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A comparison of electronic and traditional cigarette butt leachate on the development of Xenopus laevis embryos

Tatiana Tatum Parker[a,*], James Rayburn[b]

[a] Department of Biological Sciences, Saint Xavier University, 3700 West 103rd St., Chicago, IL, 60655, United States
[b] Department of Biology, Jacksonville State University, 700 Pelham Rd. N, Jacksonville, AL, 36265, United States

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ABSTRACT

Potential developmental toxicities of three different cigarette butt leachates were evaluated using the frog embryo teratogenesis assay–Xenopus (FETAX). Xenopus laevis embryos were exposed to regular cigarette butt (RCB), menthol (MCB) and electronic (ECB) in concentrations ranging from 0 to 4 butts/l for RCB and MCB and 0–10 butts/l for ECB. The embryos were from stage 8 to 11 and were exposed for a 96-h period in static renewal test conditions. Median lethal concentration (LC50), malformation (EC50), non-observed adverse effect concentration (NOAEC), and lowest observed adverse effect concentration (LOAEC) were calculated. Results from these studies suggest that each tested leachate is teratogenic for X. laevis embryos. The lowest LC50 was determined for ECB exposure at 17.9 cigarette butts/L. The LC50 value was the highest with RCB and MCB having LC50 s of approximately 1 cigarette butt/L. There were notable EC50 differences with RCB having the highest and ECB the lowest. The NOAEC and LOAEC levels for RCB and MCB were below 1 cigarette butt/L for both mortality and malformations; over 8 butts/L for ECB mortality and over 4 butts/L for malformations. From these results, we conclude that RCB leachate is the most toxic compound, while MCB leachate has the higher teratogenicity. ECB leachate has the lowest toxic and teratogenic effects on embryos but there were still noticeable effects. The results confirmed that the FETAX assay can be useful in an integrated biological hazard assessment for the preliminary screening for ecological risks of cigarette butts, and electronic cigarettes, in aquatic environment.

Cigarette butts are, collectively, the most common form of litter in the world. Approximately 5.6 trillion cigarettes are smoked every year worldwide. In the United States of America, cigarette waste constitutes an estimated 30% of the total litter (by count) on U.S. shorelines, in waterways, and on land [1]. The current method for measuring of how many cigarette butts are finding their way into streams, rivers, and coastal environments is the International Coastal Cleanup Day, which is organized annually by the Ocean Conservancy. The event involves more than 500,000 volunteers picking up debris from beaches, rivers, and streams around the world. The volunteers complete Marine Debris Data Cards that indicate the quantity and type of litter they pick up. During the 2013 international cleanup, 2,043,470 cigarette butts were collected, making this the most common debris item. Cigarette butts have topped the list in all International Coastal Cleanup Days since that category was added to the data cards as a separate item in 1990 [2].

The filters in one pack of 20 cigarettes weigh 0.12 ounces (with no tobacco attached) and displace a volume of 10 mL. Based on these figures, the cigarette butts collected in 2013 weighed approximately 766 lbs and displaced a volume of 1022 l. Owing to the ubiquitous nature and magnitude of cigarette butts discharged into the environment, studies have been undertaken to determine whether cigarette butt waste can exert ecotoxic effects in aquatic environments [3,4]. The environmental impact of cigarette butt waste is related to both their persistence in the environment and potential toxic effects [5] and their chemical composition – containing over 4000 different chemicals [4]. Most of these chemicals are toxic and often leach into aquatic ecosystems, thereby threatening water supply sources and aquatic animals [6,7] such as water fleas [3], which inhabit freshwater environments, and mosquito larvae [8].

Electronic cigarettes (EC) have, since their market introduction in 2004, gained a wide audience – as of 2013, there are several million e-cigarette users globally Michael Felberbaum (11 June 2013). Various reasons or factors have contributed to increased EC use; including the perception that vaping is a safer or healthier alternative to smoking conventional (tobacco) cigarettes. But the impact

* Corresponding author.
E-mail address: tatum@sxu.edu (T.T. Parker).

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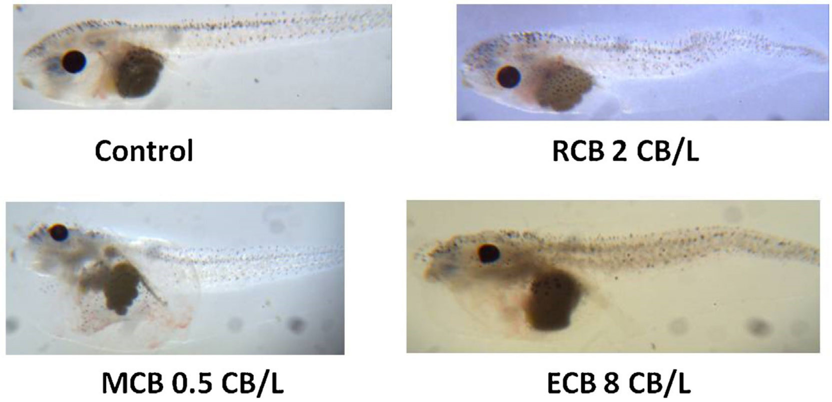

Plate 1. Control embryo top left. Top right panel is RCBL (2CB/L) and the embryo exhibits minor malformations of head, gut and overall embryonic delayed development. The RCBL embryo also exhibited significant tail abnormalities. Bottom left is MCBL (0.5 CB/L) and the embryo exhibits severe edema in the heart, craniol-facial, and abdomial areas. There are also significant gut and facial abnormalities. Bottom Right panel is ECBL at 8 CB/L and the embryo exhibits some tail abnormalities. The embryo has moderate to severe edema, heart and gut abnormalities. The embryo also has facial abnormalities as well.

of EC use on the environment is unknown. EC deliver aerosol by heating a liquid solution known as e-liquid that usually contains nicotine, propylene glycol, glycerin, and flavorings. The EC combine the fluid chamber with a heating element, vaporizing the e-liquid. Since EC do not release secondhand smoke, they are touted by some as being a safe alternative, for users and bystanders, to traditional (analog) cigarette. But researchers have analyzed the impurities in both e-liquid [9,10,11,12], and the composition of the emitted vapor [13,14,15,16]. Goniewicz et al. [13] measured 0.03–0.57 μg of lead per e-cigarette in emitted vapor. Williams et al. [16] found metals (e.g., lead, nickel, and silver), silicate beads, and nanoparticles in e-cigarette aerosol. There is far less data regarding the environmental impact of EC disposal. While one EC is typically equivalent to 40 traditional cigarettes, greatly reducing related waste byproducts, some research has been conducted to determine whether or not EC waste should be classified as hazardous [17]. Krause and Townsend [17] found that some e-cigarettes may be classified as hazardous waste for lead leaching, though they did observe a large degree of variability among brands and products. They did stress that the rate of consumption and disposal of these products is assumed to be higher than many other electronic products.

In order to derive water quality guidelines or conduct hazard assessments, toxicity data for a number of aquatic species is the minimum needed, so it is important to determine not only the toxicity of traditional cigarette butt leachate but also the toxicity of electronic cigarette related waste. In this study, we examined acute toxicity, teratogenic development, chronic and sublethal effects of cigarette butt leachate and EC leachate using Frog Embryo Teratogenesis Assay – Xenopus (FETAX). FETAX is an inexpensive alternative toxicity test system that can be used to evaluate ecological hazards from both complex environmental mixtures and pure chemical products [18]. This bioassay is a four-day, whole embryo-larval developmental toxicity screening assay that used embryos of the South African clawed frog, *Xenopus laevis*. The FETAX system is capable of monitoring acute, chronic, developmental, and behavioral toxicity for ecological and human health hazard assessment. With over 300 validation test compounds, the predictive accuracy of the FETAX model with conventional mammalian test systems is approximately 85% [19]. This allows for the examination of not only acute toxicity, but teratogenic development and chronic, sublethal effects on growth.

The specific aim of this study was to determine how different electronic cigarette butt leachate (ECBL) were compared to traditional cigarette butt leachate in terms of environmental (aquatic) toxicity, teratogenicity and impact on growth to embryos of *Xenopus laevis*. We hypothesized that the ECBL would be less toxic than the traditional cigarette butt leachate.

The objectives of this study were to determine the acute tocity (96-h LC50 and 96-h EC50) and lowest observed effect concentration (including the minimum concentration to inhibit growth) of regular cigarette butt (RCBL), menthol (MCBL), and electronic cigarette butt leachate (ECBL) to *Xenopus laevis* embryos.

1. Materials and methods

L-cysteine, human chorionic gonadotropin (HCG), NaCl, $NaHCO_3$, KCl, $CaCl_2$, $CaSO_4$, and $MgSO_4$ and other laboratory supplies were obtained from the Sigma Chemical Co., St. Louis, MO. Other supplies including 60 × 15 plastic Petri dishes, 10 mL disposable pipettes, transferable pipettes, and a 1 mL syringe were obtained from Fisher Scientific Supplies, location. Xenopus laevis frogs were obtained from Xenopus I, Inc. The Regular and menthol cigarettes contained 2.8% and 2.7% nicotine respectively, and the electronic cigarette cartridges contained 2.4% nicotine.

1.1. Cigarette butt collection, electronic cigarette purchase, and leachate preparation

Cigarette butts (CB) were collected from naturally smoked cigarettes, defined as cigarettes that were smoked by people, extinguished in cigarette disposal units and collected within 24 h. The electronic cigarettes were purchased, punctured, and then used discarded cigarette butts. We only tested one type of e-cigarette, as well as one brand of regular and menthol cigarette. The CB were selected randomly and placed in to clean plastic bags for transport to the laboratory. The collected CB were immediately processed as described below for leachate production.

Cigarette butt leachates (CBL) were made by soaking 3 butts in 300 mL FETAX solution for 1 h to produce the leachate stock solution of 10 CB/L. FETAX solution was prepared by adding 625 mg NaCl, 96 mg NaHCO3, 30 mg KCl, 15 mg CaCl2 60 mg CaSO4*2H2O, and 75 mg MgSO4 per liter distilled water [19,20]. CBLs were made fresh for RCBL, MCBL, ECBL for each replicate experiment.

1.2. Animal care and breeding

Animal care and breeding was performed according to the specifications set forth in the ASTM's Standard Guide for Conducting the Frog Embryo Teratogenesis Assay-Xenopus [19]. Animal care and use was performed in accordance with the requirements of