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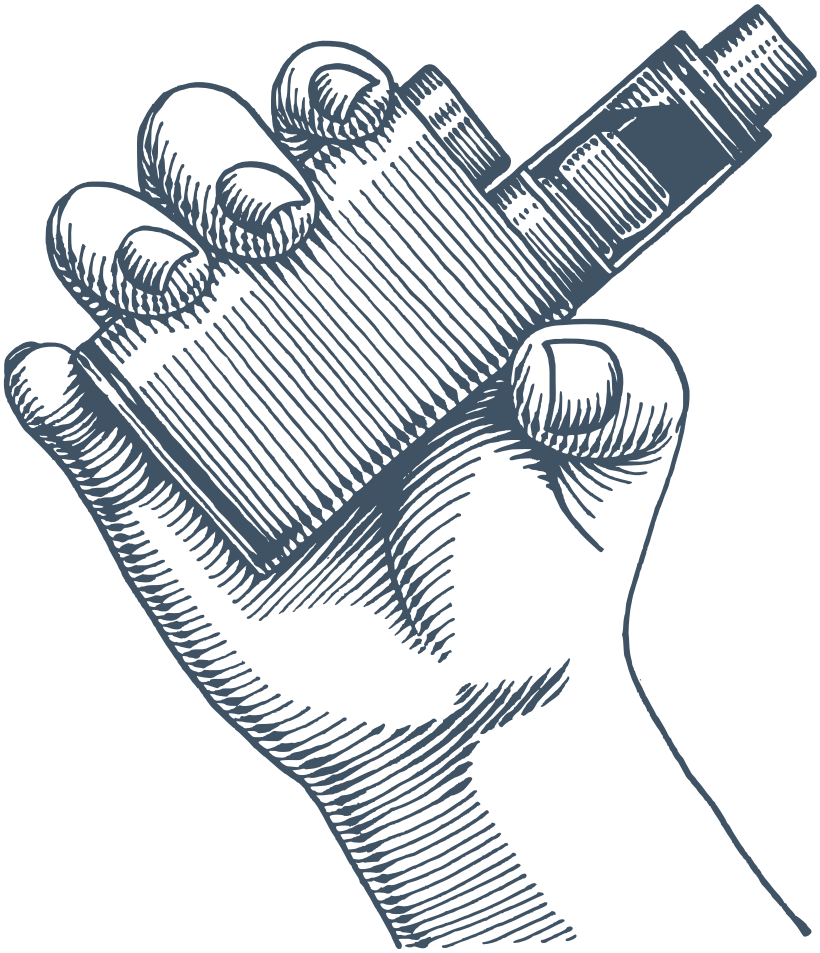


SAFETY ASSESSMENT OF E-CIGARETTES IN THE EU

Authors:
Pradeep Kumar and Shrutismita

1. Introduction

Electronic cigarettes (EC) are defined as products that can be used for the consumption of a nicotine-containing aerosol via a mouthpiece, or any component of that product, including a cartridge, a tank, and the device without a cartridge or tank [1]. EC and heated tobacco products (HTP) are becoming popular alternatives to cigarettes in several countries. For example, since their introduction, HTPs have partially replaced cigarettes in Japan, while the prevalence of EC has risen in the United States and other countries. EC aerosols are generated from the heating of liquids, and HTP aerosols are generated from the heating of tobacco at temperatures far below the combustion temperatures observed for cigarettes. Consequently, lower concentrations of harmful and potentially harmful constituents (HPHC) in their aerosols are measured when compared to mainstream smoke from reference or commercial cigarettes [2]. An EC is a part of an emerging class of electronic nicotine-delivery systems (ENDS) that aerosolize nicotine and produce a vapor that emulates that of tobacco cigarettes but purportedly has fewer traditional harmful substances than second-hand smoke [3]. For the first time, EU Directive Article 20 of the Tobacco Products introduces a comprehensive regulatory framework for ECs with a focus on safety, quality, consumer protection, and collection of information. EC are recent products in the EU market, and evidence concerning their potential risks and benefits is emerging [1].

2. Background and mechanism

The EU Commission and Member States are monitoring scientific evidence, user profiles, and market developments regarding all EC, given their increased usage. Open questions for the use of EC are:

- Adverse health effects (short- and long-term effects) caused due to EC
- Role of EC as a gateway to smoking/the initiation of smoking (mainly
- focusing on young people)
- Role of EC in harm reduction/cessation of traditional tobacco smoking [1]

Currently, there are five generations of EC in the EU [1]

- First-generation devices: cig-like devices have the most physical resemblance to traditional cigarettes. They afford the least amount of user control over heating. Nicotine delivery is not as efficient as compared to newer devices.
- Second-generation models: larger, enable voltage adjustment by users, and higher-capacity lithium-ion rechargeable batteries.
- Third generation: Removable and rechargeable large batteries (external) with user control (both voltage and wattage) tanks containing more e-liquid that is heated at higher temperatures. These models often contain sub-ohm resistance heating coils that aid users in generating relatively large aerosol volumes.
- Fourth generation: Enable control over the temperature of the heating coil.
- Fifth generation: Use changeable, nicotine salt-based liquid cartridges and temperature regulation to produce an aerosol as an alternative to traditional cigarettes [1].

3. Safety requirements of EC as per EU

EC regulated as medicines may be made available in strengths and volumes greater than those permitted under the Tobacco and Related Products Regulations (TRPR) (i.e., containing more than 20 mg/ml nicotine, more than 2 ml for single use cartridge/disposable products or more than 10 ml for refill containers) and follows the EU MDR safety regulation 2002. Lesser volumes or strengths fall under consumer products [4].

For assessing the safety, the toxicological consequences of heating and vaporizing the formulation of nicotine and excipients during the normal use of the product need to be considered. For example, particular concern has been raised in studies about the presence of acrolein and other carbonyls, such as formaldehyde and acetaldehyde, that can be produced as a consequence of the thermal decomposition of glycerol and propylene glycol. Analytical chemistry data should be used to confirm the compounds present in the vapor produced by an e-cigarette device under its normal operating conditions . Information on the potential toxicity of any degradation products at relevant exposure levels and all routes of exposure should be provided [4].

Example for Point of Departure (PoD) selection:

Glycerol: The most appropriate study for PoD derivation is the rat experiment. The main effects consisted of local irritation when rats were exposed to 662 mg/m^3, 6 hours/day, 5 days/week for 13 weeks reported squamous metaplasia of the epithelium lining of the epiglottis. No toxic effects were reported at an exposure concentration of 165 mg/m^3. No systemic effects were reported in this study or in a study with rats exposed to concentrations of up to 3910 mg/m^3, 6 hours/day, 5 days/week for 14 days. Hence, the No Observed Adverse Effect Level of 165 mg/m3 for a 6-hour exposure was chosen as PoD for glycerol [5].

4. Exposure Assessment

Due to the large number of devices and liquids present in the market and also a large variation in individual exposures due to the variability in concentrations in the inhaled aerosol, duration of exposure, frequency of exposure events (electronic cigarette use sessions), and the frequency of inhalation during sessions of electronic cigarette use are great challenges for the exposure assessment for users of EC and those exposed to exhaled air from these users.

Aerosol characteristics play a major role in exposure assessment, composed of droplets of e-liquids, which contain mainly propylene glycol, glycerol, nicotine, water, flavorings (if added), and small amounts of by-products of thermal decomposition of some of these constituents. Inhaled aerosol is highly concentrated and contains mainly submicrometric-size particles. Electronic cigarette aerosol is composed of droplets of e-liquids surrounded by air and a mixture of aerosols. Based on laboratory simulation, a 10-puff session would result in inhaling 2.5–72.5 mg e-liquid, with 37–69% of aerosol being < 4 µm in size (highly respirable). For e-liquid containing 20 mg/mL nicotine, this would be an intake of 0.08–1.45 mg nicotine/session. Data on total puff volume and nicotine intake can contribute to the development of a standard protocol for laboratory testing of electronic cigarette products [1].

4.1 Primary exposure

The compounds identified in the aerosols inhaled by users of EC originate from the liquids used or directly from the electronic cigarette device or indirectly from chemical reactions . It is noted that, in view of the rapidly changing nature of electronic devices used, some exposure data may not apply anymore or may only be valid in specific EU countries .

The relevant compounds for the risk assessment in EC aerosols are mainly the solvent carriers (glycols and glycerol), nicotine, flavorings, nitrosamines (TSNAs), by-products of thermal decomposition of some of these constituents, notably carbonyls, and metals originating from the device [1].

4.2 Second-hand exposure

EC users partially exhale harmful components because electronic cigarettes are only active when users take a puff and do not produce aerosol when no puff is being taken. Therefore, they do not emit harmful compounds when no puff is taken, unlike tobacco cigarettes. Nevertheless, non-users may be exposed to exhaled air following a puff [1].

5. Hazard Identification

Most of the ingredients and additives used in traditional cigarettes and other tobacco products, along with nicotine and its derivates, were among the most used ingredients in e-liquids. Some of them are included in the 15 priority chemical substances list by the scientific committee on emerging and newly identified health risks in its Opinion Tobacco Additives 1, used by the EU Commission. These include nicotine, solvent carriers (propylene glycol, ethylene glycol, and glycerol), tobacco-specific nitrosamines (TSNAs), volatile organic compounds (VOCs), phenolic compounds, flavorings, and tobacco alkaloids can be found in the aerosols of EC. In addition, the aerosols also contain pyrolysis products of the liquids (i.e., aldehydes, free radicals, and reactive oxygen species, furans) and metals originating from the heated device.

These above-mentioned ingredients can be toxic, affecting different target organs and with different mechanisms involved. Additionally, reactions between the ingredients can also occur, leading to other chemical formations, such as aldehydes, among others. [1]. No harmonized classification was present for most of the ingredients listed in e-liquids and aerosol components to identify the hazard, and the toxicological profile has not been thoroughly investigated. Hence, based on the comparison between measured exposure levels in aerosols and health-based guidance values, the overall weight of evidence for the risk of respiratory tract carcinogenicity due to long-term cumulative exposure to nitrosamines, acetaldehyde, and formaldehyde, is weak to moderate [1].

Nicotine is a parasympathomimetic alkaloid that stimulates the heart rate and blood pressure at low doses and also acts on the gastrointestinal tract and the central nervous system. The dose route and duration of administration determine whether there will be a stimulating or an inhibiting effect on blood circulation. At toxic doses, central stimulation is followed by inhibition, e.g., central inhibition of respiration. Concerning the intoxication of humans, estimates range from 60 mg from self-testing up to more recent estimates of 0.5–1 g of ingested nicotine, corresponding to an oral lethal dose 50 of 6.5–13 mg/kg. According to the harmonized classification and labeling approved by the EU, nicotine is fatal if swallowed, in contact with skin, if inhaled, and is toxic to aquatic life with long-lasting effects [1]. Toxicological data on some endpoints (i.e., CMR [6], cardiovascular, respiratory system, among others) and effects on the health of consumers when inhaled, along with data on any addictive effect, information on the nicotine doses and uptake when consumed under normal or reasonably foreseeable conditions are required for the nicotine formulations along with excipients [7].

6. Risk assessment

Results of exposure assessments will be compared against the safety comparator for each ingredient/impurity/degradant present in the EC aerosol. Risk assessment was performed based on measured aerosol concentrations (below table) and the identified hazards and human health impacts. In addition, a comparison is made to the list of compounds recommended to be measured in the ECs aerosol, according to the tobacco and electronic cigarette industry for regulatory submission under the TPD and to the list of the European Association for the Co-ordination of Consumer Representation in Standardisation [1].

Nicotine exposure may induce effects on the respiratory tract since the alveolar concentrations calculated are higher than or comparable to effect concentrations in human volunteer studies, showing coughing and constriction of the airways. Systemic effects on the cardiovascular system are considered possible since the absorbed doses are higher than effect levels in human volunteer studies with nicotine, showing changes in heart-beat and systolic blood pressure. There may be a risk for adverse effects on the fetus for heavy users since the absorbed doses calculated were slightly lower than effect concentrations in a study with monkeys [1].

Table 1: Reported maximum concentrations of compounds in EC aerosols

Compound	Maximum median aerosol concentration (µg/l)
Nicotine	2000
Propylene glycol	97000
Glycerol	71000
Formaldehyde	470
Acetaldehyde	70
acrolein	50
diacetyl	220
Acetoin	nm
NNN3	0.0038
NAT3	0.0012
NAB3	0.0001
NNK3	0.0017
Cromium	0.0067
Manganese	0.0083
Cobalt	0.091
Nickel	0.343
Copper	0.133
Zinc	0.0014
Cadmium	1.22
Tin	0.03
Lead	nm
Arsenic	nm

nm: not measured; NNN = N'-nitrosonornicotine, NAT = N'-nitrosoanatabine, NAB= N'-nitrosoanabasine, NNK =4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone

6.1 Second-hand exposure

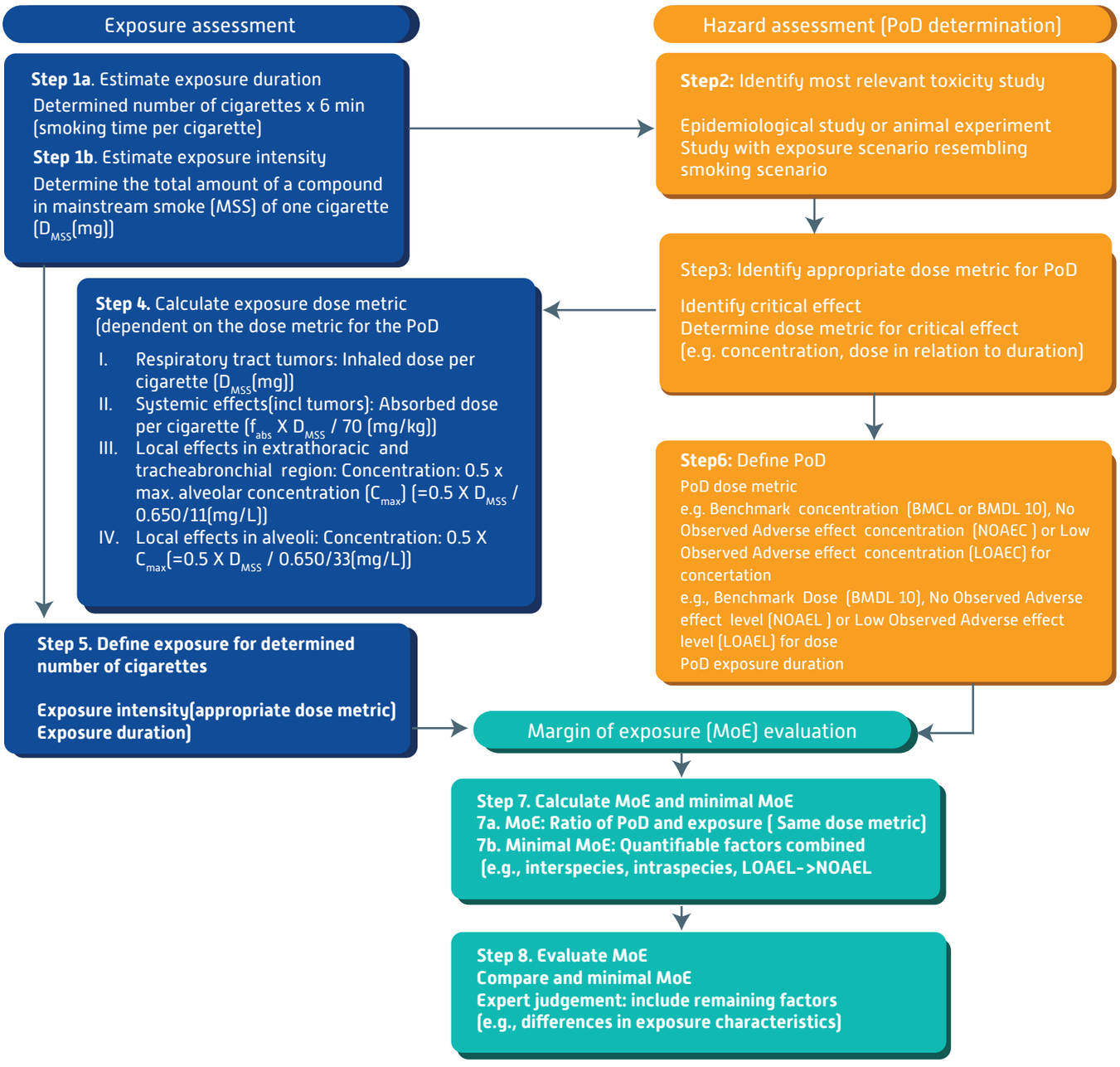
The exhaled breath was collected from EC/e-liquid combinations from 17 volunteers during usage, and the levels of contaminants were measured. Subjects took a specified number of puffs and exhaled onto a trapping device immediately after each puff via a mouthpiece. Samples of control breath without EC were obtained from each subject at the start of the experiment. Analysis of exhaled aerosol is summarized in the below table , providing information on second-hand exposure. The maximum levels will be used in specific exposure scenarios for the risk assessment [1].

Table 2: Chemical analysis of exhaled aerosol. The columns with ranges and medians list average amounts recovered in the first exhaled breath after inhaling a puff.

Components of EC	n	range min	max	Median	Unit
Carrier liquid and nicotine nicotine	17	<LOQ	2140	108	ng
Propylene glycol	17	<LOQ	127	<LOQ	µg
7Glycerol	17	<LOQ	<LOQ	<LOQ	µg
Aldehydes					
Formaldehyde	4	<LOQ	<LOQ	<LOQ	µg
Acetaldehyde	4	<LOQ	<LOQ	<LOQ	µg
Acrolein	4	<LOQ	<LOQ	<LOQ	µg
Nitrosamines1					
NNN	9	<LOQ	111	29	pg
NAT	9	<LOQ	40	14	pg
NAB	9	<LOQ	8	2	pg
NNK	9	<LOQ	71	15	pg
NDMA equivalent total TSNAs	9	<LOQ	77	28	pg
Metals	3	<LOQ	2.92	<LOQ	ng
copper	3	<LOQ	<LOQ	<LOQ	ng
all other metals					

LOQ: limit of quantification; n: No.of subjects; NNN = N'-nitrosonornicotine, NAT = N'-nitrosoanatabine, NAB= N'-nitrosoanabasine, NNK =4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone;ng:nanogram; µg:microgram

Fig 1.Stepwise, pragmatic approach for the risk assessment of individual components in EC aerosols (5)



The dose metric to be used depends on the mode of action of the chemical, toxicokinetics, and dynamics, which could be the concentration in the aerosol in different regions of the respiratory tract, inhaled dose per time interval, absorbed dose per time interval, or a cumulative dose over partial or total lifetime [1].

Users Assessment

Light user: 15 inhalations per day, 1 puff per 4 minutes, with a total daily use duration of sixty minutes

Average user: 60 inhalations per day, 1 puff per 2 minutes with a total daily use duration of 120 minutes

Heavy user: 500 inhalations per day, 2 puffs per minute with a total daily use duration of 240 minutes

For local effects on the respiratory tract, the margin of exposure (MoE) was based on the approximate maximum median alveolar concentration calculated from the puff dose, the volume/puff (70 ml), a low absorption rate (30%) and the dilution rate in the lungs. With respect to the latter: the aerosol concentration in the respiratory tract will be reduced since, together with the

puff, air will be inhaled. For systemic effects, the MoE was based on the calculated total absorbed daily dose. On the hazard side, a suitable animal experiment can be chosen to derive the PoD [1].

Risk assessment was proposed based on the MoE approach. The choice of an appropriate dose metric, such as inhaled concentration and absorbed dose, depends on the type of effect. Temporal characteristics also should be considered in the final step of the MoE approach [5]. The minimal value required for the MoE to come to a conclusion of no or low concern depends on the hazard information available and on the exposure characteristics, and thus, will be different for different scenarios [1].

Natural impurities in nicotine are nicotine-N-oxides, cotinine, nornicotine, anatabine, myosmine, anabasine, and β-nicotyrine which are not considered as carcinogens along with nicotine. Other impurities like N-Nitrosonornicotine, nitrosamine ketone and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol are suspected to cause cancer in humans; this is the area where there is no specific process for conducting safety assessments mentioned; based on the levels in aerosol concentration their safety will be addressed [1]

7. Conclusion

An average reduction in concentrations of more than 90% among given lists of HPHCs has been observed in the aerosol of commercial HTPs or EC against their concentrations in commercial cigarette smoke [2].

Research performed in a European context and focused on EU policy needs is still limited and needs to set out requirements for nicotine-containing liquids, including prohibiting certain additives.

8. References

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Authors

Adunoothala Pradeep Kumar, M.Pharm
Associate Manager - Toxicology
Safety & Medical Affairs

Shrutismita, M.tech.
Associate Manager - Toxicology
Safety & Medical Affairs

ClinChoice Inc.
1300 Virginia Drive, Suite 408
Fort Washington, PA 19034 USA
Phone: +1 215 283 6035
Fax: +1 215 283 6382
Email: info@clinchoice.com

ClinChoice
No. 420 Fenglin Road
Floor 11
Xuhui District
Shanghai 430000, China
Email: bd@clinchoice.com

ClinChoice Limited
Suite G48
268 Bath Road, Slough, SL1 4DX
Berkshire, United Kingdom
Phone: +44 1628 566121
Email: europe@clinchoice.com

ClinChoice LLC
3 Barbusse, 0028
Yerevan, Armenia
Phone: +374 60 541515
Email: europe@clinchoice.com

ClinChoice Private Ltd.
#729 8th Main
11th Cross, J.P. Towers
JP Nagar 3rd Phase
Bangalore - 560 076
Karnataka, India
Phone: +91 80 2658 5551
Email: info@clinchoice.com

ClinChoice Inc.
17th Floor
Wynsum Corporate Plaza
22 F. Ortigas Jr. Road, Ortigas Center
Pasig City, - 1605
The Philippines
Phone: +632 8633 5176
Email: info@clinchoice.com

ClinChoice K.K.
THE GATE NIHONBASHI Bld.
3FHigashi Nihonbashi 3-4-1,
Chuo-ku
Tokyo 104-0031 Japan
Phone: +81 3 6228 7360
Email: japan@clinchoice.com

ClinChoice Canada Inc.
2 Robert Speck Pkwy
Mississauga, ON L4Z 1H8,
Canada
Phone: +1 215 283 6035
Email: info@clinchoice.com

CROMSOURCE
A ClinChoice Company
Via Giorgio De Sandre, 3
37135 Verona - Italy
Phone: +39 045 485 9279
Email: cromsource@cromsource.com