TOXICITY OF CIGARETTE BUTTS AND THEIR CHEMICAL COMPONENTS TO THE MARINE AND FRESHWATER FISHES, ATHERINOPS AFFINIS AND PIMEPHALES PROMELAS

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ABSTRACT OF THE THESIS

Toxicity of Cigarette Butts and their Chemical Components to the Marine and Freshwater Fishes, *Atherinops affinis* and *Pimephales promelas*

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Master of Public Health with a concentration in Environmental Health

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Cigarette butts are the most common form of litter worldwide, as an estimated 4.5 trillion cigarette butts are deposited somewhere into the environment every year. With cigarette consumption on the rise globally, along with the increasing popularity of bans on indoor smoking, the global environmental burden of cigarette waste may increase in the years to come. Many chemical products are used during the course of growing tobacco and manufacturing cigarettes, the residues of which may be found in cigarettes consumed and therefore in the butts discarded. These include pesticides, herbicides, insecticides, fungicides, and rodenticides. Additionally, over 4,000 chemicals may also be introduced to the ambient environment via combusted cigarette particulate matter (tar) and mainstream smoke. These include chemicals such as carbon monoxide, hydrogen cyanide, nitrogen oxides, polycyclic aromatic hydrocarbons, ammonia, acetaldehyde, formaldehyde, benzene, phenol, argon, pyridines, and acetone, over fifty of which are known to be carcinogenic to humans. Furthermore, chemicals such as arsenic, nicotine, polycyclic aromatic hydrocarbons, and heavy metals have been found to leach into the environment from cigarette butts littered along roadsides and in laboratory studies.

Using standard acute fish bioassays, cigarette butts were analyzed for aquatic toxicity. The LC50 for leachate from smoked cigarette butts (with remnant tobacco intact) was approximately 1.1 cigarette butts/L for both the marine topsmelt (*Atherinops affinis*) and the freshwater fathead minnow (*Pimephales promelas*). Leachate from smoked cigarette filters (remnant tobacco removed), was less toxic, with LC50 values of 4.1 and 5.5 cigarette butts/L, respectively for both fish species. Lastly, 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. Consequently, toxicity of cigarette butt leachate was found to increase with smoking the cigarette, and again with leaving remnant burnt tobacco intact. Additionally, the marine topsmelt was found to be more sensitive to most cigarette butt leachates than was the freshwater fathead minnow. This study represents the first in the literature to investigate the toxicity of cigarette butts to fish, and will assist in assessing the potential ecological risks of cigarette butts to the aquatic environment.

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CHAPTER 1

INTRODUCTION

Littered cigarettes are ubiquitous in the environment, and with global cigarette consumption currently on the rise, the global environmental burden of cigarette litter could become greatly exacerbated. Aquatic ecosystems, such as shorelines and waterways, may be most vulnerable, as the majority of land-based litter is ultimately deposited in aquatic environments. Cigarettes contain thousands of chemicals, many of which are known to be carcinogenic to humans, and are capable of leaching from littered cigarettes. Due to the chemical composition of cigarettes, and the magnitude at which they are deposited into the environment as waste, it is important to study the potential for cigarette waste to exert ecotoxic effects on aquatic environments. This study seeks to gain a better understanding of the impact cigarette waste may have on marine and freshwater environments by performing acute toxicity bioassays with marine and freshwater fish.

1.1 Background on Cigarette Litter

The global environmental burden of cigarette litter is significant, as an estimated 4.5 trillion cigarettes are littered every year (Litter Free Planet, 2009). In fact, cigarette butts are the most common form of litter in the world and are the most prevalent debris item collected along waterways during the Ocean Conservancy's International Coastal Cleanups, a status which has been maintained since 1990 (Table 1.1) (Ocean Conservancy, 2010). In the United States, an estimated 360 billion cigarettes were consumed in 2007 (United States Department of Agriculture [USDA], 2007). Of course, not all cigarette butts are littered, but it has been estimated that 1 in every 3 cigarettes are discarded as waste (City of Tacoma, 2010). Given that the weight of 20 cigarette filters is 0.12 ounces (Register, 2000), the waste from U.S. consumption in 2007 alone would weigh at least 45 million pounds. This figure accounts for weight of the filter alone and does not include weight of remnant tobacco. Furthermore, cigarette butts comprise an estimated 30% of the total litter (by count) along U.S. shorelines, waterways, and on land (Litter Free Planet, 2009), and Keep America Beautiful reported that

Table 1.1. Top Ten Marine Debris Items

Rank	Debris Item	Number of Debris Items	Percentage of Total Debris Items
1	cigarettes/cigarette filters	2,189,252	21%
2	bags (plastic)	1,126,774	11%
3	food wrappers/containers	943,233	9%
4	caps, lids	912,246	9%
5	beverage bottles (plastic)	883,737	9%
6	cups, plates, forks, knives, spoons	512,517	5%
7	beverage bottles (glass)	459,531	4%
8	beverage cans	457,631	4%
9	straws, stirrers	412,940	4%
10	bags (paper)	331,476	3%
Top Ten Total Debris Items		8,229,337	80%
Total Debris Items Worldwide		10,239,538	100%

80% of all marine debris originates from land-based sources (2010). Given the vast quantity of cigarette waste, and that the majority of it ends up in aquatic ecosystems, the impact it may have on these systems is of great concern.

Bans on indoor smoking may exacerbate outdoor cigarette butt litter. Residents, business owners, and politicians all reported an increase in the quantity of cigarette butts littered after bans on indoor smoking took effect in local cities (Andrews, 2004; KHBS, 2004; Niolet, 2010). For example, Keep Britain Tidy released a study in 2007 stating that there has been a 43% increase in the number of littered cigarettes attributable to a recent ban on indoor smoking. In response to the environmental impact of cigarette butt litter, several policy changes to aid in the mitigation of cigarette butt waste have been offered. Those suggested by Novotny, Lum, Smith, Wang, & Barnes (2009) include the addition of a warning label on a pack of cigarettes that informs the smoker of the non-biodegradability of filters. This study also suggested a deposit/return strategy, wherein the consumer pays a deposit when a pack of cigarettes is purchased and that deposit is refunded when the pack is returned with the cigarette butts. Other suggestions are to implement a cigarette butt waste litter fee to fund environmental cleanup and/or research on cigarette butt waste, litigation that holds cigarette companies responsible for environmental impacts of their products, fines on cigarette manufacturers that are proportional to the amount of cigarette waste found, mandatory filter biodegradability, banning disposable filters, and consumer education and responsibility (Novotny et al., 2009).

1.2 Composition and Evolution of Cigarettes

A typical discarded cigarette butt consists of three components: unsmoked remnant tobacco (including a partially smoked/charred tobacco tip), the filter of a filtered cigarette, and a paper wrap. Each of these components of the discarded cigarette butt presents its own concern.

1.2.1 Tobacco

Cultivated tobacco, *Nicotiana tabacum*, is a member of the nightshade family (Solanaceae) and is grown in over 100 countries for tobacco products. Approximately 80% of all tobacco grown worldwide is used to produce cigarettes (Food and Agriculture Organization [FAO], 1990). China is the world's largest producer of tobacco, while India, Brazil, and the U.S. are also quite prominent (Table 1.2) (FAO, 2004). There are many chemicals in unburned processed tobacco, approximately 23 of which have been identified as carcinogenic (International Agency for Research on Cancer [IARC], 1987, 1995). Some are naturally-occurring in the tobacco plant, others are agricultural chemical residues from growing the tobacco, while others still are additives and flavorings used in the cigarette manufacturing process. Analysis has found over 4,000 compounds to be the result of burning tobacco (Hoffmann & Hoffmann, 1997; Li, Banyasz, Parrish, Lyons-Hart, & Shafer, 2002).

Table 1.2. Leaf Production '000 Tonnes

	Actual 2000	Projected 2010
World	6,138	7,160
China	2,299	2,972
India	595	685
Brazil	521	585
USA	408	527
EU (15)	315	301
Zimbabwe	205	233
Turkey	194	269
Indonesia	167	120
USSR (Former Area)	117	70
Malawi	108	138

1.2.2 Filters and Paper

Prior to the 1950s, few cigarettes (2%) were filtered (Borio, 2007). However, growing concerns over scientific evidence of the cause-effect relationship between smoking and deleterious diseases, such as lung cancer encouraged cigarette companies to manufacture and promote a ‘safer cigarette’ (United States Department of Health and Human Services [USDHHS], 1981). The cigarette was redesigned and a new filter was added to most cigarettes. Filters were found to absorb and remove vapors and various constituents of cigarette smoke, such as tar and nicotine, in machine-measured analyses (National Cancer Institute [NCI], 2001). These low-yield cigarettes were considered a significant advancement toward making a ‘safer cigarette’. Sales of filtered cigarettes escalated and by the 1960s approximately 50% of all cigarettes sold in the United States had filters (Borio, 2007). Despite evidence that smokers who switched to low-yield brands did not significantly decrease their exposure to tar and nicotine (NCI, 2001), filtered cigarettes continued to increase in popularity, now constituting 98% of the market (Medicine Online, 2007). The filter of a filter-tipped cigarette is comprised of cellulose acetate fibers (USDHHS, 1989), a plastic extremely slow to degrade in the environment, with degradation under ideal conditions estimated as upwards of 18 months (Ach, 1993). These fibers, each approximately 20 μ 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 (Norman, 1999; Pauly, Mapani, Lesses, Cummings, & Streck, 2002). Most cigarette filters are surrounded by two layers of paper and/or rayon wrapping, the porosity of which acts to control the amount of airflow through the filter. Regular cigarettes have less porous wraps to inhibit airflow, while light cigarettes have more porous wraps to allow for more airflow, which reduces smoke yields relative to regular cigarettes. Cigarette paper also contains many chemicals, including glues to hold the paper together and alkali metal salts of organic acids (e.g. sodium acetate) in order to maintain burning while the cigarette is being smoked (Norman, 1999).

1.3 Statement of the Problem

An estimated 5.6 trillion filtered cigarettes were consumed worldwide in 2002 and nine trillion are projected for 2025 (Mackay, Eriksen, & Shafey, 2006). With global cigarette consumption on the rise and an already estimated 4.5 trillion cigarettes littered every year worldwide (Litter Free Planet, 2009), the global environmental burden of cigarette litter could be greatly exacerbated in the coming years, unless there is change in the manner in which global populations dispose of cigarette butts. Keep America Beautiful reported that 18% of all litter is ultimately deposited in waterways (2010) and cigarette butts constitute an estimated 30% of the total litter (by count) along U.S. shorelines, waterways, and on land (Litter Free Planet, 2009). Due to the ubiquitous nature and magnitude of cigarette butts discharged into the environment, studies are needed to determine whether littered cigarette butts might exert ecotoxic effects when they enter aquatic environments.

1.4 Purpose of the Study

This study represents the first in the literature to investigate the toxicity of cigarette butts to marine and freshwater fishes, and will assist in assessing the potential ecological risks of cigarette butts to the aquatic environment. This research supports findings from previous studies that cigarette butts are toxic to aquatic organisms. Determining the toxicity of cigarette butts to fishes will assist in understanding the environmental burden of cigarette butt waste so that regulatory policies and approaches to disposal of cigarette butt waste may be better justified and designed.

1.5 Theoretical Bases and Organization

Cigarettes and cigarette smoke contain thousands of chemicals, many of which are known to be acutely toxic to animals and carcinogenic to humans. Cigarette filters are specially designed to absorb various harmful constituents of cigarette smoke. Were these chemicals capable of leaching out of cigarette filters, they may exert ecotoxic effects after entering aquatic environments. All previous studies used non-vertebrate species (i.e. daphnids) for testing the toxicity of cigarette butts in water, whereas similar studies investigating the toxicity to marine and freshwater fishers have not been performed. Fish are ecologically important organisms, and often used as bioindicators of healthy aquatic systems. Therefore, it is important to determine the toxicity of cigarette butt leachate to fish. In this study, we investigated the toxicity of cigarette butts (smoked filters with and without remnant tobacco, as well as unsmoked filters without tobacco) to the marine fish, the topsmelt (*Atherinops affinis*), and to the freshwater fish, the fathead minnow (*Pimephales promelas*) to better understand the impact of cigarette waste on both marine and freshwater ecosystems. Appendix A contains definitions of many technical terms presented.

1.6 LIMITATIONS OF THE STUDY

- Toxicity of cigarette butt leachate was analyzed with 2 species, both of which were fish, and no other similar research has been yet conducted with other species. Therefore, there are no other studies with which to directly compare our findings. To expand on this analysis, specific chemicals, known to occur in cigarettes (e.g. nicotine), were studied in the literature and, from that, some comparisons were possible.
- Cigarettes contain a vast array of chemicals. No single chemical was isolated for analysis in the cigarette butt leachate at this time. Future studies to identify specific toxins are planned.
- Cigarette butts were not quantified based upon weight, rather upon number alone. Cigarette weight would provide a more exact description of the concentration of cigarette butts (per liter of water) that were used to produce the cigarette leachate tested.
- Some tests were completed with artificially-smoked cigarettes and then repeated with naturally-smoked cigarettes. Consequently, there may be variability in the toxicity of the cigarette butt leachate due to the different methods of smoking.

CHAPTER 2

LITERATURE REVIEW

Information regarding the chemical composition and combustion products of cigarettes, as well as government regulation of tobacco, is detailed below. This study also researched previous studies that analyzed the toxicity of cigarette butts with aquatic bioassays.

2.1 Agricultural Residues, Additives, Flavorings and Government Regulation

Many chemicals in unburned cigarette tobacco are from products used during the course of growing tobacco and manufacturing cigarettes, the residues of which may be found in cigarettes prepared for consumption (Hoffmann & Hoffmann, 1997; Sheets, 1991). These include pesticides, herbicides, insecticides, fungicides, and rodenticides to control pests, as well as humectants to keep tobacco moist (Glantz, Slade, Bero, Hanauer, & Barnes, 1996). Natural herbs and spices, essential oils, and artificial flavors have also been added to cigarettes to curb or intensify the flavor of tobacco leaf (Triest, 1979). In 1994 a list of 599 flavoring ingredients, sugars, and processing aids used by 6 major U.S. tobacco companies was released to the public (Doull, Frawley, & George, 1994; R.J. Reynolds Tobacco Company, 1994; T.R. Staff Report, 1994). Although the maximum percentage used is reported, exact quantities of these chemicals in cigarette tobacco are still unknown. The vast majority of additives listed in the 1994 release were flavors commonly used by the food and beverage industries and declared “Generally Recognized as Safe” (GRAS) for use in food, either by the U.S. Food and Drug Administration (FDA) or by the Flavor and Extract Manufacturers’ Association (FEMA) (USDHHS, 2000). However, materials on this list are deemed safe when they are in a product that is eaten, not in one that is burned and inhaled as smoke. A material considered safe when eaten is not necessarily safe in other forms and while these additives may be considered safe, their combustion products may be toxic.

Additives have been reported to constitute 10% of the weight of the tobacco in a cigarette and 4% of the total weight of the cigarette (Register, 2000). Additives make cigarette smoke more palatable and appealing to the consumer. Humectants, for example, increase shelf life, and along with sugars, aid in the dissolution of nicotine into tar droplets, making smoke milder and easier to inhale. Diethylene glycol (DEG), commonly used as an automotive antifreeze, was introduced to cigarettes as a humectant in the 1930s (Glantz et al., 1996). This ingredient was used for approximately 50 years without contest and it was only after efforts from public relations in the 1980s that DEG was finally deemed not GRAS by FDA and FEMA. DEG was not included in the list of additives released by the tobacco industry in 1994 (R.J. Reynolds Tobacco Company, 1994), therefore we can conclude that it is no longer used. This sequence of events, however, contradicts the usual expectation for consumer products that safety is established *before* a product is used.

Flavorings and aromas are additives that impart variety and complexity to the cigarette. Cocoa, for example, is a flavor that has long been an ingredient in American blend cigarettes and has been shown to increase the carcinogenicity of cigarette smoke condensate (NCI, 1977; USDHHS, 1981; Roemer & Hackenberg, 1990). Other additives, such as ammonia compounds, increase the delivery of nicotine, and menthol and eugenol act as anesthetics, numbing the throat, making it easier to inhale cigarette smoke (Glantz et al., 1996). Once again, materials deemed “Generally Recognized as Safe” (GRAS) by the FDA or FEMA are done so by considering their use in food. The concern is that many additives form new compounds when they are burned, and while an additive may be safe in a product that is eaten, it may not be safe in a product that is burned and inhaled as smoke. These combustion products (or pyrolysis products) possess new properties and may be toxic even if their unburned counterparts are not.

Agricultural chemicals that may be found in cigarette tobacco include sucker control agents, rodenticides, fungicides, insecticides, and various other pesticides. Sucker control agents, such as MH-30 (maleic hydrazide) and Penar (dimethyldodeclamine acetate), curb the number of new shoots growing from the tobacco plant in order to keep the nicotine content of tobacco leaves high (Glantz et al., 1996). Pesticides prevent, destroy, or mitigate numerous pests. Pesticides are of concern because of the potential toxicity of their residues as well as inconsistent government regulation between countries. The United States Environmental Protection Agency (USEPA) regulates which specific pesticides may be used on tobacco crops, as well as how they are used (USEPA, 2010). They perform routine testing for the purpose of keeping this list of acceptable pesticides up-to-date. However, USEPA does not regulate pesticide residues on tobacco, as is required for human foods and animal feed crops. Rather, the United States Department of Agriculture (USDA), mainly for the purpose of trade equity, is responsible for regulating such tobacco pesticide residues (Dairy and Tobacco Adjustment Act, 1983). In this case, regulation is in the form of testing only for those pesticides that were *banned* for use in the United States in 1989 (these pesticides may be used in other countries from which the United States imports tobacco) as well as testing imported and domestic tobacco to ensure that acceptable pesticide residues have not exceeded maximum levels (United States Government Accountability Office [USGAO], 2003). Through testing, the USDA has found a small percentage of imported and domestic tobacco to exceed such residue limits. However, the USDA has not reevaluated nor updated their list of pesticides subject to regulation since 1989, even though pesticides used on tobacco have changed in the past 21 years and over 30 pesticides have been prohibited for tobacco use in the United States since 1989 (USGAO, 2003). Most of the pesticides the USDA regulates are organochlorine pesticides that persist in the environment and accumulate in the tissues of animals (USEPA, 2010). Several of these, such as DDT and toxaphene, have been banned in the United States prior to 1989. However, if a pesticide was *acceptable* before 1989, the USDA would not be required to test for residues of that pesticide. For example, the USEPA cancelled the use of lindane on all tobacco crops in 2000 (Kegley, Hill, Orme, & Choi, 2010). Lindane is a highly persistent organochlorine pesticide and is possibly still used in other countries. The USDA does not currently regulate pesticide residues of lindane because it was still approved for use on tobacco in 1989, the last time USDA evaluated the list of pesticides it would regulate.

Such agricultural chemicals have been found to be present in cigarette smoke. For example, Dane, Crystal, & Kent (2006) found three previously undetected pesticides (flumetralin, pendimethalin, and trifluralin) in both mainstream and sidestream cigarette smoke. Cigarette filters are theoretically designed to absorb various constituents of cigarette smoke, and if these harmful chemicals in cigarette tobacco are transferred to cigarette smoke, they could also be retained by cigarette filters. Pesticides are manufactured to effectively kill target organisms at relatively low doses, and were such chemicals to leach from cigarette filters, they could potentially impart toxicity to the aquatic environment.

2.2 Combustion Products of Tobacco

As stated earlier, the combustion products of tobacco, agricultural residues, flavorings and additives may be quite different and more harmful to human and environmental health than their unburned counterparts. The American Health Foundation (AHF, 1990; Glantz et al., 1996) has identified many cigarette tobacco additives to be potentially toxic when used under conditions of heating or burning. That is to say that the combustion products of these additives may lead to the formation of carcinogens. For example, the combustion products of amino acids and licorice root extract (containing glycyrrhizin) used as additives to cigarette tobacco are known to be carcinogenic (AHF, 1990; Glantz et al., 1996). In fact, over 4,000 chemicals may also be introduced to the ambient environment via the combustion of cigarette tobacco, over 50 of which are known to be carcinogenic to humans (Hoffmann & Hoffmann, 1997). Cigarette smoke is a complex mixture of gases, cigarette particulate matter (tar), and submicron-sized solid particles that are suspended in cigarette smoke (Harris, 1996). These include chemicals such as carbon monoxide, hydrogen cyanide, nitrogen oxide, benzene, formaldehyde, polycyclic aromatic hydrocarbons, ammonia, acetaldehyde, phenol, argon, pyridines, and acetone (Hoffmann & Hoffmann, 1997). Cigarette tar is a catch-all term for the particulate phase components of cigarette smoke, except for water and alkaloid compounds such as nicotine (Center for Disease Control and Prevention [CDC], 2010) and is comprised of organic and inorganic compounds, many of which are carcinogenic (Harris, 1996).

2.3 Environmental Contamination from Cigarette Butt Litter

A study performed by Moriwaki, Kitajima, & Katahira (2009) found that arsenic, nicotine, polycyclic aromatic hydrocarbons (PAHs), and heavy metals are released into the environment by littered "roadside waste" cigarette butts. Roadside waste was collected in a Japanese suburb over a four month period, and the distribution, quantity, and types of waste were studied, as well as the environmental loading of pollutants from roadside waste. Overall, 690 different items were littered per month, the most common of which were cigarette butts at an average rate of 150 cigarette butts/km/mo. To analyze the roadside waste cigarettes, ICP atomic emission microscopy was used for heavy metals, LC/MS was used to measure nicotine, and PAHs were quantified with an HPLC/fluorescence detector. Arsenic and nicotine were eluted from roadside waste cigarette butts at concentrations of 0.041 mg/L and 3.8 mg/L, respectively. Environmental loading of heavy metals, such as lead, copper, chromium, and cadmium, as well as PAHs from littered cigarette butts, was also confirmed (Table 3.1) (Moriwaki et al., 2009).

Table 3.1. Content of Polyaromatic Hydrocarbons (PAHs) in Roadside Waste Cigarette Butts and Roadside Soil

PAHs	Concentration (mg/kg wet)		Load potential (mg/km/month)[1]
	Cigarette butts	Roadside soil	
Fluorene	0.028	0.01	0.0023
Phenanthrene	0.078	0.14	0.0063
Anthracene	0.071	0.0058	0.00057
Pyrene	0.091	0.36	0.0074
Benzo(*a*)anthracene	0.026	0.084	0.0021
Chrysene	0.044	0.11	0.0035
Benzo(*b*)fluoranthene	0.031	0.088	0.0025
Benzo(*k*)fluoranthene	0.015	0.055	0.0012
Benzo(*a*)pyrene	0.031	0.12	0.0025
Dibenzo(*a,h*)anthracene	0.0065	0.016	0.00053
Benzo(*g,h,i*)perylne	0.031	0.093	0.0025
total	0.39	1.1	0.032

[1] Values of load potential were calculated using the quantity of cigarette butts per month, concentration of PAHs, and length of sampling course (3.2 km).

2.4 Cigarette Butt Leachate Bioassays

Previous studies have shown chemicals that leach from cigarette butts can be acutely toxic to aquatic organisms (Micevska, Warne, Pablo, & Patra, 2006; Register, 2000; Warne, Patra, Cole, & Lunau, 2002). Register (2000) followed the USEPA's 1996 "Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids" protocol to perform toxicity bioassays of cigarette butts. Cigarette butt leachate was prepared by allowing cigarette butts to soak in deionized water for one hour. Register (2000) 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 greater than 16 cigarette butts/L (48-hr LC50), respectively. This test took place over a 48-hour period, and survival was the single endpoint.

Warne et al. (2002) prepared cigarette butt leachate by placing cigarette butts in water and shaking for one hour. Leachates from smoked cigarette butts, smoked cigarette filters, and unsmoked cigarette tobacco were found to be acutely toxic to the freshwater cladoceran *Ceriodaphnia* cf. *dubia* at 0.05, 0.15, and 1.7 cigarette butts/L, respectively (48-hr EC50 (immobilization)), and to the marine bacterium *Vibrio fischeri*, at 0.6, 1.25, and greater than 970 cigarette butts/L, respectively (30-min EC50 (bioluminescence)). *Ceriodaphnia* cf. *dubia* is a species that conforms (cf.) with, but is not identical to, the US species *Ceriodaphnia dubia*. This test took place over a 48-hour period and the sub-lethal effect, immobilization, was the single endpoint. The assay of *V. fischeri* took place over a 30 minute period and the sub-lethal effect, bioluminescence, was the single endpoint.

Lastly, Micevska et al. (2006) followed USEPA 1993c protocols to perform daphnid bioassays and New South Whales Environmental Protection Agency (NSWEPA) 2001 protocols to complete bacterium bioassays. Cigarette butt leachate was prepared by shaking cigarette butts in water for 24 hours. Smoked cigarette butt leachate from nineteen different brands of smoked cigarette butts were found to be toxic to *Ceriodaphnia* cf. *dubia* at concentrations between 8.9 and 25.9 mg butts/L (units of current study 0.03 – 0.08 butts/L) (48-hr EC50 (immobilization)) and to *Vibrio fischeri* at concentrations between 104 and 832 mg butts/L (units of current study 0.3 – 2.7 butts/L) (30-min EC50 (bioluminescence)). This study also completed Toxicity Identification Evaluation (TIE) phase I and preliminary phase II tests using USEPA (1991, 1993a, 1993b) protocols. Nicotine and ethylphenol were identified as the most likely causative toxicants in cigarette butt leachate. However, the concentrations of these chemicals in the leachate were not measured.

2.5 NICOTINE

Approximately 0.6 – 3.0% of tobacco (by dry weight) is comprised of nicotine (Hoffmann & Hoffmann, 1998). Tobacco has also commonly been used as an organic pesticide due to the toxic effects of nicotine it contains (Rodgman & Perfetti, 2008). In fact, nicotine has been used as a pesticide since the 15th century and became popular in the United States in the 1940s and 50s. However, popularity has dwindled and it has recently been requested that the last nicotine pesticide product in the United States have its registration canceled (USEPA, 2008). Research has shown nicotine to be extremely dangerous to human health. Nicotine can be absorbed by the body through several routes including the skin, lung, gastrointestinal tract, and oral cavity (Yildiz, 2004) and is known to be acutely toxic to animals and humans (Karaconji, 2005). Furthermore, nicotine is extremely addictive as it interacts with specific receptors in the brain and initiates metabolic and electrical activity (USDHHS, 1988). An average cigarette yields approximately 1 – 2.3 mg of absorbed nicotine (Benowitz & Jacob, 1984; Benowitz, Jacob, Denaro, & Jenkins, 1991) and, in this low concentration, acts as a stimulant in mammals and is the main determinant responsible for tobacco dependence. Furthermore, nicotine content in cigarettes has shown to have increased over the years, as a study conducted in 2007 found an average increase in cigarette nicotine content of 1.6% per year between 1998 and 2005 (Connolly, Alpert, Wayne, & Koh, 2007).

2.6 Ethylphenol

Ethylphenol is used in many ways. In the tobacco industry, it is used as a flavoring agent. In other industrial sectors, it is used in the production of resins, varnishes and rubber, and is an intermediate for pharmaceuticals and dyes (Hazardous Substances Data Bank [HSDB], 2009). It has been reported that ethylphenol is present in cigarette smoke (Clark & Bunch, 1996) and contributes to the smokey taste in cocoa powder (Bonvehi & Coll, 1998), a common flavoring used in cigarette tobacco (R.J. Reynolds Tobacco Company, 1994; Triest, 1979). It is capable of biocentration in aquatic organisms (HSDB, 2009), but does not appear to be a significant toxicant in the limited number of toxicological studies completed (Florin, Rutberg, Curvall, & Enzell 1980; Hoechst Celanese Corporation, 1986; Takahashi et al., 2006; Thompson, Perera, & London, 1995). Thompson et al. (1995) identified an LC50 for ethylphenol, using rat liver microsomes, at 150 mg/L. This is considered to be a relatively high concentration of ethylphenol (Rayne & Eggers, 2007) and the concentration of ethylphenol in mainstream cigarette smoke has been found to range between 0.98 – 1.90 mg/cigarette for 4-ethylphenol and 2.67 – 6.30 mg/cigarette for 3-ethylphenol (Clark & Bunch, 1996). Although the concentration of ethylphenol in mainstream smoke of a single cigarette is less than the LC50 for ethylphenol, this does not eliminate ethylphenol as a potential toxicant in cigarette butts. Cellulose acetate, the major component of cigarette filters, has shown to effectively remove phenols from cigarette smoke (Baggett & Morie, 1973; George & Keith, 1967; Hoffmann & Wynder, 1963; Spears, 1963). Consequently, ethylphenol may be present in the cigarette filter at much higher concentrations than is present in cigarette smoke.

CHAPTER 3

METHODOLOGY

This study followed the United States EPA standards to complete aquatic toxicity testing of cigarette butt leachates with marine and freshwater fishes, *Atherinops affinis* and *Pimephales promelas*.

3.1 Design of the Investigation

Toxicity tests on marine and freshwater fish were performed with three different cigarette leachates:

1. Leachate from smoked cigarette butts with 1 – 2 cm of remnant tobacco intact. This test was performed twice, once with artificially-smoked cigarettes and again with naturally-smoked cigarettes;
2. Leachate from smoked cigarette filters, 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, without tobacco. This test was performed once.

Four different brands of test cigarettes (Camel, Kool, Marlboro, and American Spirits) were purchased new and artificially-smoked to control for variability and to decrease the risk of contamination from external sources. Cigarettes were smoked at the University of California, San Francisco, according to ISO Standard 3308:2000 using a TE10z smoking machine (Teague Enterprises, 530-406-88931237 E. Beamer, Suite E Woodland, California 95776). 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 persons, extinguished in cigarette disposal units, and collected within 24 hours of deposition.

To produce the highest leachate concentration, cigarette butts were allowed to soak in dilution water (diluted mineral water for freshwater tests and natural seawater for marine tests), prepared according to USEPA protocol (USEPA, 2002), for 24 hours. Diluted mineral water consisted of eight parts nanopure deionized water for every two parts Perrier sparkling mineral water. Following overnight aeration, the mixture 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 bioassay lab. Seawater was held in a flow-through system with a 20-µm in-line fiber filter and chiller unit. A 0.5x dilution series was then performed to obtain subsequent lower concentrations. Concentrations for the smoked cigarette butts (with remnant tobacco) tests were 4, 2, 1, 0.5, 0.25, 0.125 cigarette butts/L. Concentrations for the smoked cigarette filters (without tobacco) tests were 8, 4, 2, 1, 0.5, 0.25, 0.125 cigarette butts/L. Concentrations for the unsmoked cigarette filters (no remnant tobacco) tests were 16, 8, 4, 2, 1, 0.5 cigarette butts/L. All tests were run with laboratory controls comprised solely of clean dilution water of either seawater or diluted mineral water.

3.2 Test Species

Toxicity tests were performed with the marine fish, the topsmelt (*Atherinops Affinis*), and the freshwater fish, the fathead minnow (*Pimphales promelas*), both of which are EPA-accepted species for acute toxicity testing. Institutional Animal Care and Use Committee (IACUC) animal subjects approval can be viewed in Appendix B. The fathead minnow is widely distributed across North America and is tolerant to a variety of water chemistry conditions. The topsmelt is common along the coast of Southern California and, due to their tolerance of varying levels of salinity, may be found in both marine and estuarine environments. Fish were provided by Aquatic Bio Systems in Fort Collins, Colorado, specializing in the culturing of organisms for aquatic toxicity testing. Fish were received two to three days prior to test initiation to allow for acclimation. Once fish were received, they were immediately transferred to a clean aquarium where they received two water renewals and feedings per day as well as continuous light aeration. Water quality parameters (DO, temperature, pH, and salinity/conductivity) were measured on a daily basis to ensure a controlled environment. Fish received a light cycle of sixteen hours light and eight hours dark. Any fish that died during this acclimation period were immediately removed from the aquarium. At initiation, fathead minnows were 12 – 14 days old and topsmelt were 7 – 15 days old.

3.3 TREATMENT

Toxicity test methods followed standardized protocols published in "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms" (USEPA, 2002). There were 4 replicates for every concentration, each replicate containing five fish, for a total of 20 fish per concentration. Tests using smoked cigarette butts (with remnant tobacco) and unsmoked cigarette filters (no tobacco) had six leachate concentrations plus a laboratory control; therefore 140 fish were used for these tests. Tests with smoked cigarette filters (no remnant tobacco) were comprised of seven leachate concentrations plus a laboratory control, resulting in 160 fish used for these tests. Each replicate consisted of five fish, placed in a plastic test chamber, filled with 250 ml of water. Fish were fed *Artemia* (brine shrimp) prior to initiation and again after 48 hours of testing. All tests received continuous light aeration, with a light cycle of sixteen hours of light and eight hours darkness. Water quality parameters (pH, conductivity, salinity, dissolved oxygen, and temperature) and survival counts were recorded on a daily basis, until test termination at 96 hours, to ensure a controlled environment. Conductivity/salinity and pH were measured and recorded to ensure that they stayed relatively consistent among days. Water was to have a DO content between 6 and 9 mg/L at initiation and at the 48-hour renewal (described below), and was never allowed to fall below 4 mg/L during testing. Temperature was to remain between 20±1°C for marine 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 meter to measure temperature and conductivity/salinity. All tests received a water renewal at 48 hours of testing, that involved siphoning off 80% of the water from each test chamber (200 ml) and then replacing it with 200 ml of new water of the corresponding concentration. Water quality parameters were measured and recorded for renewal water prior to performing the renewal to ensure a controlled environment.

3.4 DATA ANALYSIS PROCEDURES

Survival was the single endpoint evaluated, and data were analyzed to identify the median lethal effect concentration (LC50), the concentration of cigarette butt leachate resulting in 50% mortality. Mean survival in the laboratory controls must be 90% or greater in order to be deemed acceptable. LC50 values were determined with the Trimmed Spearman-Kärber method (USEPA, 2002), using Comprehensive Environmental Toxicity Information System (CETIS) v1.6.3revE, Tidepool Scientific Software. To determine whether there were statistically significant ($p < 0.05$) differences in the toxicity of cigarette butt leachates, dose-response curves were compared with an F test (Motulsky & Christopoulos, 2003) using Prism version 4.02, GraphPad Software, Inc.

CHAPTER 4

RESULTS

Data were analyzed to identify the LC50 for each cigarette butt leachate tested.

4.1 TOXICITY OF LEACHATE FROM SMOKED CIGARETTE BUTTS

Leachate from smoked cigarette butts, with 1 – 2 cm of tobacco intact, was found to be acutely toxic to both the saltwater topsmelt *(Atherinops affinis)* and the freshwater fathead minnow *(Pimephales promelas)*. An LC50 of 1.1 cigarette butts/L of water was obtained for both species. The dose-response curve for the topsmelt is shown in Figure 4.1 and for the fathead minnow in Figure 4.2. 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 dose-response curves for this test were not found to be statistically different ($p > 0.05$) when comparing artificially-smoked versus naturally-smoked cigarette leachates for both fish species (see Appendix C, Figures C.1 and C.2).

4.2 TOXICITY OF LEACHATE FROM SMOKED CIGARETTE FILTERS

Leachate from smoked cigarette filters, with all remnant tobacco removed, was also found to be acutely toxic to topsmelt at the concentration of 4.1 cigarette butts/L (Figure 4.1), and to fathead minnows at 5.5 cigarette butts/L (Figure 4.2). These findings are consistent with findings published in Register (2000), who found an approximate 4 to 5-fold increase in toxicity from smoked cigarette butt leachate (with remnant tobacco) as compared to smoked cigarette filter leachate (no remnant tobacco). The toxicity tests for smoked cigarettes with all remnant tobacco removed were performed three times, once with artificially-smoked cigarettes and twice with naturally-smoked cigarettes. The different methods of smoking the cigarette yielded different results. Dose-response curves for both species were found to be statistically different ($p < 0.05$) when comparing artificially-smoked

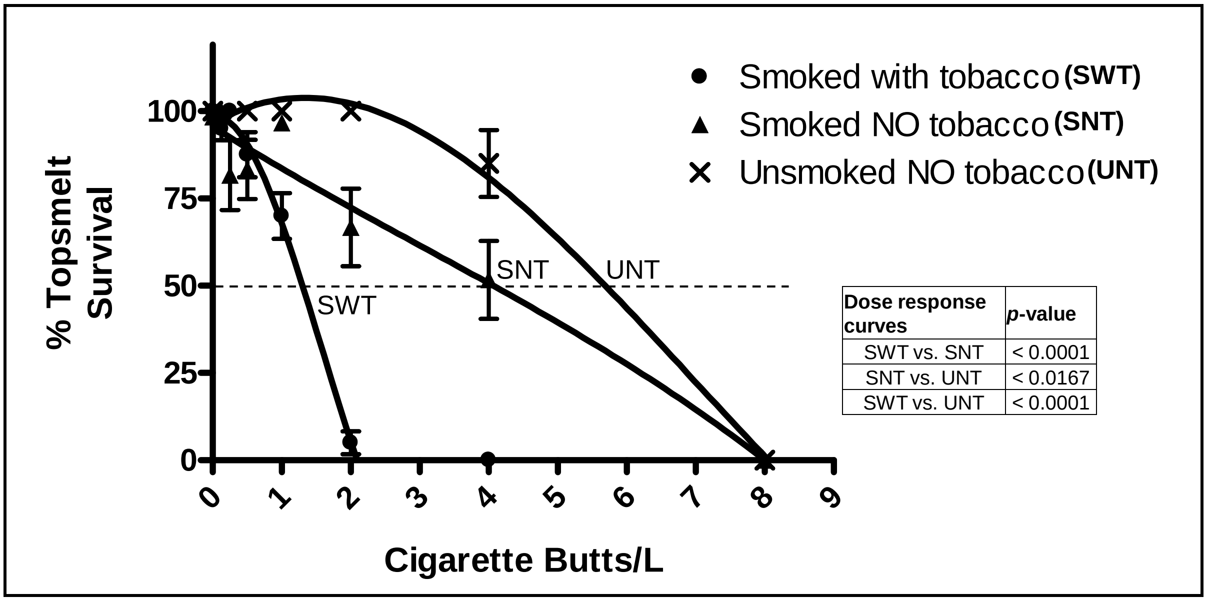

Figure 4.1. Dose-response curves for topsmelt (*Atherinops affinis*). Error bars represent one standard error of the mean. Dose-response curves are significantly different ($p < 0.05$).

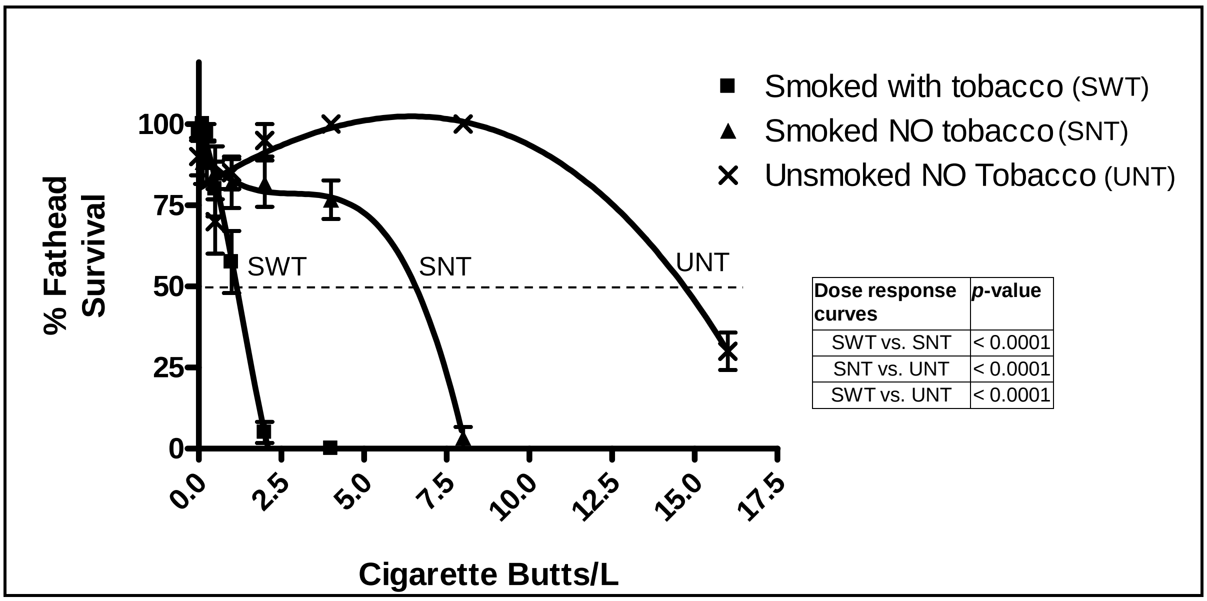

Figure 4.2. Dose-response curves for the fathead minnow (*Pimephales promelas*). Error bars represent one standard error of the mean. Dose-response curves are significantly different ($p < 0.05$).

versus naturally-smoked cigarette leachates (Figures C.3 and C.4). Both fish species exhibited a greater toxic response to the artificially-smoked cigarette leachate than to the naturally-smoked cigarette leachate. The reasons for the discrepancy between artificially-smoked and naturally-smoked cigarette leachates with this test are unclear.

4.3 TOXICITY OF LEACHATE FROM UNSMOKED CIGARETTE FILTERS

Leachate from unsmoked cigarette filters, with all remnant tobacco removed, was found to be acutely toxic to both fish species with an LC50 value of 5.1 for the topsmelt (Figure 4.1) and 13.5 cigarette butts/L for the fathead minnow (Figure 4.2).

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CHAPTER 5

DISCUSSION

Implications of the findings and comparisons to previous research are detailed below.

5.1 SMOKED CIGARETTES

Toxicity of leachates from smoked cigarettes are discussed and compared.

5.1.1 Smoked Cigarette Butts (with Remnant Tobacco)

Table 5.1 summarizes the LC50s and EC50 identified for smoked cigarette butt leachate (with remnant tobacco). Results of a previous study found that smoked cigarette butt 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 (Micevska et al., 2006). Given that the mean weight of a single smoked cigarette butt used in this study was approximately 310 mg (Micevska et al., 2006), it can be calculated that smoked cigarette butt leachate was found to be acutely toxic to daphnids at between 0.03 and 0.08 cigarette butts/L (48-hr EC50 (immobilization)). A study conducted by Warne et al. (2002) supports this finding, as a similar EC50 (48-hr (immobilization)) of 0.05 cigarette butts/L was identified, using the same test species, but only testing the smoked cigarette tobacco (no filter). However, a study conducted by Register (2000) 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-hr LC50). In comparison, our current study found smoked cigarette butt leachate to be less toxic to topsmelt and fathead minnows, than to daphnids tested in previous studies, as the LC50 (96-hr) for fish was identified as 1.1 cigarette butts/L.

5.1.2 Smoked Cigarette Filters (No Remnant Tobacco)

Table 5.1 summarizes the LC50s and EC50 identified for smoked cigarette filter leachate (no remnant tobacco). Register (2000) found leachate from smoked cigarette filters (no remnant tobacco) to be toxic to *D. magna* between 1 and 2 cigarette butts/L

Table 5.1. Toxicity Summary: LC50 and EC50 Values for Leachates from Unsmoked Cigarette Filters (No Tobacco), Smoked Cigarette Filters (No Remnant Tobacco), and Smoked Cigarette Butts (with Remnant Tobacco)

	Cigarette Butts/L		
Species	Unsmoked; No Tobacco	Smoked; No Tobacco	Smoked; With Tobacco
Topsmelt LC50	5.1	4.1	1.1
Fathead minnow LC50	13.5	5.5	1.1
Daphnid (*D. magna*)[1] LC50	> 16	1.0 – 2.0	0.125 – 0.25
Daphnid (*C.* cf *dubia)*[2] EC50	N/A	N/A	0.03 – 0.08
Daphnid (*C.* cf *dubia*)[3] EC50	1.70	0.15	0.05

[1] Courtesy: Register (2000)
[2] Courtesy: Micevska et al. (2006)
[3] Courtesy: Warne et al. (2002)

(48-hr LC50). Warne et al. (2002) found daphnids to be more sensitive to leachate from smoked cigarette filters (no remnant tobacco), as leachate was found to be toxic to *C.* cf. *dubia* at approximately 0.15 cigarette butts/L (48-hr EC50 (immobilization)). Compared to the current study, fish were found to be less sensitive to the leachate than daphnids in previous studies, with LC50s of 4.1 and 5.5 cigarette butts/L for the topsmelt and fathead minnow, respectively.

5.1.3 Differing Sensitivities to Pesticides and Metals

The reason for the greater sensitivity of daphnids to smoked cigarette butt leachate, as 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. Pesticide residues remaining in unsmoked tobacco may help explain the decreased fish sensitivity to cigarette leachate, as compared to daphnids. Pesticides are manufactured to effectively kill target organisms (i.e. insects) at relatively low doses, so as to be rendered harmless to organisms that are not targeted (e.g. fishes). In effect, pesticides are expected to be more toxic to water fleas (i.e. *D. magna* and *C. dubia*) than to fish (*P. promelas* and *A. affinis*) (Maki, 1979). A 2006 study performed by Dane et al. confirmed that pesticides occur in cigarette smoke and found three previously undectected pesticides (flumetralin, pendimethalin, and trifluralin) in both mainstream and sidestream cigarette smoke. This finding supports the possibility that pesticides could also be retained by the cigarette filter.

Were such pesticides to leach out of cigarette filters, they have shown they would be more toxic to daphnids than to fishes (Maki, 1979). Furthermore, Micevska et al. (2006), who conducted toxicity identification evaluations (TIEs) on cigarette butt leachates, found that nicotine may play a significant role in causing the toxicity observed in daphnids. Nicotine is an antiherbivore chemical derived from the tobacco plant *Nicotiana* sp. It has commonly been used as an insecticide (Rodgman & Perfetti, 2008). Daphnids, specifically *D. magna* and *C.* cf. *dubia*, are largely herbivorous and detritivorous and have shown to be more susceptible to nicotine than fishes (Konar, 1977). Consequently, it is possible that the difference in sensitivity to cigarette leachate observed between daphnids in previous studies and fish in the current study may be due to the presence of nicotine, as well as to the residues of other pesticides, in cigarette leachate.

Metals have also been identified as a potential agent causing toxicity in cigarette leachate (Micevska et al., 2006). LeBlanc (1984) found that for eleven metals tested, daphnids were considerably more sensitive than fishes. Additionally, when comparing the chronic toxicities of chemicals to *D. magna* and *P. promelas* to those observed in a previous study (Maki, 1979) results were found to be similar, as daphnids were found to be much more sensitive to the chronic toxic effects of metals than fishes. This may explain why the acute toxicity of cigarette butt leachate was observed at lower concentrations in previous studies for *D. magna* (Register, 2000) and *C.*cf. *dubia* (Micevska et al., 2006; Warne et al., 2002) compared to the current study analyzing fish toxicity.

5.1.4 Chemical Additives

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 alone can prolong shelf life, ammonia may enhance the delivery of nicotine, and menthol and eugenol effectively numb the throat (Glantz et al., 1996). In fact, 599 additives were in use by major American cigarette companies in 1994 (Doull et al., 1994; R.J. Reynolds Tobacco Company, 1994; T.R. Staff Report, 1994). While these chemicals may be deemed GRAS, their combustion products may not, and many of these chemicals may be harmful to humans when smoked. For example, the major humectants used for cigarettes are glycerol, diethylene glycol, and/or propylene glycol, and may be carcinogenic to humans

(Hoffmann & Hoffmann, 1997). Little is known about the environmental fate of such additives found in cigarette butt leachates and how they may impact aquatic organisms. Given that many cigarette additives are carcinogenic to humans, it may follow that they are also toxic to aquatic organisms. Chemical additives are a significant component of cigarettes, as they comprise approximately 10% of the cigarette by weight (Keithly, Wayne, Cullen, & Connolly, 2005), and could possibly serve to impart toxicity to aquatic organisms.

5.2 Toxicity of Leachate from Unsmoked Cigarette Filters (No Tobacco)

Table 5.1 summarizes the LC50s and EC50 identified for unsmoked cigarette filter leachate (no tobacco). One surprising result of our study was that leachate from unsmoked cigarette filters, with all remnant tobacco removed, was found to be acutely toxic to both topsmelt and fathead minnows. With LC50 values of 5.1 and 13.5 for topsmelt and fathead minnows, respectively, fish exhibited sensitivity to the leachate that was in line with daphnids in previous studies. Warne et al. (2002) found leachates from unsmoked cigarette filters to be acutely toxic to the freshwater cladoceran *Ceriodaphnia* cf. *dubia* at 1.7 cigarette butts/L (48-hr EC50 (immobilization)) and Register (2000) found leachates from unsmoked cigarette filters to be acutely toxic to the freshwater cladoceran *Daphnia magna* at higher concentrations of >16 cigarette butts/L (48-hr LC50).

5.3 Comparison of Topsmelt and Fathead Minnow

Topsmelt exhibited more sensitivity to the leachate than fathead minnows. The greater hardness of saltwater, compared to freshwater, may provide some explanation as to why topsmelt were more sensitive to the leachate. Increasing the hardness of water made nicotine more toxic to fishes;.adding a weak alkali or a basic salt to the water decomposed any unavailable nicotine compounds more rapidly, effectively releasing a larger quantity of bioavailable nicotine into the water, increasing toxicity (Konar, 1977). Saltwater (with salinity at approximately 34 ppt) has a $CaCO_3$ content in excess of 1000 mg/L, whereas the freshwater or diluted mineral water used in this study (with salinity < 0.5 ppt) has a much lower $CaCO_3$ content between 80-100 mg/L (Nautilus Environmental, personal communication, 2009). It is possible that any unavailable nicotine became bioavailable more quickly in saltwater, delivering a higher concentration of nicotine more rapidly to the topsmelt. This may serve to elucidate the difference in sensitivity between the two species of fish, as the topsmelt was more sensitive to the cigarette leachate than the fathead.

5.4 COMPARING DOSE-RESPONSES

Both fish species exhibited statistically different dose-responses to the different cigarette leachates, as reported by the *p*-values in Figures 4.1 and 4.2. There was a trend of increased toxicity with the smoked cigarettes and again with leaving remnant tobacco intact. Consequently, leachate from unsmoked cigarette filters was least toxic, leachate from smoked cigarette filters (no remnant tobacco) was more toxic, and leachate from smoked cigarette butts (with remnant tobacco) was most toxic. 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 (Iskander, 1985), our results show that the chemicals solely in the smoked filter still exert considerable toxicity to fishes. Some possible explanations for this trend of increased toxicity with smoked cigarettes are that smoking may create new, more toxic chemicals. For example, PAHs, furans, and benzene are all toxic products of combustion and have all been found to occur in cigarette smoke (Hoffmann & Hoffmann, 1997). Smoking may also act to change the solubility of compounds in cigarette butts, making them more bioavailiable. For example, PAHs found is cigarette smoke are capable of bioaccumulating in the tissues of fish (Agency for Toxic Substances and Disease Registry [ATSDR] 1995). The act of smoking may also increase the concentration of toxicants in the cigarette butt as more chemicals are pulled through and retained by the filter as smoking continues. It would seem reasonable to suggest that a combination of the events are occurring and could aid in explaining the increased toxicity found with the smoked cigarettes. 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 was comprised of unburned tobacco as well as a burnt tobacco tip and including such remnant tobacco effectively exacerbated toxicity. The chemicals in smoked cigarette butts (with remnant tobacco) may be significantly greater and different from those retained within the filter itself, the former of which 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.

5.5 Risk Assessment

Despite the mounting evidence on the toxicity of cigarette butt leachates to various organisms, it is difficult to assess the risk that littered cigarettes 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 on aquatic organisms (Warne et al., 2002). 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.

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CHAPTER 6

SUMMARY AND CONCLUSION

Cigarette butts are the most common form of litter in the world, and this study represents the first to show that cigarette butt leachate is toxic to representative marine and freshwater fish species, topsmelt (*Atheriops affinis*) and fathead minnow (*Pimephales promelas*). The LC50 for unsmoked cigarette filter (no tobacco) leachate was found to be 5.1 and 13.5 cigarette butts/L for the topsmelt and the fathead minnow, respectively. The LC50 for smoked cigarette filter (no remnant tobacco) leachate was found to be 4.1 and 5.5 cigarette butts/L for the marine topsmelt and the freshwater fathead minnow, respectively. The LC50 for smoked cigarette butts (with remnant tobacco) leachate was found to be approximately 1.1 cigarette butts/L for both the topsmelt and the fathead minnow. Consequently, cigarette filter leachate was found to increase in toxicity to fish after cigarettes had been smoked, compared to unsmoked cigarette filters, and even more so when remnant burnt tobacco was left intact. The topsmelt (*Atherinops affinis*) exhibited more sensitivity to most cigarette leachates than did the fathead minnow (*Pimephales promelas*). Additional research is needed to explore the actual risks that cigarette waste has on freshwater and marine environments, the fate of such chemicals in aquatic environments, as well as their potential for bioaccumulation.

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APPENDIX A

DEFINITION OF TERMS

Definition of Terms

Artificially-smoked cigarettes	Cigarettes that have been smoked on a machine
$CaCO_3$	Calcium Carbonate
Cigarette	Cigarette particulate matter
Carcinogenic	Causing cancer
EC50	50% effect concentration; the concentration resulting in immobilization of 50% of the test population.
GRAS	General Recognized as Safe
Hardness of water	Relates to mineral content, such as calcium and magnesium
LC50	50% lethal concentration; the concentration resulting in mortality of 50% of the test population.
Mainstream smoke	Cigarette smoke that is inhaled and exhaled by a smoker
Naturally-smoked cigarettes	Cigarettes that have been smoked by persons
PAHs	Polycyclic Aromatic Hydrocarbons
ppm	parts per million
ppt	parts per thousand
Sidestream smoke	Cigarette smoke from the smoldering cigarette tip
TIE	Toxicity Identification Evaluation
Toxic	The ability of a substance to cause to damage to an exposed organism

APPENDIX B

IACUC ANIMAL SUBJECTS APPROVAL

Graduate and Research Affairs
Division of Research Affairs
San Diego State University
5500 Campanile Drive
San Diego CA 92182 · 8220
Phone: 619 · 594 · 5938
Fax: 619 · 594 · 4109

SAN DIEGO STATE UNIVERSITY

February 19, 2009

Richard Gersberg
Graduate School of Public Health
San Diego State University

APF#: 09-01-002G
Title: Toxicity of Cigarette Butts and their Chemical Components to Marine and Freshwater Fish. Student research for Elli Slaughter.
Subject: IACUC Approval

Dear Professor Gersberg:

The project referenced was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) in accordance with the requirements pertaining to animal subjects protections within the Public Health Service Policy and USDA Animal Welfare Regulations on **February 18, 2009**. Approval carries with it the understanding that you will contact the Committee promptly to report any unanticipated or serious adverse events, to obtain authorization to implement any proposed changes to the protocol, to document a change in your affiliation with SDSU, and/or to report study completion. **Any proposed changes to the protocol, including the addition or deletion of personnel, must be submitted on an amendment form, reviewed and approved by the IACUC before those changes can be implemented. In addition, approval is only valid provided personnel working on this protocol have completed all training requirements.**

The category of use for this study is E as indicated on the APF. Housing will be identified as "non-standard" in section 5 of the APF.

Delete any reference to housing the fish at Nautilus Environmental on the APF. It is understood the fish will be housed at SDSU, HT, Room 214. If you would like to change the housing location, an amendment form must be submitted, reviewed and approved by the IACUC prior to initiating the change.

Contact Tony Slimp, OLAC Manager (tslimp@sciences.sdsu.edu or x45421) or Kent Osborn, Veterinarian (kent.osborn@mail.sdsu.edu or x45421)for any housing facility inspection requirements and/or if you require any assistance with animal care.

Protocol approval is valid for up to three years provided you submit annual renewals to the IACUC for review. Your 1st year renewal is due **February 9, 2010.** The IACUC office will send you a reminder to renew your protocol; however, **it is your responsibility to submit a completed Renewal Form at least four weeks in advance of the renewal date**.

For questions related to this correspondence, please contact Christine Cook in the IACUC office at (619) 594-0905 or e-mail iacuc@mail.sdsu.edu. To access the IACUC review materials, relevant policies and guidelines related to the involvement of animal subjects in research, please visit the IACUC web site at http://gra.sdsu.edu/research/iacuc. To access password protected documents, use "pi" as the username and "pi_123" as the password.

Sincerely,

Greg Harris, Ph.D.
Chair, Institutional Animal Care and Use Committee

GH:clc

Copy to: SDSU Research Foundation, State of California Tobacco Related Disease Research, Grant Pending.
Elli Slaughter, Graduate School of Public Health

Protocol Expiration Date: February 9, 2012

APPENDIX C

ADDITIONAL FIGURES

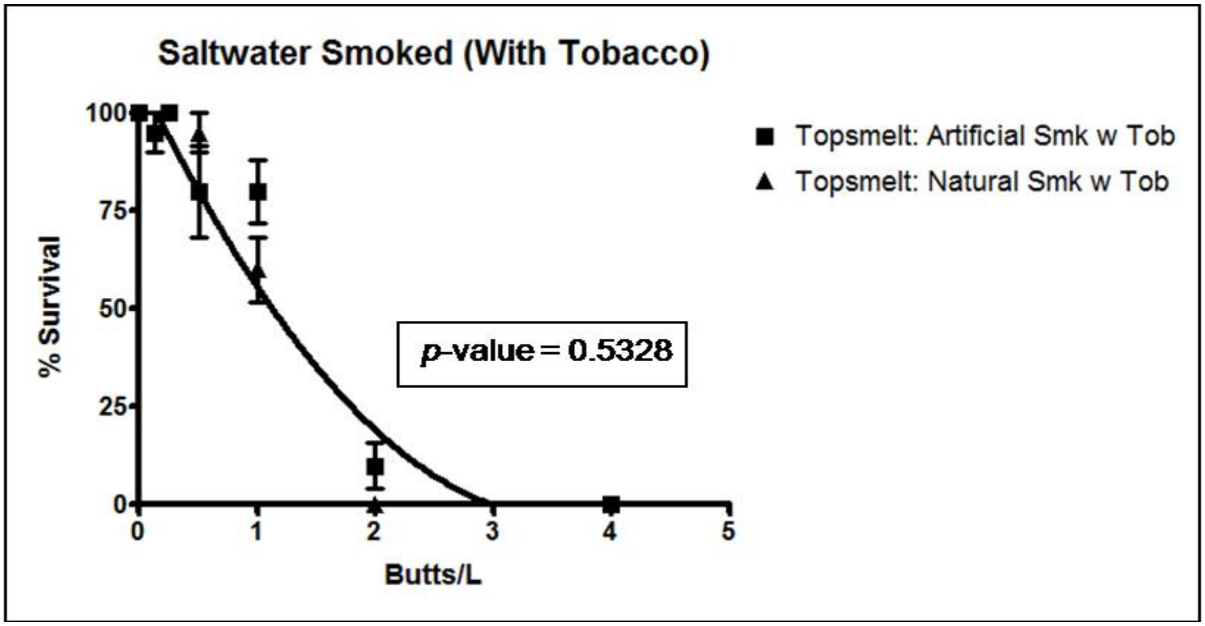

Figure C.1. Artificially-smoked and naturally-smoked cigarettes with tobacco were not found to be statistically different for the topsmelt.

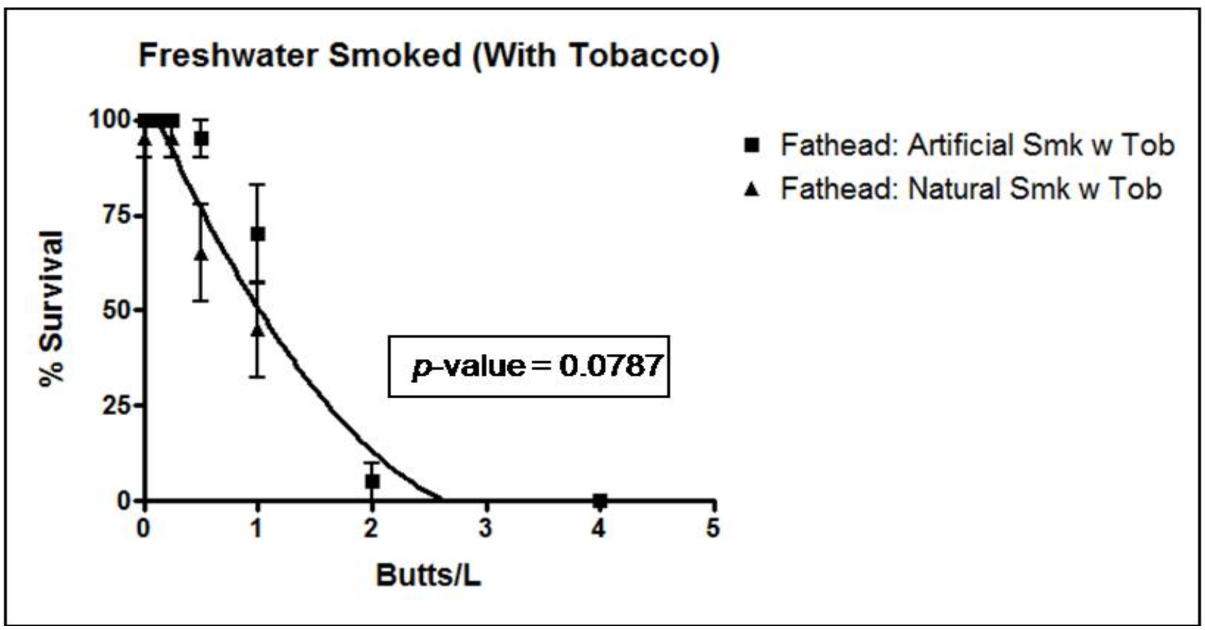

Figure C.2. Artificially-smoked and naturally-smoked cigarettes with tobacco were not found to be statistically different for the fathead minnow.

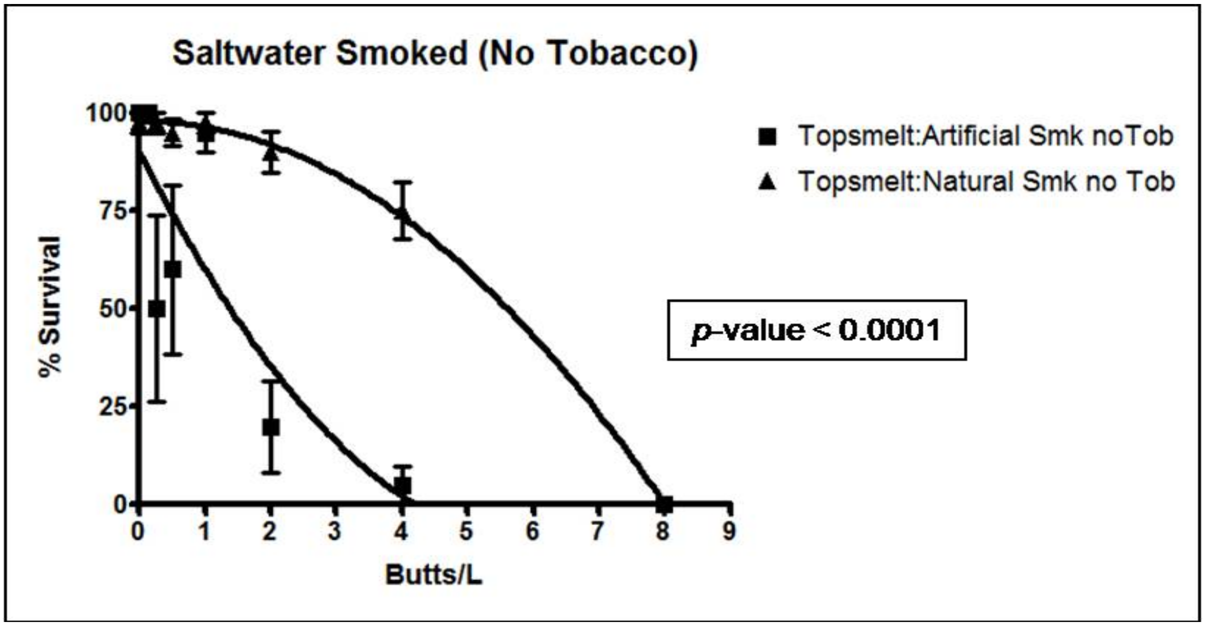

Figure C.3. Artificially-smoked and naturally-smoked cigarettes without tobacco were found to be statistically different for the topsmelt.

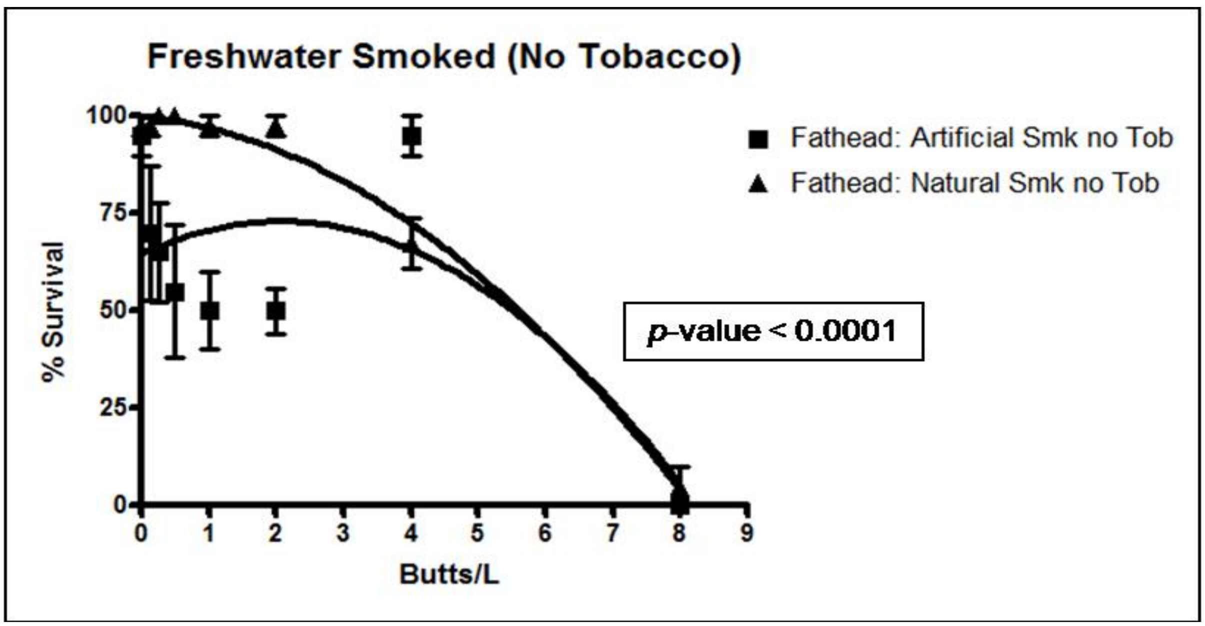

Figure C.4. Artificially-smoked and naturally-smoked cigarettes without tobacco were found to be statistically different for the fathead minnow.