine	13,925-00-3	0.05	106
1-indone	83-33-0	0.04	108
3-methylpentanoic acid	105-43-1	0.04	113
N-methylsuccinimide	1121-07-9	0.04	124
quinoline	91-22-5	0.02	130
3-ethylpyridine	536-78-7	0.02	140
3-pyridinol, 2-methyl-	1121-25-1	0.01	233
4-cyclopentene-1,3-dione	930-60-9	0.01	265
6-methyl-3,5-heptadiene-2-one	1604-28-0	0.01	357
2-furancarboxaldehyde, 5-methyl-	620-02-0	0.01	516

[a]Compounds are listed in order of average peak area abundance, from most abundant to least abundant. Peak area percentage was calculated by dividing the compounds peak area by the sum total peak area of all 722 compounds isolated during analysis. Area rank describes the rank order of each compound relative to all 722 compounds isolated during analysis.* Indicates that the compound does not have a known tobacco-related source in the literature. Compound names written in italics denote aromaticity of the compound.

https://doi.org/10.1021/acs.chemrestox.3c00167
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the NIST Mass Spectral Library but were unable to obtain an authentic standard for confirmation. The findings of King et al., 2021, corroborate the findings of this study, in which nicotine, cotinine, nornicotyrine, myosmine, and 2,3′-dipyridyl were confirmed to be present in the smoked cigarette leachate.[40]

In terms of the structure, 63% of confirmed compounds are aromatic. While individual compounds were not quantified in smoked cigarette leachate, as this analysis was intended to be qualitative in nature, chromatographic peak area was used to observe the abundance of compounds relative to each other. To do so, the average peak area of each individual compound was summed in order to obtain the total chromatographic peak area. While 722 individual compounds were observed, the top 25 most abundant compounds comprised nearly 80% of the total chromatographic peak area, while the top 3 most abundant compounds, nicotine, diacetin, and triacetin, comprise nearly 63% of the total chromatographic peak area. As several compounds were much more abundant in smoked cigarette leachate than others, it would be reasonable to assume that these compounds are the most likely candidates to be found at detectable levels in the environment.

Of the identified compounds, nicotine; triacetin; phenol; 2-methylpyridine; m-cresol; acetophenone; benzonitrile; 1-indone; 4-oxoisophorone; 2-furanmethanol; 2-cyclohexenone; 2-cyclopenten-1-one, 2,3-dimethyl-; 2-cyclopenten-1-one, 2-methyl-; and isoquinoline have been previously identified in rivers and bays.[41−49] Additionally, cotinine and myosmine have been identified as photochemical degradation products of nicotine in wastewater treatment plant effluent water.[50] The results of our analysis indicate that discarded cigarette butts are a point of entry into the environment for these previously observed pollutants.

Fish Exposure to Cigarette Butt Leachate. There was a significant effect of exposure on fish body mass [χ^2 (1) = 5.3, p = 0.021] that lowered individual fish body mass by 0.13 g ± 0.050 standard errors, corresponding to a mean percent decrease of 18%. Note the random effect of the test chamber was not statistically significant, as determined by linear models of fish mass as a function of the test chamber for the control [F(1,40) = 3.9, p = 0.057] and exposure [F(1,41) = 0.12, p = 0.73] groups (Figure 1 with data in Table S4). The reduced weight of the exposed rainbow trout may be attributable to the 28 day leachate exposure. Wright et al., 2015, reported a significant decrease in the relative growth rate (−33% mean weight ±2% standard error of the mean.) of marine ragworms exposed for 96 h to a leachate concentration of 8 cigarette filters/L.[19] Similarly, sea urchin larvae (*plutei*) exposed to cigarette butt leachate show a reduction in body size in comparison to unexposed *plutei*.[51] Other studies reported a reduction in earthworm mass exposed to imidacloprid, a neonicotinoid insecticide structurally similar to nicotine. It was hypothesized that the reduced mass was attributable to decreased feeding, reduced assimilation efficiency, or the implementation of an energetically unfavorable detoxification pathway.[52,53]

Identified Chemicals in Exposed Rainbow Trout. Nicotine, nicotyrine, myosmine, and 2,2′-bipyridine were identified as unique to the 0.5 CB/L sample group, and their presence was confirmed using authentic standards. Initially, only nicotine and nicotyrine were identified using the nontargeted analysis criteria described above (Figure S2). Since both compounds are tobacco alkaloids, we hypothesized that other tobacco alkaloids might be present at low abundance. We manually searched the GC×GC/TOF-MS data for the 28 primary tobacco alkaloids (nicotine-related compounds) and related isomers identified previously in the smoked cigarette leachate.[54] Myosmine and 2,2′-bipyridine were found to have been initially excluded since they were below a S/N of 100 in all three triplicate samples.

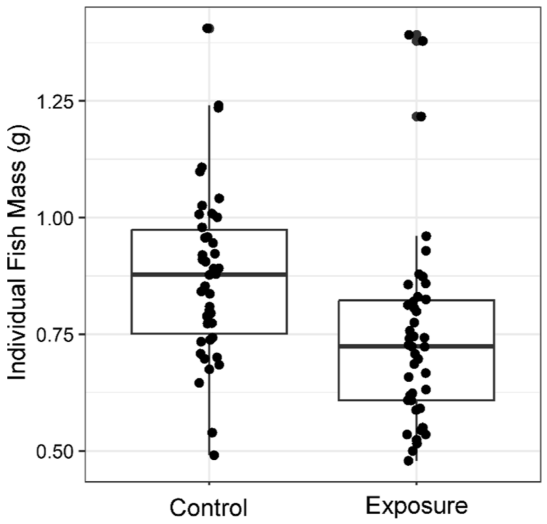

Figure 1. Control and exposed (0.5 CB/L) rainbow trout masses from the 28 day cigarette leachate definitive exposure test, χ^2 (1) = 5.3, p = 0.021 (see the main text). Bold horizontal lines correspond to the median, boxes correspond to the interquartile range (IQR), and whiskers flag potential outliers and extend to the smallest and largest values that are <1.5 × IQR from the 25th and 75th percentiles, respectively.

Compound Concentrations. Tissue concentrations and aqueous concentrations in the leachate for the four compounds (nicotine, nicotyrine, myosmine, and 2,2′-bipyridine) identified in the rainbow trout are listed in Table 2. Aqueous concentrations were quantified in the 10 CB/L leachate solution (n = 3, reported in Table S5), then estimated concentrations were determined for the 0.5 CB/L leachate used for the exposure experiment. Although detected in the fish tissue, 2,2′-bipyridine was nondetected at 10 CB/L. Therefore, we used 1/2 LOQ of bipyridine to estimate its concentration at 0.5 CB/L. In results, the rank-ordered concentrations in the aqueous leachate were nicotine > myosmine > nicotyrine >2,2′-bipyridine, while the rank ordered concentrations in the fish were nicotine > myosmine >2,2′-bipyridine > nicotyrine.

Although this experiment did not focus on the measurement of the bioconcentration factor (BCF), the EPA's CompTox Chemical Dashboard was used to predict BCF values from their chemical structure,[55] as shown in Table 2. Interestingly, nicotine was the most abundant, and myosmine was the second most abundant in the fish tissue and leachate, but their estimated BCF values from this study and predicated studies were relatively low. This suggests that their bioaccumulation potential is relatively lower compared to 2,2′-bipyridine and nicotyrine.

It is important to note that metabolism may be a contributing factor to bioaccumulation potential for myosmine and nicotyrine because both compounds are known nicotine metabolites via the cytochrome P450 pathway[56−60] (Figure 2) and therefore have the potential to bioaccumulate with or without direct exposure.

https://doi.org/10.1021/acs.chemrestox.3c00167
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Table 2. Concentration and Bioconcentration Factors of the Four Chemicals Identified in Cigarette Leachate and Exposed (0.5 CB/L) Fish Tissue[a]

compound name	testing concentration (CB/L)	average concentration per tissue weight (ng/g)	average concentration per lipid weight (ng/g)	average concentration in 0.5 CB/L leachate (ng/mL)[b]	comp tox predicted BCF median (range)	comp tox experimental or predicted log K_{ow} median (range)
nicotine	0.5	466 (±114)	97,707 (±20,539)	1727 (±21)	6.52 (4.46-12.0)	1.17 (experimental)
nicotyrine		55.4 (±12.6)	11,605 (±2228)	0.657 (±0.090)	34.4 (5.78-5730)	1.78 (0.840-2.22) (predicted)
myosmine		94.1 (±5.2)	19,825 (±910)	6.54 (±0.10)	3.08	0.817 (predicted)
2,2′-bipyridine		70.8 (±3.8)	14,941 (±1123)	0.133[c]	39.7 (5.37-115)	1.50 (experimental)
all compounds	0.0 (lab control)	nd	nd	nd		

[a]For each average concentration, $n = 3$. Standard deviation is provided in parentheses, and nd = non-detect. Predicted BCF values from the USEPA CompTox Chemicals Dashboard models. If multiple models were available, the median BCF and log K_{ow} value and range are shown, otherwise the single value is provided. CompTox provided either experimental or predicted log K_{ow} values, as described. [b]The 0.5 CB/L leachate concentrations were estimated using the analyte's concentration measured at 10 CB/L. [c]Estimated based on 1/2 LOQ.

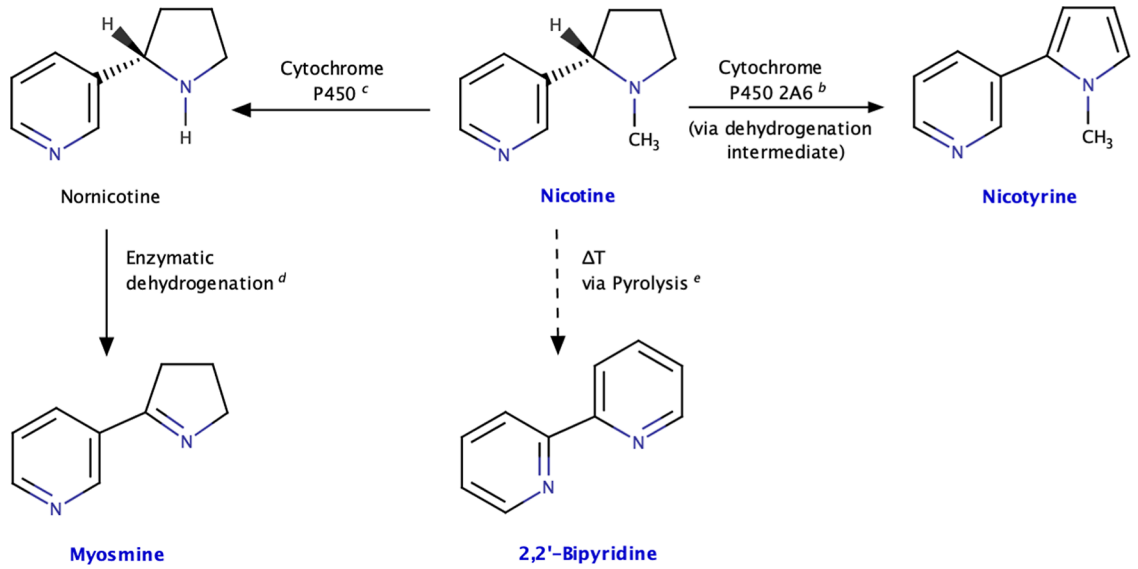

Figure 2. Overview diagram of the four organic tobacco alkaloids confirmed in the rainbow trout tissue following 28 day definitive exposure to 0.5 CB/L leachate. (a) Structures sourced from CompTox (USEPA, 2020).[65] (b) Pathway source: (Kramlinger et al., 2012, 2013).[66,67] (c) Pathway source: (Hukkanen et al., 2005).[68] (d) Pathway source: (Bush et al., 1993; Leete and Chedekel, 1974).[69,70] (e) Pathway source: (Schmeltz and Hoffmann, 1977).[71]

Few studies have previously explored the potential of chemicals associated with discarded smoked cigarettes to bioaccumulate in aquatic species. Wright et al., 2015, observed the bioaccumulation of nicotine in the polychaete worm *H. diversicolor* (ragworm). The authors reported an average nicotine tissue concentration of 1901 ng/g at a 0.5 CB/L exposure level, while this study reports an average nicotine tissue concentration of 466 ng/g at the same exposure level.[19] However, there are differences in the study designs, as well as species differences in physiology and metabolism. The rainbow trout is a vertebrate and utilizes a liver to regulate uptake, absorption, and excretion of environmental contaminants, while the marine worm lacks a liver. The presence (or absence) of a liver greatly affects an organism's ability to metabolize nicotine (or other contaminants) and is vital in reducing toxicity.[61] The lack of a liver may reduce the ragworm's ability to excrete nicotine, leading to a higher tissue concentration compared to the rainbow trout.

Another distinction is the nicotine concentration measured in the 0.5 CB/L leachate of the two studies. We measured an average nicotine concentration of 1727 ng/mL, while Wright et al., 2015, determined a concentration of 23.5 ng/mL. The difference may be attributed to the method for producing the leachate. We used machine-smoked cigarette butts in their entirety, including the outer paper, filter, and any tobacco remnant. Wright et al., 2015, removed the outer paper and any excess tobacco prior to leachate production. The nicotine content leached by the included tobacco remnant likely accounts for the difference in aqueous nicotine concentrations.

To the best of our knowledge, no other studies have examined the bioaccumulative potential of chemicals associated with smoked cigarette leachate via nontargeted analysis in edible fish. The nontargeted analysis enabled identification of the major leachate contaminants, nicotyrine and nicotine, in rainbow trout. Following a reverse search for other tobacco alkaloids, we identified two additional compounds, myosmine and 2,2′-bipyridine. Their presence in smoked cigarette leachate warrants further investigation as myosmine has been shown to exhibit sublethal effects in human and animal studies.[62] Nicotyrine inhibits nicotine metabolism and reduces its clearance rate.[63] Further investigation of 2,2′-bipyridine in terms of bioaccumulative potential may be of particular interest as it was undetectable in smoked cigarette leachate, yet was found in all fish tissue samples in the exposure group,

https://doi.org/10.1021/acs.chemrestox.3c00167
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suggesting a high BCF. Nicotine and its metabolites have been frequently detected in wastewater treatment plant influents and effluents and may therefore be pseudopersistent in the environment and are bioavailable.[64]

Additionally, bioconcentration of chemicals from smoked cigarette leachate may vary among fish species and other aquatic organisms. Further investigation is necessary using different fish species exposed to smoked cigarette leachate, to measure transfer through food webs, and to further assess the toxicity of the tobacco alkaloid contaminants. Nevertheless, these findings contribute to a growing body of research that confirms the potential for exposure and subsequent toxicity of discarded tobacco product waste to animals and potentially to humans. According to the environmental precautionary principle, such exposure may be justifiably prevented through environmental policy even without obvious large-scale adverse human health outcomes.[2]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.chemrestox.3c00167.

> Chemical analysis materials, smoked cigarette leachate preparation, data analysis flowchart, rainbow trout 28 day cigarette butt leachate exposure rangefinder test, D0 rainbow trout weight data, GC×GC/TOF-MS instrument conditions, rainbow trout 28 day cigarette butt leachate exposure definitive test, organic compound/tobacco alkaloid concentrations in 10 CB/L leachate, and estimated concentrations in 0.5 CB/L leachate (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This project was funded by the California Tobacco Related Disease Research Program (TRDRP: 23RT-0014H and T30IP0974CHS). We especially acknowledge the late TRDRP program officer Anwer Mujeeb for his encouragement and support of tobacco product waste research.

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