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Risk Assessment of E-Liquid Components and Their Reactions to Heating

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Abstract : Electronic cigarette (e-cig) is a new phenomenon in the world, which is considered as an alternative for overcoming dependency on cigarette but is also criticized for its safety. The link between the triggering factor and e-cig safety mechanism and risk is not yet clear and so far there has been no theoretical basis that verifies e-cig as a less-harmful substitute to cigarette. A fundamental study needs to be conducted, covering a toxicological analysis of e-liquid components and assessment of their degradation potential due to heating.
A study on 9samples of e-liquid available in Bandung shows that nearly all e-liquid manufacturers list substances categorized as food grade as the ingredients (USP propylene glycol, USP glycerin, artificial/natural flavoring/sweeteners, distilled water).However, the result of HPLC reversed-phase chromatography shows that the substance profiles of 7out of 9e-liquid samples underwent product degradation due to e-cig device heating, in term of increase and decrease in substance concentration.
Keywords : Risk Assessment, E-Liquid, Heating.

Introduction

Electronic cigarette (e-cig) is a new phenomenon in the world including Indonesia. Its presence is considered by some as an alternative for overcoming dependency on cigarette**,** but itis also criticized for its safety factor and health impact on its users. E-cigarette is a smoking simulation device through inhalation of nicotine vapor, propylene glycol, glycerine, and flavorings.

Studies on the safety and risk of the chemical contents of e-cig vapor found no presence of acrolein, phenol (respiratory organ irritant), and *PAH* (carcinogenic substance), but they found acetaldehyde and

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formaldehyde (carcinogenic substance and respiratory organ and eye irritant) in small amounts, traces of TSNAs/Tobacco-specific nitrosamines (carcinogenic substance originating from tobacco nicotine) which is equal to NRT/nicotine replacement therapy, carbon monoxide, VOC (volatile organic compounds, which is mucous irritant and may cause headache and nausea), diethylene glycol, tadalafil, rimonabant, and metals such as cadmium, nickel, and lead in e-cig vapor/aerosol[1-8]. Study shows that e-cig vapor pollution has minimal impact on room air quality, and the vapor chemical compound analysis shows no health risk[9]. Clinical study on e-cig shows moderate symptoms (mouth and throat irritation, dry cough, throat inflammation)[10], increase in parameters of acute inflammation condition (white blood cells, neutrophil, and lymphocyte)[11,12]is not observed, serious adverse impact on health is not observed[13]. E-cig consumption by volunteers who have increased blood pressure after consuming cigarettes resulted in the drop of systolic and diastolic blood pressure and their heart pressure due to the low nicotine levels in e-cig[14]. The above information shows that e-cig can be a far less harmful substitute to cigarettebecause it prevents its users from getting exposed to toxic chemicals from cigarette burning and toxic contaminants of tobacco processing[15-17].

However, the unclear link between the triggering factor and e-cig safety mechanism and risk, and the lack of theoretical basis that verifies e-cig as a less harmful substitute to cigarette necessitate a further systematic, fundamental, and comprehensive study to build a solid theoretical foundation[18,19].

Experimental

Based on the criteria used for e-liquid sample, i.e. frequently used e-liquids with the cheapest price and most expensive price in Bandung, 9 brands of e-liquids were collected and Tobacco Heating System, *Tobacco Stick* (Philips & Morris) as a control.

A. Assessment on the results of the toxicology analysis of the constituent substance composition of the e-liquid.

The assessment was performed according to the approach stated in *Risk assessment approach for E-cigarette flavours*by Costigan et al (2014)which included[20]:

- Analysis on whether the ingredients written down on the package of the e-liquid are food grade;
- Further analysis on whether those ingredients are carcinogenic, mutagenic, toxic to reproduction, or teratogenic (CMR) or may cause sensitization of the respiratory tract; and
- Toxicology analysis of the substance, if substances with CMR nature were found.

This screening resulted in an analysis output to show whether the ingredients can or cannot be used as the ingredients of the e-liquid.

B. Assessment on the degradation potentials of the e-liquid components when exposed to heating:

Each e-liquid sample was analyzed for its substances using the High Performance Liquid Chromatography (HPLC) Reversed Phase Gas Chromatography, which is one of the analysis techniques and chromatography separation for liquid using a high-pressure pumping system. HPLC is used to separate components in liquid sample based on the difference in it affinity to liquid phase in the column. Analyses were performed using HPLC include qualitative and quantitative analyses. In this study, the qualitative analysis showed the amount of component mix in the sample and an increase or a reduction in the concentration of the component in the sample after heating.

Sample Preparation:

1. E-liquid sample without heating: 0.1 mL of sample was pipetted and diluted with 1 mL of 10% methanol. The solution was homogenized and ready to be injected into HPLC.

2. E-liquid sample with heating: the e-liquid sample was heated using the e-cigarette device and the vapor produced was sucked in by a vacuum and trapped using 1:10 of 10% methanol. The diluted vapor was ready to be injected into HLPC.

Chromatography Conditions (HPLC):

- Column: LichrocartC18 (5µm)
- Moving phase: Phosphate Buffer, PH 6.8:Methanol (35:65)
- Flow rate: 0.6 mL/minute
- Injection Volume: 20 µL
- Detector: UV 259 nm

Result and Discussion

A. Assessment on the results of the toxicology analysis of the constituent substance composition of the e-liquid:

Nine e-liquid 'brewers'(9 brands) that were collectedattached written label that the ingredients used werefood grade, including USP Propylene Glycol,USP Glycerin Natural/Vegetable, Artificial/Natural Flavoring/Sweeteners,Distilled Water.

Some important notes regarding the e-liquid components above are [21,22]:

1. Propylene Glycol (PG):

Many authors agreed and stated that PG is commonly used in food and drug industries (McCormick/flavoring, toothpaste, cough syrup, hand sanitation liquid, lotion, cosmetics, etc.). The health risk due to e-liquid that contains PG tends to be limited to throat irritation. However, chronic exposure to PG in room air for children has the possibility to worsen or induce rhinitis, asthma, eczema, and allergic reactions. The acute and chronic respiratory effects include reduced lung function which is only observed in people who are exposed to PG-containing theater fogs for years. It also has a drying effect, leading to occasional dryness of the mouth and throat of e-cigarette users. PG in e-liquid has a possibility to be oxidized by heating during use; however, in a very high extreme temperature, this substance forms methyl glyoxal, formaldehyde, and acetaldehyde that potentially disturb health by triggering hypertension, asthma, chronic obstructive lung diseases, pneumonia lipoid, arrhythmia of the heart, pneumonia, congestive heart failure, disorientation, hypotension, irritation of mouth and throat, coughing, nausea, and vomiting due to the nature of PG that is easily soluble in water. There are only few studies analyzed the health impact of e-cigarette vapor in regards of the safety of PG. It is only mentioned that a short exposure to PG causes irritations of the eyes, skin, and respiratory tract and that a long exposure to PG will affect the central nervous system. If swallowed, PG may cause lactate acidosis and seizure. Data on toxicity in human show that the administration of 500 mg PG on the skin for 7 days will lead to mild irritation. ACGIH, IARC, NTP, or CA Stated that PG is not listed as a carcinogen and, in general, PG does not affect fertility or reproductive organ functions.

2. Glycerin:

E-liquid uses glycerol from the purification of vegetable glycerin, which is a non toxic substance. This substance has alcohol primary groups and a secondary alcohol group that may experience oxidation. Glycerin is a result of fat acid separation and is frequently used in cosmetic industries as, among others, a substance to control shampoo consistency, mouthwash, and toothpaste. In tobacco processing, glycerin is an important part of the liquid sprays to the tobacco leaves before they are grinded and packaged. Since 1959, glycerin is considered safe by FDA. Glycerin is easily digested and is not toxic; it is metabolized together with carbohydrates. When heated in an extreme high temperature, glycerin will decompose into an aldehyde carbonyl complex that is regarded as a carcinogenic substance, acrolein, and carbon oxides. This, nevertheless, is still controversial due to a report that glycerin is not degraded/changed under 900^{o}C heating. Observations on the impact of glycerol on the activity of the e-cigarette users show traces of respiratory tract irritation and increased heart rate of an average 8 times higher after 5 minutes, which is followed by a comfortable feeling. In a high temperature, glycerol vapor may cause irritation or irritation of mucous membrane. An exposure to concentrated glycerin on the skin leads to mild irritation effect, dehydration, rash, and, rarely, allergic reaction in sensitive individuals. Glycerin is not considered as carcinogenic and does not have any adverse effect on reproductive organs.

3. Flavors:

The flavor used in e-liquid is diacetyl, a substance that has been linked to bronchiolitis obliterans (a condition known as "popcorn lung"). Dyacetil is usually used as an artificial flavor in butter. E-liquid also uses thujone (α-/ß-diastereomers) and ethyl vanillin flavors. The danger of this substance to health may include acute poisoning, skin irritation, serious eye irritation, and specific target organ toxicity. Symptoms related to the toxicology characteristics of Ethyl Vanillin are severe eye irritation, respiratory tract irritation, and skin irritation. Ethyl Vanillin is not considered as carcinogenic, teratogenic, or causing adverse effects on reproductive parameters.

B. Assessment on the degradation potentials of the e-liquid components when exposed to heating:

Each component or compound has a specific retention time in the HPLC system and the size of the component peak shows the amount of concentration in the sample. The table below describes the concentration of sample components under the above chromatography condition.

In sample 1, it was observed that before heating the chromatogram of the sample showed 6 components with relatively low dominant peaks, meaning that these components had quite low concentrations. After heating, the chromatogram showed several new peaks. The concentration of the pre-heating components seemed to be drastically increased, which was marked by the peak height and peak size in the chromatogram. This means that, qualitatively, there was a degradation of components of the product along with increased concentration of the components.

In sample 3, it was observed that before heating, the chromatogram peaks were quite various based on the overlaps of several peaks (more than one component had a close retention time that before the peak was totally separated, another peak was already formed). After heating, a new peak that was not detected in the pre-heating chromatogram appeared. Based on the change in the peak size, the majority of the components have an average of two times increase. This shows that, qualitatively, there has been a degradation of the product components by heating and that the concentration increases twice for each component.

In sample number 4, it was observed that before heating there were quite many components detected in the chromatogram. After heating, a significant change happened to the peak retention time of 6.3, which was a reduction of around 50% of the peak size. The number of components that experienced reduction and increase in the peak size was the same, showing that qualitatively there is no degradation of the product due to heating.

In sample 6, the component with a retention time of around minute 6.3 was seen to have the biggest peak size. However, after heating, there was a peak reduction of around 50% with the appearance of new peaks. This shows that, qualitatively, there is a thermal degradation of the e-liquid components.

In sample 10, there were not many components seen before heating and some peaks were not separated totally with 2 dominant peaks. After heating, there was a reduction in the sample composition shown by the reduction of the peak size. A new peak was detected at retention time 25.7 with a quite low composition. This shows, qualitatively, that a reduction in component concentration happens but with product degradation due to heat and formation of a new component.

All producers of the e-liquid samples collected in Bandung City claimed that they use food grade ingredients that, toxicologically, are not considered as carcinogenic-teratogenic-mutagenic-genotoxic substances and do not affect fertility functions. Hence, they seem to be able to meet the health safety requirements for e-liquid components.

The HPLC chromatography analysis on e-liquid samples from Bandung shows that in general the heated e-liquid samples were degraded when compared to the pre-heating samples, except for several samples that, after observation, were seen not producing new component, including THS (Phillip Morris International) as control.

The results of the overall risk assessment on e-liquid show that the 9 samples collected in Bandung,Indonesia attached written labels claimed that they used food grade ingredients. However, the confirmation analysis using risk assessment of reaction products and thermal breakdownproducts shows contrary results with a possibility that 7 samples may use non-food grade ingredients that were degraded by heating when they are used with the e-cigarette device. The degradation leads to a formation of a new substance and increaseor decreasethe concentration of other e-liquid components.

In conclusion, our study has shown that e-liquid manufacturer or brewer would demand quality control procedures by official authorities similar to other consumer products such as tobacco cigarettes to ensure their safety for the consumers.

Table Sample component concentration before and after heating

Sample	Before Heating	After Heating
1	In the chromatogram, it was shown that the sample was separated into 6 components with dominant peaks. Based on the peak height of each component that was relatively low, the components had a quite low concentration	After heating, some new peaks appeared in the chromatogram. The concentration of the components previously detected increased drastically, which was obvious from the peak height, especially the size of the peak.
2	In the chromatogram, there are 6 peaks detected with concentration variation shown by the size of the peak of each component. The peak size during the retention time of minute 9.3 shows the biggest composition of the sample.	Changes were seen after heating in the form of increased peak size for each component, meaning that the concentration in the sample increased. The size of the change was different for each peak. Another significant change was the biggest component in the sample shown by the retention time peak of minute 5.5.
3	The peaks shown in the chromatogram were quite various. There were some overlapped peaks which were probably due to the presence of more than one component that had close retention times so that before the peak was totally separated, another peak was already formed.	In the chromatogram, a new peak that was not detected in the pre-heating chromatogram appeared. Based on the change in the peak size, the majority of components had an increase of average two times higher. Reduction in the peak size was seen in the retention time component in minute 7.2 and 15.9.
4	Components detected in this chromatogram were quite many. The highest composition was shown by a peak in the retention time of around minute 6.3. The remaining components had almost similar compositions.	A significant change happened to a peak in the retention time of minute 6.3 with a reduction of the peak size of around 50%. The number of components that experienced a decrease and in increase in peak size was the same.
5	There were some overlapped peaks in the retention time around minute 5 to minute 11. This might be due to the similar polarity of many of the sample components, hence close retention times.	Overlapped peaks were still seen in the chromatogram after heating. To avoid it, further purification or modification of HPLC system conditions would be needed. It was difficult to interpret the overlapped peaks. In general, with heating, the majority of component peak sizes reduced.
6	The component in the retention time of minute 6.3 has the biggest peak size. Peak overlapped was seen in the component with a retention time of minute 9.	Reduction of around 50% happened in the peak time of the retention time in minute 6.3. After heating, new peaks appeared in the retention time of minute 11.3, 15.9, 23.3.
7	Sample composition was dominated by the peak in the retention time of minute 5.6 and 6.4. Other component peaks were detected in a very small concentration.	The peak size during the retention time of minute 5.6, 6.4, and 9.9 experienced a quite significant increase. The peak at the retention time of minute 23.4 experienced a reduction in the peak size that it was not detected by the detector.
8	The peak size at the retention time of minute 6.5 was the biggest, meaning that the biggest composition for this sample was found in the compound with a retention time of 6.5. The compounds that could be read in this sample were only 4 compositions.	A new peak with a retention time of 5.6 appeared with a quite big peak size. Othe compositions of this sample tended to be reduced, which was marked by the reduction in peak size.
9	Peak overlaps was seen in around retention time of 5.5 that it was difficult to integrate the peak size in an accurate enough manner. The component with the retention time of 6.5 had a very big peak size that it reached 50% of the sample composition.	A quite significant reduction was seen at the retention time of 9.6. In general, all components experienced reduction in the peak size except for the composition in retention time of 25.7 that experienced an increase.
10	There were not many components in this	In general, the composition in the sample was reduced by

	sample. Some peaks could not be totally separated. There were two dominant peaks in the sample composition.	heating, which was marked by the reduction in the peak size. A new peak appeared, which was detected at a retention time of 25.7.

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