Inhalation Toxicity Report for the Aerosol Generated by the Operation of Green Smoke Electronic Cigarettes

Prepared for:
Smoke Free Alternative Trade Association

Prepared by:
Arlene L. Weiss, MS, DABT, ERT
Rudolph J. Jaeger, PhD, DABT, ERT
Environmental Medicine Inc.

September 12, 2012

This report is divided into five sections. Section I comprises the introduction and background. Section II contains the toxicological data available for the compounds detected in the aerosol generated from the Green Smoke brand of electronic cigarettes. Section III discusses the extent of the scientific literature concerning the potential for adverse health effects of electronic cigarette smoking. Section IV reports on the components not found in the electronic cigarette aerosol as compared to the smoke of conventional tobacco burning cigarettes. Section V consists of the Summary and Conclusions.

Section I: Introduction and Background

Environmental Medicine Inc. (EMI) was asked to create a toxicological profile for the inhalational exposure to an aerosol[1] generated from Green Smoke brand of electronic cigarettes with specific reference to the major components detected, namely propylene glycol, nicotine, and glycerin. In addition, other tentatively identified compounds are incorporated into the profile. Specific toxicity endpoints such as cardiovascular disease, chronic obstructive pulmonary disease (COPD) and lung cancer among other morbidities are included. Specific references to these endpoints are included as they are causally associated with conventional tobacco cigarette smoke[2], referred to as tobacco burning cigarettes (TBCs).

EMI was provided with an analysis done by Chemir Analytical Services (report date 6/6/11). They analyzed the air drawn through an electronic cigarette for the presence of the three major components of the aerosol: propylene glycol, nicotine, and glycerin (see Table 1) using Solid Phase Microextraction (SPME) gas chromatography/mass spectrometry (GC/MS). Several other low concentration solvents were also tentatively identified using SPME GC/MS and are included in this profile. They also analyzed the air drawn through an electronic cigarette for the presence of combustion products, tar, tobacco specific nitrosamines and carbon monoxide using GC/MS. Propylene glycol, glycerin and nicotine were also detected in a methanol extraction of the sample "absolute tobacco cartomizers" by GC/MS.

Green Smoke brand of electronic cigarettes were used in Chemir's analysis. EMI was provided with the starter kits for two brands of electronic cigarettes, the Green Smoke electronic cigarettes (www.greensmoke.com) and v2cigs (www.v2cigs.com) (See Figure 1 and Figure 2). The electronic cigarette, which is also referred to as an electronic nicotine delivery system, is an electronic device that emulates a regular cigarette and carries a nicotine containing aerosol when puffed by the user. Each cartridge contains a manufacturer specific nicotine and flavor solution in propylene glycol and/or glycerin (Goniewicz et al. 2012; Trehy et al. 2011). In addition to the cartridge, there is a heating

[1] An aerosol is a suspension of fine solid particles or liquid droplets in a gas. In general, the terms gas and vapor are often used as fungible terms. However, scientifically, a vapor is a specific type of gas. Vapor refers to a gas whose molecules have gained energy and vaporized from a substance when at room temperature is either a solid or a liquid. For example, water at room temperature is a liquid, but when it becomes a gas (for example, when boiled) it is referred to as water vapor.

[2] Smoke is referred to as a collection of airborne solid, liquid, and gases emitted when a material undergoes pyrolysis or combustion.

element to vaporize the solution and a microprocessor with an air flow sensor that activates the heating element when the electronic cigarette is puffed. There is a rechargeable battery or other power source (USB adapter). Some brands have an LED diode that mimics the appearance of a burning cigarette fire cone. The purpose of this cigarette is to provide the user with an experience that is mechanically similar to smoking conventional cigarettes and that does not contain any tobacco specific toxins or products generated from the combustion of tobacco as it occurs in conventional cigarette smoking.

It is widely accepted in the scientific community that inhaling the complex mixture of products in tobacco smoke from tobacco burning cigarettes is responsible for the adverse health effects of cigarette, pipe, and cigar smokers including cancer, cardiovascular disease and pulmonary disease. These adverse effects occur over time by mechanisms that include DNA damage, inflammation, and oxidative stress (US Department of Health and Human Services 2010; Pappas 2011; American Council on Science and Health 2005). With respect to cancer etiology, tobacco derived cigarette smoke contains diverse carcinogens including n-nitrosamines, 1,3-butadiene, and benzo(a)pyrene being the important ones because of their carcinogenic potency and levels in cigarette smoke (US Department of Health and Human Services 2010; U.S.National Institutes of Health 2001). The tobacco specific nitrosamines and polycyclic aromatic hydrocarbons (PAH) are the two groups of compounds present in tobacco smoke that are thought to be responsible for the carcinogenic potency of cigarette smoke (Hecht 1998; World Health Organization 2010; Jenkins et al. 2000). The two major nitrosamines are N'-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (U.S.National Institutes of Health 2001). Benzo(a)pyrene, a common product of combustion, is used as the marker for carcinogenic PAHs (World Health Organization 2010).

With respect to pulmonary disease and chronic obstructive pulmonary disease (COPD) etiology, a designation that reflects underlying damage and structural abnormalities in the lung's airways and alveoli, two mechanisms which include oxidative stress and protease-antiprotease imbalance are triggered by the inhalation of combustion products of tobacco smoke directly into the lungs of active and passive smokers (US Department of Health and Human Services 2010; Fabbri et al. 2006; Archontogeorgis et al. 2012; Agusti and Barnes 2012).

With respect to cardiovascular disease etiology, cigarette smoking leads to endothelial injury and dysfunction in coronary and peripheral arteries, it produces a chronic inflammatory state that contributes to the atherogenic disease process, and leads to an increased risk of thrombosis (US Department of Health and Human Services 2010; Cobble 2012).

One other toxicological endpoint is worth mentioning, that is the reproductive and developmental effects from exposure to tobacco smoke. Within cigarette smoke, carbon monoxide is the toxicant found in the highest concentrations and its major effect is to deprive the fetus of oxygen by binding to hemoglobin (US Department of Health and Human Services 2010).

Currently, there are over one billion smokers' worldwide (World Health Organization 2011). As such, cigarette smoking is a global epidemic that poses a substantial health burden with associated costs. It is widely accepted in the scientific community that the risk of serious disease diminishes rapidly after quitting and life-long abstinence from smoking tobacco is known to reduce the risk of lung cancer, heart disease, stroke, and chronic lung disease (Lightwood and Glantz 1997; US Department of Health and Human Services 1990).

While the primary benefit of tobacco smoking is nicotine delivery, the major health burden results almost entirely from inhaling the combustion products of burning tobacco. There now exists the potential for tobacco harm reduction with the substitution of lower risk nicotine products for smoking (Wagener et al. 2012). Epidemiological evidence suggests that smokeless tobacco which delivers nicotine causes about one one-hundredth the health risk of smoking conventional cigarettes (namely, those products that burn tobacco) (Phillips and Heavner 2009).

Section II: Toxicology

In this section, the three major components are discussed first, followed by a discussion of the other compounds that were tentatively identified by Chemir in their report. The components are listed in decreasing order with the component that has the highest relative percent area listed first (see Table 1). The three major components present in the aerosol generated by the electronic cigarette are propylene glycol, nicotine, and glycerin.

Propylene glycol

According to the Chemir analysis (see Table 1), the aerosol contained 1, 2 -propylene glycol (CAS# 57-55-6) at 84% of the relative percent area. It was the most abundant component detected by Chemir. Propylene glycol is an aliphatic alcohol and has many uses. It is generally recognized as safe (GRAS) as a food ingredient by FDA[3] (American Chemistry Council's Propylene Oxide/ Propylene Glycol Panel 2001; www.fda.gov 2012b). It is used as an anticaking agent, antioxidant, dough strengthener, flavoring agent and solvent. It has been used as a tobacco ingredient for over 30 years as a solvent/vehicle for adding flavors to tobacco (R.J.Reynolds Tobacco Company 1988). In electronic cigarettes, propylene glycol is the liquid solvent/vehicle in which nicotine is carried. The process is started by taking a puff on the electronic cigarette which in turn starts the heating process. With heating, the nicotine/propylene glycol solution vaporizes.

One study reported human patients with expiratory airflow disorders were treated with an aerosol of isoproterenol in 40% propylene glycol by volume. They found that the mist was well tolerated and no adverse clinical reactions were noted. The authors recommended propylene glycol as an appropriate vehicle for routine administration of bronchodilator drugs (Cohen and Crandall 1964; Lakind et al. 1999).

Propylene glycol has low systemic toxicity in experimental animals (Lakind et al. 1999). It is not acutely toxic and does not irritate the skin (ATSDR 2008). Propylene glycol is

[3] Food and Drug Administration

primarily metabolized to lactic acid and pyruvic acid, both of which are normal constituents of the citric acid cycle , an energy generating process in humans and other mammals (Lakind et al. 1999).

Other investigators have concluded that propylene glycol is not a systemic toxicant when administered by inhalation (Suber et al. 1987). A subchronic 90 day nose-only inhalation study in rats exposed to propylene glycol at varying concentrations revealed no treatment related histological changes to the trachea, larynx, or lung (Suber et al. 1987). Other inhalation studies testing propylene glycol in rats and monkeys did not observe treatment related effects on respiratory physiology, clinical chemistry, hematology, gross pathology or respiratory tract histology (Robertson et al. 1947).

Propylene glycol was not mutagenic in the Ames assay and did not produce chromosomal breaks, sister chromatid exchanges or micronuclei formation in mammalian cells (R.J.Reynolds Tobacco Company 1988; American Chemistry Council's Propylene Oxide/ Propylene Glycol Panel 2001). Animal studies given propylene glycol orally in their diet found no increase in dermal or systemic tumor formation and no effects on growth rate, fertility, kidney function or blood counts following inhalation exposure in rats and monkeys were observed (Robertson et al. 1947; R.J.Reynolds Tobacco Company 1988). Similarly, reproductive and teratogenicity studies of animals fed propylene glycol were negative (ATSDR 2008). Expert review panels have concluded that results of laboratory studies demonstrate that propylene glycol does not pose a risk of cancer (American Chemistry Council's Propylene Oxide/ Propylene Glycol Panel 2001).

One early study demonstrated hemodynamic responses to propylene glycol administered to animals by intravenous administration. However, they also reported that no hemolysis or hemodynamic effects were noted in animals following inhalation exposure to propylene glycol (MacCannell 1969).

In summary, propylene glycol is considered non-toxic to humans following exposure by inhalation and poses no increased risk of adverse effects. There is no evidence in the published scientific literature that exposure to propylene glycol causes cancer, cardiovascular disease, or pulmonary disease.

Nicotine

The second most abundant constituent of the aerosol is tobacco-derived nicotine. According to the Chemir report (see Table 1), nicotine (CAS# 54-11-5) was present at 7.4% relative percent area. It is well known in the scientific community that the major toxicity of cigarette smoke including cancer, COPD and cardiovascular disease are derived from the combustion products of burned tobacco and not *per se* the nicotine present in the tobacco (Le Houezec et al. 2011; US Department of Health and Human Services 2010; Russo et al. 2011b). However, nicotine is the substance in tobacco that is responsible for its powerful addictive action and its effects are mediated through diverse actions at multiple nicotine receptors in the brain (US Department of Health and Human Services 2010; Russo et al. 2012; Russo et al. 2011a; Benowitz 2010). According to smokers, the major reason for smoking is to satisfy the powerful nicotine addiction, thus

rendering it extremely difficult for smokers to quit smoking. In addition, pharmacologic reasons for nicotine use are enhancement of mood, either directly or through relief of withdrawal symptoms, and augmentation of mental or physical functions (Benowitz 2010).

Although nicotine use may be implicated in some forms of cardiovascular disease, the use of nicotine or other medications to facilitate smoking cessation in people with known cardiovascular disease produces far less risk than the risk of continued smoking (US Department of Health and Human Services 2010; Benowitz and Gourlay 1997). Nicotine itself is not considered to be a carcinogen, however, recent evidence suggests that nicotine may be involved in certain aspects of tumor promotion (Cardinale et al. 2012; Russo et al. 2012).

The components of the aerosol as generated by cartridges and refill solutions from three other companies, namely NJOY, Smoking Everywhere, and Johnson Creek were evaluated by FDA using GC/MS and with the HPLC gradient method (Johnson Creek Enterprises 2009; Trehy et al. 2011; Westenberger 2009). The authors reported that nicotine content labeling was not accurate with some manufacturers and that nicotine was not always present in the electronic cigarettes. Nicotine related impurities in cartridges and refills were found to vary by manufacturer (Trehy et al. 2011). It is important to note that these three cartridges and /or refill solutions were not the ones evaluated in this report.

Glycerin

According to the Chemir Report, glycerin (CAS# 56-81-5) is present in the aerosol at 4.7% relative percent area and is the third most abundant component. Glycerin, also known as glycerol and glycerine, but more properly known as propane-1,2,3-triol is a clear colorless oily liquid (Emsley 1994). Glycerin is a natural constituent of both animals and plants. Glycerol has myriads of uses in pharmaceutical and consumer goods. It is present in but not limited to skin lotions, mouthwashes, cough medicines, and drug solvents. Pharmaceutically, glycerin has a long history of usage in drugs applied topically, inhaled, and ingested (R.J.Reynolds Tobacco Company 1988). It is also used in foods and beverages and serves as a humectant[4], solvent, and sweetener, and may also help to preserve foods. Glycerin is a GRAS substance (Generally Recognized as Safe) for use in foods by the Food and Drug Administration (www.fda.gov 2012a).

Glycerin has been tested in animals for acute, subacute, chronic effects and in *in vitro* using genetic toxicology assays. Glycerol has no potential to alter DNA (R.J.Reynolds Tobacco Company 1988). Inhalation exposure of glycerin to animals (rats) at very high concentrations had no biological effect on signs of toxicity or morbidity, body weight, diet consumption, mortality and hematology parameters (R.J.Reynolds Tobacco Company 1988). Glycerin was negative in a battery of assays including the Ames assay, sister chromatid exchange, chromosomal aberrations in CHO cells, HGPRT gene mutation assay and unscheduled DNA synthesis.

[4] Humectants are compounds that are used to promote the retention of moisture

In summary, glycerin is considered non-toxic to humans following exposure by inhalation and poses no increased risk of adverse effects. There is no evidence in the published scientific literature that exposure to glycerin causes cancer, cardiovascular disease, or pulmonary disease.

Other low concentration tentatively identified components

According to the Chemir analysis (Table 1), all of the other tentatively identified components are less than 1% with the exception of ethyl alcohol present at approximately 1.1%. It is important to note that theses compounds were only tentatively identified by Chemir with GC/MS. These tentatively identified compounds were never compared to reference standards (ALW personal communication with Chemir project leader 8/29/12). The actual components may be these compounds or a structurally similar compound.

It is well known and accepted in the scientific community that tobacco smoke from TBCs is a mixture of more than 5000 chemicals. Many of these are toxic, mutagenic and carcinogenic. One recent publication based on an extensive literature search for known smoke components and their human inhalation risks (Talhout et al. 2011) provided a list of 98 hazardous tobacco smoke components. These authors included components with potential carcinogenic, cardiovascular, and respiratory effects since they are the three major smoking related causes of death. A comparison made by EMI of the results of the compounds detected by Chemir (Table 1) from the aerosol of the Green Smoke brand of electronic cigarettes tested revealed that none of these compounds were listed in this published citation of the 98 hazardous smoke components (Talhout et al. 2011). This is not surprising since the nearly all of the compounds are generated from the combustion of tobacco in TBCs and are not generated from electronic cigarettes as no tobacco is burned (Campagna et al. 2012). Notwithstanding this, these compounds were detected in such small relative amounts (see Table 1) that are not likely to cause any increased risk of cancer or morbidity.

Another published study performed an evaluation on the toxicity of 95 ingredients added to experimental cigarettes that burn tobacco (Gaworski et al. 2011; Coggins et al. 2011c; Coggins et al. 2011b; Coggins et al. 2011d; Coggins et al. 2011a; Coggins et al. 2011e). These include aliphatic and aromatic carboxylic acids, aliphatic carbonyl compounds, aromatic carbonyl compounds, essential oils and resins, and carbohydrates and natural products. In the massive study, they analyzed the mainstream smoke chemistry, performed bacterial mutagenicity testing, performed cytotoxicity testing, and for some of the ingredients, they were also tested in a 90 day nose only inhalation study using the mainstream cigarette smoke. Their results demonstrated that these added ingredients produced minimal changes in the overall toxicity profile of mainstream cigarette smoke. Notwithstanding this, none of these 95 ingredients tested were found in the analysis performed by Chemir of the Green Smoke electronic cigarettes.

In summary, these tentatively identified components of the electronic cigarette aerosol at the relative percentages reported by Chemir should not be considered to be competent

producing sources of toxicity. They are not likely to put a user of electronic cigarettes at any increased risk of harm or adverse effects.

Ethanol

Ethanol (CAS# 64-17-5; also referred to as ethyl alcohol) was also tentatively identified by Chemir (see Table 1) as being present in the aerosol generated from smoking an electronic cigarette at 1.106% relative percent area. Ethanol, when taken by mouth (ingestion) has been widely studied and many reports have been published; however, much less is known about the effects of ethanol following inhalation. It is known in the scientific community that ethanol in alcoholic beverages is currently considered a Group 1 carcinogen by IARC[5] (Bevan et al. 2009; Baan et al. 2007; World Health Organization 2012). It is important to note that ethanol exposure via routes other than ingestion has not yet been considered by IARC. Ethanol has been classified as an A4 carcinogenic risk by ACGIH[6] ("not classifiable as a human carcinogen") due to lack of animal data. Furthermore, it is not in the USEPA[7] carcinogen classification nor considered by NIOSH[8] to be a human carcinogen (Health Council of the Netherlands 2006).

One published study investigated the effects of occupational exposure, namely inhalation and dermal exposure to ethanol (Health Council of the Netherlands 2006). These authors found that the highest occupational exposure concentration detected in monitoring studies was 43 mg/m^3 ethanol (hairdressing salon) and 217 mg/m^3 (car spray painting) and that both of these values were below the Occupational Exposure Limit (OEL) of between 500-1000 ppm set by most countries (Bevan et al. 2009; Health Council of the Netherlands 2006). The Health Council of Netherlands (Health Council of the Netherlands 2006) concluded that in order to achieve detectable systemic concentrations of ethanol by inhalation, a sustained exposure to relatively high atmospheric concentrations of ethanol is necessary. It is therefore, unlikely that acute exposure to ethanol presents an increased risk of carcinogenesis.

With respect to chronic inhalation exposure, no studies relating to the carcinogenic effect in humans and animals from inhalational exposure could be found in the published literature. The Health Council of the Netherlands extrapolated from available oral route data to calculate the inhalation intake during an 8 hour shift at ethanol levels of 1000 ppm. However, given that the major differences between inhalation and ingestion is dose delivery and dose-rate delivery, interpretation of data following ingestion with extrapolation to inhalation can only be performed with extreme caution. They reported that inhalation exposure at the current OEL for the United Kingdom (1900 mg/m^3 or 1000 ppm ethanol over 8 hr shift) would increase total systemic levels of ethanol by 11.4 grams. This corresponds to the intake from drinking approximately 1 standard glass of alcohol (assume 10 grams ethanol per glass) and would be unlikely to overwhelm the metabolic capability of a worker (Health Council of the Netherlands 2006). In the occupational setting, the dose-rate delivery of this amount of ethanol is low and allows

[5] International Agency for Research on Cancer
[6] American Conference of Governmental Industrial Hygienists
[7] US Environmental Protection Agency
[8] National Institute for Occupational Safety and Health

for the rapid and effective elimination (zero order kinetics) for the majority of individuals. The authors of this study concluded that there appears to be little cause for concern that exposure to ethanol below the OEL would present any increased risk of cancer (Bevan et al. 2009; Health Council of the Netherlands 2006).

In summary, there is little evidence that absorption of ethanol following inhalation at current OEL would lead to any measurable increase with the risk of development of cancer (Bevan et al. 2009; Health Council of the Netherlands 2006).

Methanol

Methanol or methyl alcohol (CAS# 67-56-1) was also tentatively identified by Chemir (see Table 1) as being present in the aerosol generated from smoking an electronic cigarette at 0.185% relative percent area. It is currently used as a solvent, fuel additive, and in the synthesis of other chemicals. Methanol is metabolized to formaldehyde which is oxidized to formic acid and further to carbon dioxide. Accumulation of methanol does not occur in humans except at near fatal doses (Cruzan 2009).

Methanol is not classified for carcinogenicity by IARC, NTP[9], or by California in their Proposition 65 list. Currently, there are no studies that have reported increased cancer risk from methanol in humans. In addition, genotoxicity studies do not suggest carcinogenicity from methanol exposure. Animal inhalation studies did not find an increased cancer risk even at levels that caused a 10 fold increase in blood methanol levels. There was carcinogenic activity found in animals given high doses of methanol in drinking water that resulted in blood methanol levels up to 100 fold higher than normal levels (Cruzan 2009).

In summary, the data from human studies, genotoxicity studies, inhalation and drinking water exposure cancer studies support a conclusion that methanol is not likely to be carcinogenic in humans (Cruzan 2009).

Methylene Chloride

Methylene chloride, also referred to as dichloromethane (CAS# 75-09-2) is worth mentioning even though the relative amount found in the aerosol from electronic cigarettes is extremely low at 0.15% (relative % area) and it was only tentatively identified, but not confirmed with the use of reference standards. Methylene chloride is widely used in a variety of medical, industrial, and commercial applications which include paint stripping, solvent extraction in food processing, and it is also used as an aerosol propellant (Burek et al. 1984; Rioux and Myers 1988).

Methylene chloride is primarily metabolized to carbon monoxide and carbon dioxide, both of which are excreted in expired air. Metabolism to carbon monoxide takes place in the microsomal fraction (mixed function oxidase) of the liver and requires oxygen and energy.

[9] National Toxicology Program

One human epidemiological study revealed no adverse health effects or increased frequency of tumors in employees occupationally exposed to methylene chloride (Friedlander et al. 1978). This study investigated a large male employee population with continuous low level work exposure to methylene chloride for up to 30 years. They reported that overall mortality was similar to internal controls (Kodak Park controls) and less than expected when compared to external controls (New York State males). They also reported that there were no significant differences in any of the malignancy subgroups when compared to both internal and external control groups (Friedlander et al. 1978).

A two year inhalation study in rats and hamsters demonstrated some carcinogenicity and liver damage in rats, but not in hamsters and the hamsters lacked evidence of definite target organ toxicity (Burek et al. 1984). These authors reported that the high degree of sex and species specificity for the sarcoma response was inconsistent with the rest of their knowledge about the biology of the material. Methylene chloride was not teratogenic in either rats or mice at exposure concentrations as high as 4500 ppm (Burek et al. 1984; Hardin and Manson 1980). One study in which rats and mice were exposed to methylene chloride at concentration up to 1250 ppm did not cause significant maternal, embryonal or fetal toxicity, and was not teratogenic in either species tested (Schwetz et al. 1975). The authors also comment that their findings in the rats were inconsistent with the current knowledge of the biology and toxicology of methylene chloride in man (Burek et al. 1984).

Methylene chloride is currently listed in IARC as a Group 2B carcinogen (World Health Organization 2012; International Agency for Research on Cancer 1999). Group 2B means the agent is possibly carcinogenic to humans. This category is used for agents for which there is limited evidence of carcinogenicity in humans and less than sufficient evidence of carcinogenicity in experimental animals. It may also be used when there is inadequate evidence of carcinogenicity in humans but there is sufficient evidence of carcinogenicity in experimental animals. For methylene chloride, the later holds true. Currently, the evidence for carcinogenicity in humans of methylene chloride is inadequate.

In summary, the relative concentration of methylene chloride, if it is even present, in the aerosol generated from electronic cigarette usage is negligible and it not likely to pose any increased risk of cancer to the user.

Section III: Scientific literature concerning the potential for adverse health effects of electronic cigarette smoking

As electronic cigarettes are widely available to be ordered on the internet, new data is emerging in the peer-reviewed scientific literature delineating the potential for adverse health effects in comparison to the known adverse health effects causally associated with TBCs. Additional data is also becoming available to help understand the utility of electronic cigarettes in the marketplace. One recent study (Vansickel et al. 2012) assessed the initial abuse liability of an electronic cigarette in current tobacco smokers using a multiple-choice procedure with ten electronic cigarette puffs and varying amounts

of money, ten electronic cigarette puffs and varying number of their own brand cigarette puffs, or ten own brand puffs and varying amounts of money in twenty current tobacco smokers. They found that the electronic cigarette resulted in significant nicotine delivery, tobacco abstinence symptom suppression and increased product acceptability ratings. They concluded that electronic cigarettes can deliver clinically significant amounts of nicotine and reduce cigarette abstinence symptoms and appear to have a lower potential for abuse relative to traditional tobacco burning cigarettes (Vansickel et al. 2012).

Electronic cigarettes are popular among users for varying reasons. One study questioned 3587 participants (70% former tobacco smokers) after using electronic cigarettes containing nicotine for 3 months (Etter and Bullen 2011). The authors found that 96% of the participants reported that the use of electronic cigarettes helped them quit smoking, or reduce their smoking. Their reported reasons for using the electronic cigarette was the perception that it was less toxic than tobacco (84%), that is was cheaper than smoking tobacco cigarettes (57%), and to help them deal with situations where smoking was prohibited (39%). In addition, most ex-smokers (79%) feared that they might relapse to smoking cigarettes if they stopped using the electronic cigarettes. The authors concluded that the electronic cigarettes were used mainly by former smokers as an aid to quit smoking (Etter 2010) and to avoid relapse. They also found that the users of nicotine containing electronic cigarettes reported better relief of withdrawal and a great effect on smoking cessation than those using non-nicotine electronic cigarettes (Etter and Bullen 2011). The authors reported that the products were perceived as satisfactory, useful, efficacious, and that almost all users preferred nicotine containing electronic cigarettes (Etter and Bullen 2011) in contrast to electronic cigarettes without nicotine.

One recent 6 month prospective pilot study assessed the effect of electronic nicotine delivery (electronic cigarette) on smoking reduction and cessation in 40 regular current smokers that were unwilling to quit (ClinicalTrials.gov 2012; Polosa et al. 2011). In this pilot study, smokers attended a total of 5 study visits (baseline, week-4, week-8, week-12, and week-24) in which product use of electronic cigarettes, number of cigarettes smoked and exhaled carbon monoxide levels were measured at each visit. In addition, they calculated smoking reduction and abstinence rates. They found that the use of electronic cigarettes substantially decreased cigarette consumption without causing significant side effects in smokers not intending to quit. These side effects assessed were depression, anxiety, insomnia, irritability, hunger, and constipation and none were reported in this study using electronic cigarettes (Polosa et al. 2011).

A published case report demonstrated that two individuals who suffered from depression were able to quit smoking and remain abstinent for at least 6 months after taking up electronic cigarettes (Caponnetto et al. 2011). This is remarkable due to the fact that smoking may help individuals to cope with stress or medicate depressed mood as there is an association between nicotine dependence and affective disorders, particularly depressive disorders (Caponnetto et al. 2011).

Another study investigated the potential for acute effects of electronic cigarette and tobacco cigarette smoking on complete blood count (CBC) in smokers and never-

smokers (Flouris et al. 2012). They found that the CBC indices remained unchanged during the control session and during the active and passive electronic cigarette smoking sessions. However, they found that the active and passive tobacco cigarette smoking group demonstrated an increase in white blood cell, lymphocyte, and granulocyte counts for at least one hour in smokers and never smokers. They concluded that electronic cigarettes in this study did not increase the secondary proteins of acute inflammatory load as occurred in the group smoking tobacco burning cigarettes (Flouris et al. 2012).

In a recent presentation (August 2012) made by Dr. Farsalinos at the European Society of Cardiology conference in Munich (www. escardio.org) (Farsalinos et al. 2012), he compared the acute effects of using an electronic cigarette on myocardial function in comparison with the effects of regular tobacco burning cigarettes. This is the first study to investigate the effects of electronic cigarettes on cardiac function. The electronic cigarettes used (brand Norbacco) contained glycerol, propylene glycol, nicotine, and flavoring agents. It is important to note that these are the same components of the Green Smoke brand that was analyzed by Chemir. In this study, twenty tobacco smokers and 22 electronic cigarette smokers underwent haemodynamic[10] measurements, baseline echocardiogram before smoking either type of cigarette and the same parameters were measured after smoking. Their results showed that there was a significant elevation in blood pressure (+8 % in systolic pressure and +6% in diastolic pressure) and heart rate (+10%) after smoking conventional cigarettes, but only a slight increase in diastolic pressure (+4%) alone after electronic cigarette use; no change in systolic pressure or heart rate was found following electronic cigarette usage (Farsalinos et al. 2012).

With respect to cardiac function, the investigators reported that diastolic function was acutely impaired in smokers (4 parameters adversely affected), but found no difference in diastolic function observed after electronic cigarette use. They concluded that the absence of combustion products from not burning tobacco may be a safer alternative to conventional cigarettes and that the substitution of electronic cigarettes for tobacco cigarettes may be beneficial to health (Farsalinos et al. 2012).

In a recent review paper on electronic cigarettes, the authors reported on clinical trials which demonstrated that the electronic cigarette products appear to be much safer than tobacco cigarettes and are comparable in toxicity to conventional nicotine replacement products currently on the market (Campagna et al. 2012). Despite concerns about the safety of electronic cigarettes, there is detailed toxicology, characterization of the major components in the vapors, and the liquid of electronic cigarettes, namely, water, glycerin, propylene glycol, and nicotine. Both propylene glycol and glycerin have undergone extensive testing (see *supra*) and are considered safe for use. In addition, nicotine has also undergone extensive testing and is currently on the market as an aid to smoking cessation as a gum, patch, and in Swedish snus[11] (Campagna et al. 2012) to deliver nicotine. Nicotine-replacement therapy has been widely used and is well tolerated without evidence of serious adverse health effects. These authors concluded that nicotine

[10] Haemodynamic parameters refer to systolic and diastolic pressure (blood pressure) and heart rate.
[11] Snus is the Swedish word for moist snuff and has replaced cigarettes as the predominant form of tobacco use in Sweden (Phillips and Heavner 2009)

per se causes minimal risk when separated from inhaling tobacco smoke (Campagna et al. 2012).

Section IV: Components not found in the aerosol generated by these electronic cigarettes

One of the objectives of the Chemir Analysis was to determine if any combustion products known to be present in the burning of tobacco such as tar, tobacco specific nitrosamines, or carbon monoxide were generated as a result of drawing air through the electronic cigarettes that they tested (Green Smoke). The results of their GC/MS analysis did not indicate the presence of any compounds related to combustion products, tar, or tobacco specific nitrosamines. They reported that a test for carbon monoxide in the aerosol generated from the electronic cigarette was below 2 ppm, the limit of detection.[12]

The tobacco specific nitrosamines (TSNA) and polycyclic aromatic hydrocarbons (PAH) are the two groups of compounds known to be present in tobacco smoke and that are thought to be responsible for the cancers that are causally associated with conventional cigarette smoking (Hecht 1998; Jenkins et al. 2000; World Health Organization 2010).

The two major tobacco specific nitrosamines found in the highest concentrations are N'-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (U.S.National Institutes of Health 2001). Both NNN and NNK are present in the highest concentration of all the TSNAs in conventional cigarette smoke at concentrations approximately 120-3,700 ng and 80-770 ng, respectively (U.S.National Institutes of Health 2001). The evidence is strong that TSNAs play an important role in cancer induction (Hecht 1998). They are also present in unburned tobacco (Hecht 1998). According to IARC, both NNN and NNK were upgraded in 2012 based on mechanistic and other relevant data to Group 1 (the agent is carcinogenic to humans) (World Health Organization 2012). It is important to note that these TSNAs are present in both cigarette smoke and in unburned tobacco but not were not found in the aerosol generated from electronic cigarettes(Chemir Report 6/6/11).

Several PAHs are generated from the combustion of tobacco. Benzo(a)pyrene is used as the marker for carcinogenic PAHs (World Health Organization 2010) and is present in cigarette smoke at concentrations ranging from 20-40 ng (U.S.National Institutes of Health 2001). Similarly, as with NNN and NNK, according to IARC, benzo(a)pyrene was upgraded in 2012 based on mechanistic and other relevant data to Group 1 (the agent is carcinogenic to humans) (World Health Organization 2012). These carcinogenic PAHs were not detected in the aerosol generated from the Green Smoke electronic cigarette (Chemir Report 6/6/11).

[12] The limit of detection is the lowest quantity of a substance that can be distinguished from the absence of the substance in the equipment used.

With respect to carbon monoxide, it is present in mainstream smoke from conventional cigarettes and is found in high concentrations. The mainstream smoke from conventional tobacco cigarettes contain approximately 14-23 mg of carbon monoxide which is roughly 2.8-4.6% (percent of total effluent) (U.S.National Institutes of Health 2001). A major effect of carbon monoxide is to deprive the fetus of oxygen by binding to hemoglobin (US Department of Health and Human Services 2010). Evidence indicates that exposure to carbon monoxide leads to birth weight deficits and may play a role in neurologic deficits (cognitive and neurobehavioral endpoints) in the offspring of smokers (US Department of Health and Human Services 2010). In addition, carbon monoxide is thought to be a contributor to cardiovascular disease and other adverse health effects with prolonged exposure to elevated concentrations (Jenkins et al. 2000). It was considered a marker for exposure to environmental tobacco smoke but due to its low sensitivity, specificity, and duration after exposure limit its utility as a marker (Benowitz 1999).

Chemir tested the aerosol for the presence of carbon monoxide. They used a Drager gas detector pump with a carbon monoxide detector tube capable of detection levels as low as 2 ppm. Ten strokes of the gas detector pump were used to simulate the process of inhalation an electronic cigarette. A color change in the tube denotes a positive reaction. A blank with a laboratory control of car exhaust was also performed. The results showed that the aerosol generated from the tobacco cartomizers did not contain carbon monoxide at concentrations above 2 ppm (the limit of detection). Using the data from studies measuring the carbon monoxide content as a percent of the total effluent of mainstream smoke from burning tobacco, the carbon monoxide is present at concentrations ranging from 28,000 ppm to 46,000 ppm per cigarette[13] (U.S.National Institutes of Health 2001). This is at least over 4 orders of magnitude higher than the limit of detection in the aerosol generated from the electronic cigarette. Given that no measurable concentration of carbon monoxide was detected in the aerosol from electronic cigarettes (Chemir Report 6/6/11), the users are at no increased risk of adverse effects.

With respect to tar, the combustion product of cigarette tobacco smoke, it is usually determined by calculating the residual in the total particulates minus the nicotine and the water. The tar comprises the remainder of the particulates generated from the combustion of tobacco. In the electronic cigarettes, no tar is present because no tobacco is burned and there is no combustion process that occurs in the electronic cigarettes.

In summary, the agents known to produce deleterious effects in tobacco smokers, including tobacco specific nitrosamines, PAHs, carbon monoxide and tar, are not present in the electronic cigarettes from Green Smoke. The users of electronic cigarettes are at no increased risk of adverse health effects as compared to the users of conventional tobacco burning cigarettes. Based on this review, we contend that the reduction in tobacco risk with the use of electronic cigarettes is substantial and positive.

[13] 2.8%-4.6% carbon monoxide mainstream effluent per cigarette

Section V: Summary and Conclusions

A. It is widely accepted in the scientific community that inhaling the complex mixture of products in tobacco smoke is responsible for the adverse health effects seen in cigarette, pipe, and cigar smokers including cancer, cardiovascular disease and pulmonary disease.

B. Chemir Analytical Services using tobacco derived extracts and producing aerosols generated from the Green Smoke brand of electronic cigarettes found that the three major components present in the aerosol were propylene glycol, nicotine, and glycerin as identified with GC/MS.

C. Propylene glycol is considered non-toxic to humans following exposure by inhalation and poses no increased risk of adverse effects. There is no evidence in the published scientific literature that exposure to propylene glycol aerosol causes cancer, cardiovascular disease, or pulmonary disease.

D. Nicotine has also undergone extensive testing and is currently on the market as an aid to smoking cessation as an inhalable medical aerosol, gum, patch, and in various non-combustion forms (smokeless tobacco products) such as Swedish snus that are used to deliver nicotine. Nicotine-replacement therapy has been widely used and is well tolerated without evidence of serious adverse health effects. Nicotine *per se* causes minimal risk when separated from inhaling tobacco smoke.

E. Glycerin is considered non-toxic to humans following exposure by inhalation and poses no increased risk of adverse effects. There is no evidence in the published scientific literature that exposure to glycerin aerosol causes cancer, cardiovascular disease, or pulmonary disease.

F. The remainder of the tentatively identified components of the electronic cigarette aerosol at the relative percentages reported by Chemir should not be considered to be competent producing sources of toxicity. They are not likely to put a user of electronic cigarettes at any increased risk of harm or adverse effects.

G. Comparison of published studies delineating approximately 98 of the known hazardous tobacco smoke components revealed that none of these compounds were present in the aerosol generated from the Green Smoke brand of electronic cigarettes.

H. There is little evidence that absorption of ethanol, if it is indeed found to be present, following inhalation would lead to any measurable increased risk of cancer.

I. The data from human studies, genotoxicity studies, inhalation and drinking water exposure cancer studies support a conclusion that methanol, if it is indeed determined to be present, is not likely to be carcinogenic in humans.

J. The amount of methylene chloride in the aerosol produced from electronic cigarette usage is negligible and it not likely to pose any increased risk of cancer to the user.

K. The first study to investigate the comparative effects of tobacco burning cigarettes versus the effects of electronic cigarettes showed that the effect of TBCs on cardiac function revealed that there was a significant elevation in blood pressure (+8 % in systolic pressure and +6% in diastolic pressure) and heart rate (+10%) after smoking conventional cigarettes. Only a slight increase in diastolic pressure

(+4%) alone after electronic cigarette use was observed and no change in systolic pressure or heart rate was found to occur following electronic cigarette usage. The absence of combustion products from not burning tobacco provides a safer alternative to conventional tobacco burning cigarettes and that the substitution of electronic cigarettes for TBCs is likely to be beneficial to health, at least with respect to cardiac function.

L. The TSNAs, including NNN and NNK, are present in both burned tobacco cigarette smoke and in unburned tobacco. The evidence is strong that they play an important role in cancer induction. These elements were not found to be present in the aerosol generated from Green Smoke electronic cigarettes.

M. Several PAHs including Benzo(a)pyrene are known to be present in cigarette smoke due to the combustion of tobacco. The evidence is strong that they play an important role in cancer induction. Noteworthy is the fact that these elements were not found in the aerosol generated from Green Smoke electronic cigarettes.

N. Carbon monoxide is a known reproductive and developmental toxicant and it also thought to be a contributor to cardiovascular disease. Carbon monoxide was not detected at the minimum limit of detection (2 ppm) in the aerosol generated from the electronic cigarette and this "finding" is at least four orders of magnitude lower than the concentration ranges observed in mainstream tobacco smoke. Given that no substantial concentration of carbon monoxide was detected in the aerosol from electronic cigarettes, the users of electronic cigarettes are at no increased risk of adverse effects from this combustion related toxicant.

O. This evaporative aerosol technology is new and unique. However, it is based upon knowledge from the peer-reviewed, published scientific data and reports which predate this systems' development. This information on health is clear on the lack of adverse health hazards that are associated with components of the aerosols generated from Green Smoke electronic cigarettes. This knowledge, coupled with the known toxicity and human health hazards including cancer, COPD and cardiovascular disease that are causally associated with tobacco burning cigarettes, permit the following to be our considered opinion that, to a high degree of toxicologic certainty, the use by adult smokers of the electronic cigarettes described herein does provide a safer alternative to those who desire to continue to consume nicotine derived from tobacco, namely persons who choose to smoke as well as those who enjoy the smoking process. Similarly, the product is a safer alternative to those persons who may choose to use this product as a means to decrease their use of tobacco burning products.

We reserve the right to amend, edit or supplement this report as new data becomes available and provides a meaningful insight into the observations that we have recorded.

Table 1: SPME GC/MS analysis of the aerosol generated from sample absolute tobacco cartomizers

COMPOUND	CAS #	MOLECULAR FORMULA	RELATIVE % AREA
methyl alcohol	67-56-1	CH_4O	0.185%
ethyl alcohol	64-17-5	C_2H_6O	1.106%
methylene chloride	75-09-2	CH_2Cl_2	0.148%
carbonic acid, dimethyl ester	616-38-6	$C_3H_6O_3$	0.108%
heptane	142-82-5	C_7H_{16}	0.632%
carbonic acid, ethyl-, methyl ester	623-53-0	$C_4H_8O_3$	0.044%
2-propanone, 1-hydroxy-	116-09-6	$C_3H_6O_2$	0.270%
propylene glycol	57-55-6	$C_3H_8O_2$	84.430%
2-propanone, 1-hydroxy-	116-09-6	$C_3H_6O_2$	0.150%
glycerin	56-81-5	$C_3H_8O_3$	4.749%
2(3H)-furanone, 5-butyldihydro-	104-50-7	$C_8H_{14}O_2$	0.078%
nicotine	54-11-5	$C_{10}H_{14}N_2$	7.574%
6-quinolinamine, 2-methyl-	65079-19-8	$C_{10}H_{10}N_2$	0.527%

Source: Chemir Analytical Services report (6/6/11)

Table 1 Re-ordered by relative % area

Compound	**CAS#**	**Molecular Formula**	**Relative % area**
propylene glycol	57-55-6	$C_3H_8O_2$	84.430%
nicotine	54-11-5	$C_{10}H_{14}N_2$	7.574%
glycerin	56-81-5	$C_3H_8O_3$	4.749%
ethyl alcohol	64-17-5	C_2H_6O	1.106%
Heptane	142-82-5	C_7H_{16}	0.632%
6-quinolinamine, 2-methyl	65079-19-8	$C_{10}H_{10}N_2$	0.527%
2-propanone, 1-hyroxy-	116-09-6	C_3H_6O2	0.270% or 0.15%
Methyl alcohol	67-56-1	CH_4O	0.185%
Methylene chloride	75-09-2	CH_2Cl_2	0.148%
Carbonic acid, dimethyl ester	616-38-6	$C_3H_6O_3$	0.108
2(3H)-furanone, 5-butyldihydro-	104-50-7	$C_8H_{14}O_2$	0.078%
Carbonic acid, ethyl, methyl ester	623-53-0	$C_4H_8O_3$	0.044%

Figure 1: Sample of Green Smoke Starter kit

Figure 2: Sample of v2cigs starter kit

Agusti,A. and Barnes,P.J. 2012. Update in Chronic Obstructive Pulmonary Disease 2011, *Am J Respir Crit Care Med* 185: 1171-1176.

American Chemistry Council's Propylene Oxide/ Propylene Glycol Panel 2001. Propylene Glycol Does Not Pose a Cancer Risk, *American Chemistry Council* 1-2.

American Council on Science and Health 2005. *America's War On "Carcinogens" Reassessing the Use of Animal Tests to Predict Human Cancer Risk*, ACSH, New York.

Archontogeorgis,K., Steiropoulos,P., Tzouvelekis,A., and et al 2012. Lung Cancer and Interstitial Lung Diseases: A Systematic Review, *Pulm Med* 1-11.

ATSDR 2008. Addendum to the Toxicological Profile for Propylene Glycol, *ATSDR* 1-15.

Baan,R., Straif,K., Grosse,Y., Secretan,B., and et al 2007. Carcinogenicity of alcoholic beverages, *Lancet Oncol* 8: 292-293.

Benowitz,N.L. 1999. Biomarkers of environmental tobacco smoke exposure, *Environmental Health Perspectives.* 107: 349-355.

Benowitz,N.L. 2010. Nicotine Addiction, *The New England Journal of Medicine* 362: 2295-2303.

Benowitz,N.L. and Gourlay,S.G. 1997. Cardiovascular Toxicity of Nicotine: Implications for Nicotine Replacement Therapy, *JACC* 29: 1422-1431.

Bevan,R.J., Slack,R.J., Holmes,P., and Levy,L.S. 2009. An Assessment of Potential Cancer Risk Following Occupational Exposure to Ethanol, *Journal of Toxicology and Environmental Health, Part B* 12: 188-205.

Burek,J.D., Nitschke,K.D., Bell,T.J., and et al 1984. Methylene Chloride: A Two-Year Inhalation Toxicity and Oncogenicity Study in Rats and Hamsters, *Fundam.Appl.Toxicol.* 4: 30-47.

Campagna,D., Caponnetto,P., Papale,G., Polosa,R., and Russo,C. 2012. The emerging phenomenon of electronic cigarettes, *Expert Review of Respiratory Medicine* 6: 63-74.

Caponnetto,P., Polosa,R., Auditore,R., Russo,C., and Campagna,D. 2011. Smoking Cessation with E-Cigarettes in Smokers with a Documented History of Depression and Recurring Relapses, *International Journal of Clinical Medicine* 2: 281-284.

Cardinale,A., Nastrucci,C., Cesario,A., and Russo,P. 2012. Nicotine: specific role in angiogenesis, proliferation and apoptosis, *Crit Rev Toxicol* 42: 68-89.

ClinicalTrials.gov 2012. Efficacy and Safety of an Electronic Nicotine Delivery Device (E-Cigarette), *Clinical Trials.Gov* 1-4.

Cobble,M.E. 2012. Coronary Heart Disease in Men, *J Fam Pract* 61: S29-S33.

Coggins,C.R., Edmiston,J.S., Jerome,A.M., Langston,T.B., Sena,E.J., Smith,D.C., and Oldham,M.J. 2011a. A comprehensive evaluation of the toxicology of cigarette ingredients: essential oils and resins, *Inhalation Toxicology* 23: 41-69.

Coggins,C.R., Jerome,A.M., Edmiston,J.S., and Oldham,M.J. 2011b. A comprehensive evaluation of the toxicology of cigarette ingredients: aliphatic carbonyl compounds, *Inhalation Toxicology* 23: 102-118.

Coggins,C.R., Liu,J., Merski,J.A., Werley,M.S., and Oldham,M.J. 2011c. A comprehensive evaluation of the toxicology of cigarette ingredients: aliphatic and aromatic carboxylic acids, *Inhalation Toxicology* 23: 119-140.

Coggins,C.R., Sena,E.J., Langston,T.B., and Oldham,M.J. 2011d. A comprehensive evaluation of the toxicology of cigarette ingredients: aromatic carbonyl compounds, *Inhalation Toxicology* 23: 90-101.

Coggins,C.R., Wagner,K.A., Werley,M.S., and Oldham,M.J. 2011e. A comprehensive evaluation of the toxicology of cigarette ingredients: carbohydrates and natural products, *Inhalation Toxicology* 23: 13-40.

Cohen,B. and Crandall,C. 1964. Physiologic benefits of "Thermo Fog" as a bronchodilator vehicle: acute ventilation response, *Am.J.Medical Sciences* 247: 57-61.

Cruzan,G. 2009. Assessment of the cancer potential of methanol, *Crit Rev Toxicol* 39: 347-363.

Emsley,J. 1994. *The Consumer's Good Chemical Guide A Jargon-free Guide to the Chemicals of Everyday Life*, W.H. Freeman and Company Limited, New York.

Etter,J.-F. 2010. Electronic Cigarettes: a survey of users, *BMC Public Health* 10: 1-7.

Etter,J.-F. and Bullen,C. 2011. Electronic cigarette: users profile, utilization, satisfaction and perceived efficacy, *Addiction* 106: 2017-2028.

Fabbri,L.M., Luppi,F., Beghe,B., and Rabe,K.F. 2006. Update in Chronic Obstructive Pulmonary Disease 2005, *Am J Respir Crit Care Med* 173: 1056-1065.

Farsalinos,K., Tsiapras,D., Kyrzopoulos,S., and et al 2012. Acute effects of using an electronic nicotine-delivery device (3-cigarette) on myocardial function: comparison with the effects of regular cigarettes, *www.escardio.org 2012* 1-7.

Flouris,A.D., Poulianiti,K.P., Chorti,M.S., and et al 2012. Acute effects of electronic and tobacco cigarette smoking on complete blood count, *Food and Chemical Toxicology* 1-4.

Friedlander,B.R., Gearbem,T., and Hall,S. 1978. Epidemiologic investigation of employees chronically exposed to methylene chloride, *J.Occup.Med.* 20: 657-666.

Gaworski,C.L., Oldham,M.J., Wagner,K.A., Coggins,C.R., and Patskan,G.J. 2011. An evaluation of the toxicity of 95 ingredients added individually to experimental cigarettes: approach and methods, *Inhalation Toxicology* 23: 1-12.

Goniewicz,M.L., Kuma,T., Pharm,M., and et al 2012. Nicotine Levels in Electronic Cigarettes, *Nicotine and Tobacco Research Advance Access* 1-9.

Hardin,B.D. and Manson,J.M. 1980. Absence of Dichloromethane Teratogenicity with Inhalation Exposure in Rats, *Toxicology and Applied Pharmacology* 52: 22-28.

Health Council of the Netherlands 2006. Ethanol (ethyl alcohol): Evaluation of the health effects from occupational exposure, *The Hague: Dutch Expert Committee on Occupational Standards* 1-186.

Hecht,S.S. 1998. Biochemistry, Biology, and Carcinogenicity of Tobacco-Specific N-Nitrosamines, *Chemical Research in Toxicology* 11: 559-603.

International Agency for Research on Cancer 1999. Dichloromethane (Group 2B), *www.inchem.org* 71.

Jenkins,R.A., Guerin,M.R., and Tomkins,B.A. 2000. *The Chemistry of Environmental Tobacco Smoke: Composition and Measurement*, Lewis.

Johnson Creek Enterprises 2009. Characterization of Liquid :Smoke Juice" for Electronic Cigarettes, *Alliance Technologies* 1-6.

Lakind,J.S., McKenna,E.A., Hubner,R.P., and Tardiff,R.G. 1999. A Review of the Comparative Mammalian Toxicity of Ethylene Glycol and Propylene Glycol, *Critical Reviews in Toxicology* 29: 331-365.

Le Houezec,J., McNeill,A., and Britton,J. 2011. Tobacco, nicotine and harm reduction, *Drug Alcohol Rev* 30: 119-123.

Lightwood,J.M. and Glantz,S.A. 1997. Short-term Economic and Health Benefits of Smoking Cessation: Myocardial Infarction and Stroke, *CIRCULATION* 96: 1089-1096.

MacCannell 1969. Hemodynamic responses to glycols and to hemolysis, *Can.J.Physiol.Pharmacol.* 47: 563-569.

Pappas,R.S. 2011. Toxic elements in tobacco and in cigarette smoke: inflammation and sensitization, *Metallomics* 3: 1181-1198.

Phillips,C.V. and Heavner,K.K. 2009. Smokeless tobacco: the epidemiology and politics of harm, *Biomarkers* 14: 79-84.

Polosa,R., Caponnetto,P., Morjaria,J.B., and et al 2011. Effect of an electronic nicotine delivery device (e-Cigarette) on smoking reduction and cessation: a prospective 6-month pilot study, *BMC Public Health* 11: 1-12.

R.J.Reynolds Tobacco Company 1988. *New Cigarette Prototypes that Heat Instead of Burn Tobacco*, Winston,-Salem, N.C.

Rioux,J.P. and Myers,R.A.M. 1988. Methylene Chloride Poisoning: A Paradigmatic Review, *J Emerg Med* 6: 227-238.

Robertson,O.H., Loosli,C.G., Puck,T.T., and et al 1947. Tests for the chronic toxicity of propylene glycol and triethylene glycol on monkeys and rats by vapor inhalation and oral administration, *J.Pharmacol.Exp.Ther.* 91: 52-76.

Russo,P., Cardinale,A., Margaritora,S., and Cesario,A. 2012. Nicotinic receptor and tobacco-related cancer, *Life Sciences* In Press: 1-6.

Russo,P., Cesario,A., Rutella,S., Veronesi,G., and et al 2011a. Impact of Genetic Variability in Nicotinic Acetylcholine Receptors on Nicotine Addiction and Smoking Cessation Treatment, *Current Medicinal Chemistry* 18: 91-112.

Russo,P., Nastrucci,C., Alzetta,G., and Szalai,C. 2011b. Tobacco Habit: Historical, Cultural, Neurobiological, and Genetic Features of People's Relationship with an Addictive Drug, *Perspectives in Biology and Medicine* 54: 557-577.

Schwetz,B.A., Leong,B.K.J., and Gehring,P.J. 1975. The Effect of Maternally Inhaled Trichloroethylene, Perchloroethylene, Methyl Chloroform, and Methylene Chloride on Embryonal and Fetal Development in Mice and Rats, *Toxicology and Applied Pharmacology* 32: 84-96.

Suber,R.L., Nikiforov,A.I., Fouillet,X., and et al 1987. Subchronic Inhalation Study of Propylene Glycol in Rats, *Toxicologist* 7: 199.

Talhout,R., Schulz,T., Florek,E., van Benthem,J., Wester,P., and Opperhuizen,A. 2011. Hazardous Compounds in Tobacco Smoke, *Int.J.Environ Res Public Health* 8: 613-628.

Trehy,M.L., Ye,W., Hadddwiger,M.E., Moore,T.W., and et al 2011. Analysis of Electronic Cigarette Cartridges, Refill Solutions, and Smoke for Nicotine and Nicotine Related Impurities, *Journal of Liquid Chromatography & Related Technologies* 34: 1442-1458.

U.S.National Institutes of Health 2001. *Risks Associated with Smoking cigarettes with Low Machine-Measured Yields of Tar and Nicotine*, NIH Publication No. 02-5057.

US Department of Health and Human Services 1990. The Health Benefits of Smoking Cessation: A Report of the Surgeon General, *CDC* 1-29.

US Department of Health and Human Services 2010. How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking - Attributable Disease, *U.S Department of Health and Human Services* 1-9.

Vansickel,A.R., Weaver,M.F., and Eissenberg,T. 2012. Clinical laboratory assessment of the abuse liability of an electronic cigarette, *Addiction* **107**: 1493-1500.

Wagener,T., Siegel,M., and Borrelli,B. 2012. Electronic cigarettes: Achieving a balanced perspective, *Addiction* 107: 1545-1548.

Westenberger,B.J. 2009. Evaluation of e-cigarettes, *FDA Department of Health & Human Services* 1-8.

World Health Organization 2010. WHO Study Group on Tobacco Product Regulation Report on the Scientific Basis of Tobacco Product Regulation, *World Health Organization* Series # 955: 1-41.

World Health Organization 2011. Tobacco Fact Sheet N339, *Who, Geneva Switzerland* 1-2.

World Health Organization 2012. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, *World Health Organization, Lyon, France* 1-33.

www.fda.gov 2012a. GRAS Substances for Glycerin and Glycerides, *Select Committee on GRAS Substances* 1-17.

www.fda.gov 2012b. GRAS Substances for Propylene Glycol, *U.S.Food and Drug Administration* 1-27.