Review Article

Critical Appraisal of Exposure Studies on E-Cigarette Aerosols Generated by High-Powered Devices

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The InExpose system manufactured by SCIREQ® is valuable equipment for conducting preclinical studies in the laboratory. It generates e-cigarette (EC) aerosol by puffing the box mod of a high-powered third-generation device (JoyeTech® EVIC Mini), with its atomizer replaced by a custom-made 70 mL tank. We examined the experimental quality of aerosol generation procedures in 40 studies selected from an extensive literature search focused on the usage of the EVIC Mini with a 0.15 Ω coil. Only 14 out of the 40 studies provided sufficient information on their aerosol generation methodology. We identified and reviewed individually 5 studies from the 14 mentioned above that also conducted a chemical analysis of the aerosol. According to our experimental results, there is full certainty that all 14 studies exposed biological systems to aerosols generated under overheating and unrealistic conditions with high aldehyde loads that follow from machine puffing a high-powered device with inappropriate airflow. Given the similarity in design and scope of all studies and the needed training to use the InExpose, we argue that this evaluation very likely applies to the remaining 26 studies that used this equipment without providing sufficient information on aerosol generation procedures. Since preclinical studies are valuable for assessing effects on in vitro and in vivo systems exposed to EC aerosols, it is important to provide full information on aerosol generation parameters and to prevent exposing these biological systems to overheated and toxin-loaded aerosols. Finally, we provide a series of guidelines on aerosol generation procedures that we believe will be useful for the operation of the InExpose and for preclinical studies in general.

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1. Introduction

There is a widespread consensus that E-cigarettes (ECs) provide smokers with a much safer nicotine delivery than combustible cigarettes, thus motivating part of the global public health community to endorse their adoption by smokers as a popular harm reduction product to substitute conventional tobacco cigarettes (CCs)[1][2] (see the opposing stance to this policy in[3]). While users of the devices ("vapers") are exposed to significantly less harmful and potentially harmful compounds (HPHCs), it is still necessary to assess the risks involved in their usage. This is a complex process involving laboratory testing of EC emissions followed by probing the biological and medical effects of the inhaled chemicals through preclinical studies (biomarkers, cytotoxicity, animal models) and clinical studies. In particular, in vitro and in vivo studies (in spite of their known limitations[2][4][5][6][7][8][9][10][11]), might provide a valuable laboratory evaluation of toxicity from exposure to EC aerosol emissions, contributing to the assessment of the safety profile of ECs.

The computerized InExpose equipment, manufactured by SCIREQ (Scireq®, Montreal, QC, Canada), is a valuable tool for the examination of biological and physiological effects of EC aerosol exposure on cell cultures and rodents[12]. The InExpose system is coupled with an ECX-JoyeTech EVIC Mini accessory that is composed of a third-generation tank device (JoyeTech® EVIC mini), with its atomizer replaced by a 70 mL custom-made tank, incorporating as well instruments for chemical analysis and biological testing of in vitro and in vivo systems (see description in[13]). EC aerosol is generated as a controller puffs this box mod that SCIREQ supplies by default, operating with a default choice of KangerTech resistances, mainly 0.15 Ω (nickel), 0.5 Ω, 1.5 Ω (Kanthal), under power and temperature control modes. The controller can draw rectangular or sinusoidal puff profiles with a maximal instantaneous peak flow of 1.675 L/min, leading to airflow rates around 1-2 L/min for puff durations around 2-4 s[12].

Considering the publicly available information supplied by SCIREQ[12][13], a recent article[14] examined in the laboratory the general functioning of the aerosol generation process of an accurately constructed simulation of the InExpose equipment described above, using the same box mod of a Joyetech EVIC mini, a similar custom-made tank, as well as the set of KangerTech coils recommended for its usage in power and temperature control modes[13]. The electric calibration revealed significant differences between the nominal coil resistance value specified by the retailer and the laboratory-measured value (for example, the 0.15 Ω resistance measured 0.2 Ω). Likewise, significant differences were found between values of power, voltage, and temperature displayed in the instrument panel of the box mod and their corresponding laboratory-measured values.

The tests in[14] were conducted by puffing the JoyeTech EVIC mini box mod in consecutive blocks, each with a sequentially increasing fixed value of supplied power. This testing determines the power range of the Optimal Regime of operation, defined by a linear relation between the mass of e-liquid vaporized (MEV in mg) and supplied power. As shown in previous research[14][15][16][17], the slope of the linear relation MEV vs W increases with increasing airflow and defines an increase in the thermodynamical efficiency of EC operation[16]. The relation MEV vs W becomes non-linear, with a very decreased slope (very low efficiency), when supplying power rises above the range of the Optimal Regime, leading to an Overheating Regime, with inefficient aerosol generation, also marking the onset of an exponential production of carbonyl byproducts (which remain in minute quantities under the Optimal Regime).

In the present review article, we rely on the results of the laboratory tests conducted in[14], (summarized in Section 2), but considering only the EVIC Mini box mod with the OCC 0.15 Ω nickel coil, since the InExpose with this coil and this box mod has been used by at least 30 of the 40 studies that we selected from an extensive literature search in Section 3 and listed in Table 1.

One of the main problems we found in most of the 40 studies using the InExpose (this problem also occurs in numerous other preclinical studies on ECs) is the scarcity of information provided by the authors on the parameters they used to generate EC aerosol with the InExpose. In fact, only 14 of the 40 studies using the InExpose provided bare minimal information on their aerosol generation parameters to potentially reproduce their results, while 16 provided very incomplete information and 10 did not report any information other than mentioning the usage of the InExpose. This information vacuum on an important technical point renders these studies unreproducible (a serious quality flaw in experimental research)[18][19].

We evaluate in full detail the aerosol generation in the 14 reproducible studies, reviewing individually 5 of them that provided information on the chemical composition of the aerosols. For this purpose, we consider the information supplied by the authors evaluated with respect to our laboratory tests conducted in[14] (summarized in Section 2) with the OCC 0.15 Ω nickel coil. Our evaluation clearly proves, with full or nearly full certainty, that at least 14 studies of the 40 selected exposed biological systems to EC aerosols (in power and temperature modes) generated under overheating conditions with high levels of carbonyl yields. We argue that there is a high likelihood that the same conclusion applies to at least 16 of the remaining 26 studies. These outcomes follow (as concluded in[14]) from the authors' generating an aerosol under an unrealistic combination of high supplied power and a low airflow around 1 L/min. The fact that these studies exposed cell lines and rodents to an overheated and aldehyde-loaded aerosol puts in doubt the utility of their reported biological effects for the assessment of health risks of ECs.

Our section-by-section plan is as follows. Section 2 provides a summary of the calibration and functionality tests on the InExpose generating aerosol by the box mod of the EVIC Mini with the 0.15 Ω coil. In Section 3, we identify a selection of 40 studies from a literature search based on the criteria described in Section 2. The studies are listed in Table 1. Section 4 provides a detailed review of 5 studies in this selection that provided full information on their aerosol generation and quantified yields of aldehyde, nicotine, and other byproducts. In Section 5, we gather and discuss the main results of the review. Section 6 summarizes a series of guidelines on aerosol generation procedures for the operation of the InExpose and offers our conclusions in Section 7.

2. Background on the e-cig aerosol generator designed by SCIREQ

EC emissions required for in vitro and in vivo testing can be generated by an additional module within the InExpose series of laboratory equipment manufactured by Scireq (Scireq®, Montreal, QC, Canada). This equipment, containing the EXC-JoyeTech E-VIC Mini kit, is composed of a JoyeTech EVIC mini mod box (the electronic components) and a third-generation atomizer (Subtank® by KangerTech) modified by a customized tank allowing it to be filled by a maximum of 70 mL of e-liquid. Although these are originally commercial products, the modification of design, such as the horizontal position of the mod box and their inclusion in another commercial product (the additional module), makes Scireq responsible for providing appropriate instructions for their use. This module can be connected to another module (a peristatic pump) to simulate inhalation.

Among the various coils marketed by Kangertech for this atomizer (0.15 Ω, 0.5 Ω, 1.2 Ω, and 1.5 Ω), the 0.15 Ω nickel coil was the most frequently found in our search, followed by the 1.5 Ω and the 0.5 Ω coils (0.15 Ω: 72%, 0.5 Ω: 10%, and 1.5 Ω: 21%), whereas the 1.2 Ω coil was not used. Each coil was examined in a recent paper[14], and their functioning limits were experimentally determined. It is also important to remark that coils are manufactured by Chinese companies for commercial use with varying quality and precision. It is also important to point out that there is no current standardized claromizer, electric resistance, and related settings for laboratory purposes, as there are standard references for e-liquids[20][21]. Thus, appropriate calibration is required to evaluate their repeatability for laboratory purposes and to fully understand and identify their functioning limits. Otherwise, setting up the conditions fixed for aerosol generation becomes a random exercise.

As shown in[14], the electric calibration and the characterizations of the energy supplied using the EVIC VTC box with the OCC 0.15Ω coil lead to the two following graphs (Figure 1), reporting the real power supplied using the power and the temperature control modes. To facilitate the visualization of the graphs, the grey shaded regions illustrate the range of conditions in both power and temperature control modes that were used in the studies we list in Table 1 (Section 3) and review in Section 4.

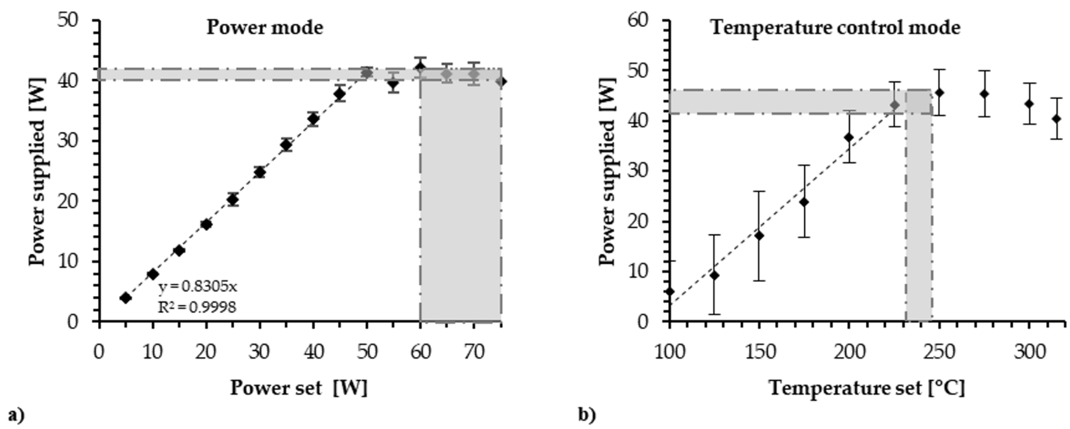

Figure 1. Calibration curve of the EVIC VTC modes using the OCC 0.15 coil and an airflow rate of 1.1L/min (a) wattage mode and b) temperature control mode). Gray boxes illustrate the range of conditions reported in the literature we list in Table 1 and reviewed in Section 4.

As an overall observation, it is necessary to stress from Figure 1 that reported conditions in the reviewed literature in both wattage and temperature control modes lead to an actual measured supplied power that tops between 40W and 46W. Additionally, the variations of power supplied through a single puff are lower in wattage mode than in temperature control mode. Figure 2 illustrates the difference between the two modes.

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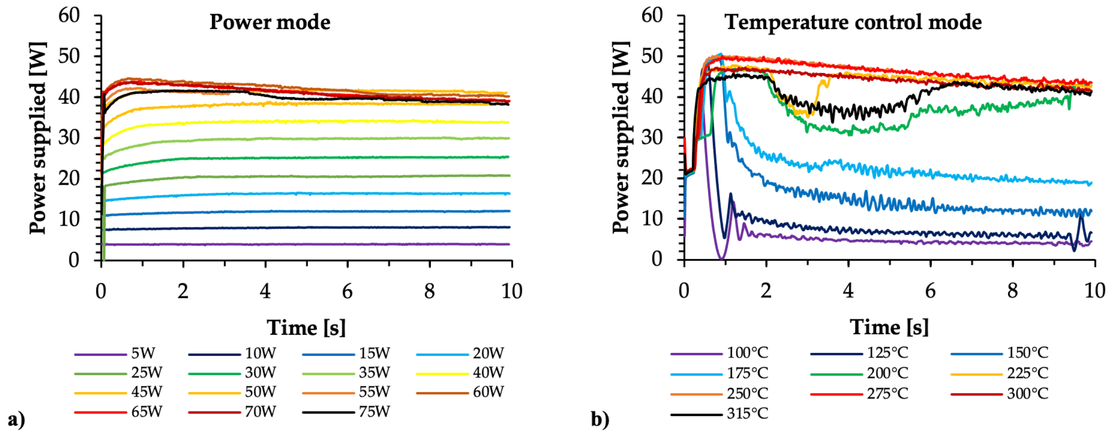

Figure 2. Calibration curve of the EVIC VTC modes using the OCC 0.15Ω coil and an airflow rate of 1.1L/min (a) wattage mode and b) temperature control mode).

The main result emerging from Figure 1 is the power profile difference between the two modes: it is almost constant in power control mode, while in temperature control mode, it rapidly reaches an initial peak and then decreases to reach a constant power. The signal is also flat in the power mode, while it is fluctuating in temperature control mode. Therefore, the power mode is more appropriate to assure the experimental repeatability required for laboratory purposes. Figure 2 emphasizes (from the gray shaded regions in Figure 1) the stability of actual supplied power around the range 40-45 W in the conditions used in the reviewed literature, which corresponds (in studies using the temperature mode) to stable temperatures close to and slightly above 300°C.

Finally, some articles reported setting up voltage values when generating aerosols with the EVIC VTC box, which supposedly were voltage values displayed on the device's instrument screen, though the authors provide no explanation of how these voltages were determined. However, we were unable to obtain voltage control from the device screen, so we assessed the power control mode in the laboratory and reported the voltage that should have been displayed on the device screen. We compared it with measured voltages and power levels. The results are presented in Figure 2, displaying the simulated screen voltage, together with the supplied voltage and power that were actually measured.

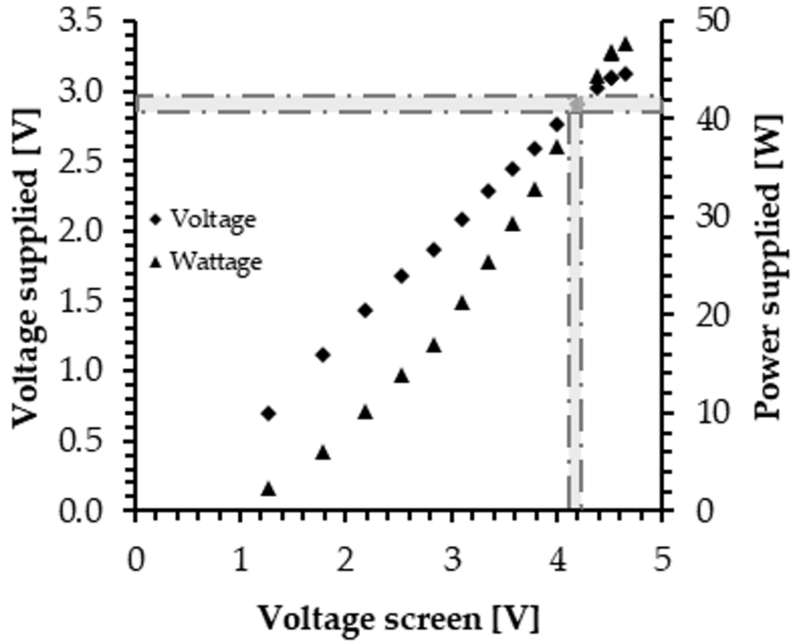

Figure 3. Calibration curve of the EVIC VTC modes using a 0.20 Ω coil (a) voltage calibration and b) resulting wattage supplied in wattage mode. Gray boxes illustrate the range of conditions reported in the following review process (4.1V and 4.2V).

Under power-controlled mode, we also observed a significant deviation between the voltage displayed on the screen and the measured supplied voltage. For example, with a 0.2 Ω coil, the measured applied voltage is about 68% lower than the one that would appear on the device screen, meaning that the 4.2V screen value corresponds to around 2.85V actually measured (and corresponds to 41W measured supplied power).

Through all the conditions reported in our reviewing process, the OCC 0.15Ω was truly used under close conditions (between 41W and 46W supplied).

The consistency between supplied power and airflow rate is a major factor in determining the range of supplied power to achieve an optimal regime characterized by efficient and consistent conditions of aerosol generation[14][15][16][17]. This efficient aerosol generation occurs when puffing a given device in the supplied power ranges that allow for a linear relation between MEV (mass of e-liquid vaporized) and power, with the width of these power ranges strongly depending on the airflow rate. This is particularly important when puffing a high-powered device, since insufficient airflow significantly narrows the power range of the optimal regime, making it easy in the laboratory to puff the device at power levels above this regime under overheating conditions that do not occur at these power levels under sufficiently high airflows.

Laboratory tests conducted in[14] have determined the optimal regime for the EVIC VTC equipped with the OCC 0.15 Ω coil to extend between 20W and 63W under an airflow of 10L/min (see Figure 4). In contrast, under an airflow rate of 1.1 L/min (recommended by the CORESTA puffing regime[22][23]), the same device and 0.15 Ω coil led to a significant narrowing of these ranges to 15-30W.

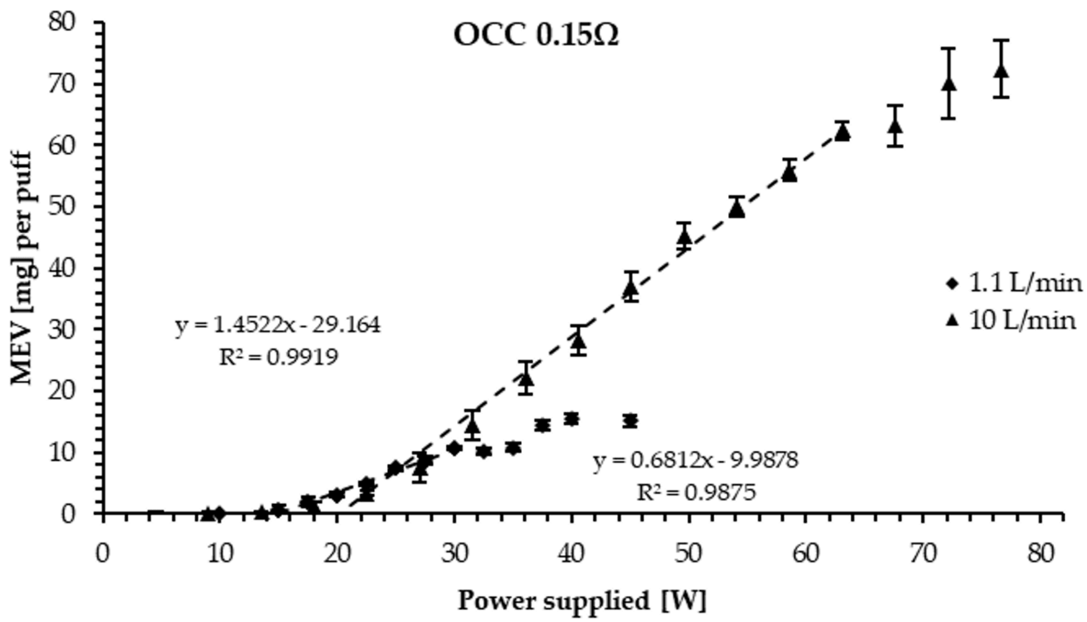

Figure 4. Functioning curves of the OCC 0.15Ω coil applying 1.1L/min and 10L/min.

Supplied power at 30W (equivalent to 35W set on the screen of the EVIC VTC box) is the maximal value for the optimal regime for the OCC 0.15Ω coil under the CORESTA puffing regime[22][23] at an airflow rate of 1.1 L/min (Figure 4). At power levels above this value under this airflow, overheating conditions initiate, producing the emergence of a gas layer surrounding the wire, indicating an ongoing thermal process known as film boiling[17][24]. At this stage, the quantification displayed in Figure 5 reveals an exponential increase in aldehyde yields resulting from glycerol dehydration[14][17], with the cotton element in the wick possibly becoming pyrolyzed as power keeps increasing. This maximal power of

30W marks an important transition in the assessment of toxicological risks induced by vaping products, a transition that is perceivable by users as a repellent sensation to be avoided[25].

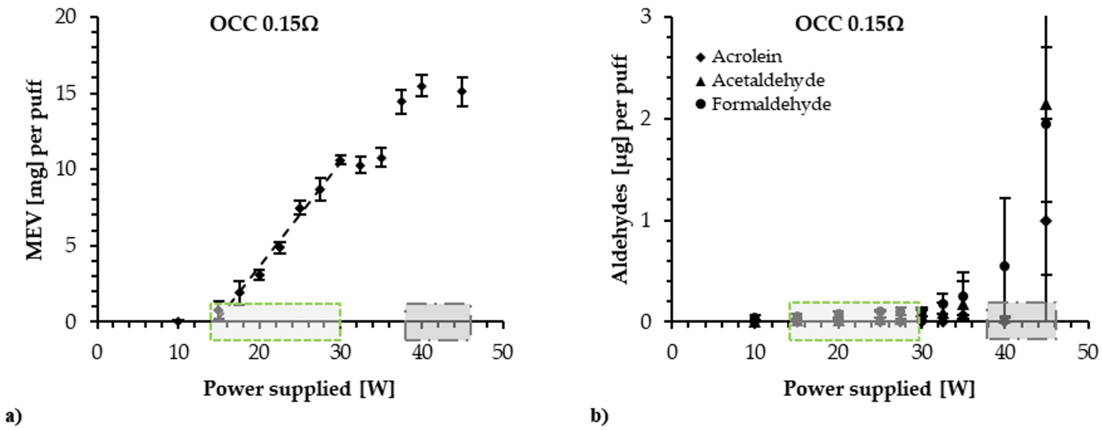

Figure 5. a) Functioning curve of the OCC 0.15Ω coil and b) the resulting aldehydes released.

The calibration and functionality tests we have presented reveal a major problem: the reviewed studies are using the InExpose with the OCC 0.15Ω coil at high powers with an inappropriately low CORESTA airflow, which implies aerosol generation in the overheating regime above the power ranges of the optimal regime. The SCIREQ documentation[12] mentions the controller is limited to draw a maximal instantaneous peak flow of 1.675 L/min, which is close to the CORESTA regime[22][23], an airflow rate that will extremely likely produce overheating conditions even at relatively lower powers. This experimental flaw was previously highlighted in two reviews of the literature dealing with the quantification of metals and organic chemicals in the aerosol[18][19] and is also worth highlighting its occurrence in in-vitro or in-vivo articles not necessarily using the SCIREQ. As pointed out in Figure 4, with an appropriate high airflow rate compatible with consumer usage (10 L/min), the reported experimental conditions of the reviewed preclinical studies would have occurred under an optimal regime, and this criticism would not apply to their aerosol generation procedures.

Considering that our calibration tests, conducted in[14], and summarized in this section, point towards near certainty that aerosol was generated under overheating conditions in studies using the 0.15Ω coil, provided by the SCIREQ equipment, we need to identify first all articles citing or referring to "SCIREQ" and then to identify their reported conditions of aerosol generation. These tasks are described in the following section.

3. Literature search and selection methodology

3.1. Selection methodology

We had to follow an atypical process for searching the literature, since we aimed at searching for papers whose experiments relied on specific aerosol generation conditions (explained in Section 2), a background material that is only mentioned and/or described in the materials and methods section of each article. Google Scholar appeared to be the most appropriate search engine for this purpose, since it can look for specific words inside the articles. However, filtering the search output must be done by hand because Google Scholar lacks an inbuilt capacity to filter the information. To illustrate our approach, we provide below a flow chart of a step-by-step description of the criteria applied to filter the articles. The key sentence we used as input (Google Scholar 11/29/2023) was "(electronic cigarette(s) OR electronic-cigarette(s) OR e-cigarette(s) OR e-cigarettes OR vaping OR "Electronic Nicotine Delivery System") AND "scireq". The results were extracted, and after suppression of duplicates, the list was finally composed of 263 references.

- The first step was to remove studies with abstracts of conferences, reviews, books, theses, or other reports.
- The next step was identifying (by title) and removing from the list articles not dealing with tobacco products (heated or combusted), since the Google Search does not restrict the titles, and many selected articles failed this search criterion even if the term "electronic cigarette" was mentioned in their introduction.
- We removed from the list articles that did not contain keywords linked to vaping.
- At this point in the selection, all papers mentioned the SCIREQ equipment, but it was not evident that they used its exposure chamber or the aerosol generator. We had to look manually in each article to find the specific equipment or instrument that was used, removing those articles that did not use the SCIREQ manufactured aerosol generation equipment.
- Once completing the above-mentioned selection, the final step was to select for more specific conditions of aerosol generation, since the SCIREQ equipment has been used with several EC devices that are outside the scope of our review, such as Juul® or Blu® pods.

However, as previously explained, aerosol generation conditions are often ill-described, with authors often providing incomplete and/or missing information[18][19]. Since we are searching for

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experiments conducted with the 0.15Ω coil at high powers, we extracted articles that contained basic descriptive terms of the device, which at least would allow for a suspicion of our targeted usage, such as “third generation device” or “ECX EVIC VTC”, the name of the device marketed by SCIREQ. At this step, we also kept articles containing the term “device” but providing no further information.

Additionally, the Subtank uses several coils that are available, and we also assumed that not all the testing conditions should be under an overheating regime. So, we applied a filter based on the electric resistance, selecting articles mentioning the 0.15Ω or 0.2Ω coils. We suspect that articles reporting a 0.2Ω coil were really using a 0.15Ω coil rounded to 0.2Ω, since SCIREQ mainly provides three coils with resistances: 0.15Ω, 0.5Ω, and 1.5Ω[12][13].

The last step in filtering the literature is linked to the power supplied. In most of the articles, this fundamental information was missing. Figure 6 displays the PRISMA chart associated with the literature search we have described.

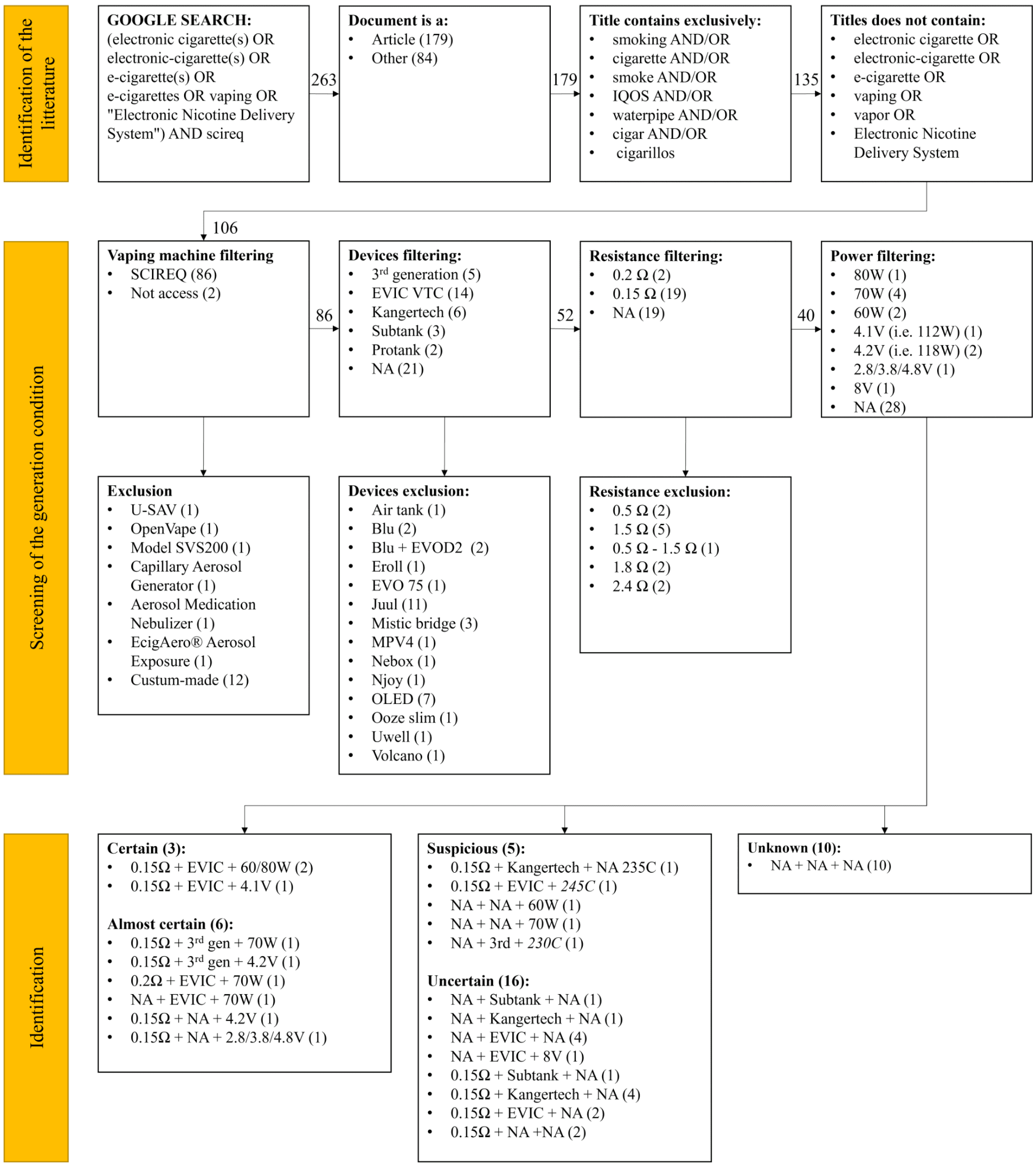

Figure 6. PRISMA chart associated with our literature search (see Section 3).

3.2. Results of the literature search

The resulting 40 papers that met the search criteria previously described were classified in Table 1. Aerosol generation parameters of the 40 revised studies using the InExpose. Power tested as declared by authors allegedly from display in the instrument screen of the box mod. 6 articles used nicotine concentrations above 30 mg/mL, 14 in]20 mg/mL;30 mg/mL], 9 between]10 mg/mL;20 mg/mL], 12 between]3 mg/mL;10 mg/mL] and 11 did not use nicotine. 20 articles were above the Tobacco Product Directive requirement of 20 mg/mL. On the grounds of our level of confidence in the identification of aerosol generation conditions in their experimental aerosol generation procedures that can be potentially associated with overheating as described in Section 2.

- “Certain” means that the device, coil, and power or voltage were fully identified.
- “Almost certain” means that one item of information was not clearly described or was missing, but the available information is sufficient to evaluate aerosol generation.
- “Suspicious” means incomplete minimal information that is insufficient to assess aerosol generation conditions. However, based on the significant similarity in experimental design and the materials and methods between these studies and the fact that operating the InExpose requires training, all of which makes it likely that the authors followed the aerosol generation procedures of the “almost certain” and “certain” categories stated above.
- “Uncertain” means that information is so restricted that it prevents the discussion of aerosol generation conditions. In all cases, the usage of InExpose or the term “SCIREQ” is mentioned, but there is no information on supplied power, and the device is at best partially identified. As argued in the previous point, it is very likely that complete information might have led to the same operating conditions as studies with more information.
- “Unknown” means a complete lack of information on aerosol generation parameters (either the device, the coil, and supplied power, or only the SCIREQ equipment is mentioned). In this case, aerosol generation conditions are unknown, but the experiments are then totally irreproducible.

	Reference	Nicotine (mg/ml)	PG/VG	Device	Coil (Ω)	Power tested (W)	Airflow (L/min)
Certain	[26]	0 and 6	50/50	EVIC	0.15	80W	2.00
	[27]	0 and 18	60/30 10%w	EVIC	0.15	4.1V	3 (60ml)
	[28]	30 and 36	30/70 and 40/60	Juul+EVIC	0.15	3V	1.10
Almost certain	[29]	50	50/50	3rd	0.15	70W	1.05
	[30]	36	50/50	3rd	0.15	4.2V	1.10
	[31]	20	30/70	EVIC	0.2	70W	1.40
	[32]	20	50/50	EVIC	0.2	70W	2.00
	[33]	12 and 18	Varied	Juul+EVIC	0.15	4.2V	1.00
	[34]	36	Varied	Pods+EVIC	0.15;0.5;1.5	2.8;3.8;4.8V	1.10
Suspicious	[35]	NA	30/70	Kangertech	0.15	235C	1.27
	[10]	24	55/45	EVIC	0.15	245C	NA
	[36]	0	50/50	NA	NA	60W	1.22
	[37]	24	50/50	NA	NA	70W	1.05
	[38]	24	50/50	3rd	NA	230C	9s
Uncertain	[39]	24	50/50	Subtank	NA	NA	10s
	[40]	0 and 18	NA	KangerTech	NA	NA	1.10
	[41]	50	NA	EVIC	NA	NA	NA
	[42]	50	NA	EVIC	NA	NA	NA
	[43]	0 and 25	100/0	EVIC	0.15	NA	1.27
	[44]	18	50/50	EVIC	NA	NA	1.10
	[45]	0 and 6	70/30	Subtank	0.15	NA	NA

	Reference	Nicotine (mg/ml)	PG/VG	Device	Coil (Ω)	Power tested (W)	Airflow (L/min)
	[46]	0 and 25	NA	Kangertech	0.15	NA	1.27
	[47]	NA	100/0	EVIC	0.15	230C 70W	1.00
	[48]	0 and 18	55/35 (10% water)	EVIC	NA	NA	NA
	[49]	0	50/50	KangerTech	0.15	NA	1.02
	[50]	0, 25, 33	Varied	Kangertech	0.15	NA	1.27
	[51]	NA	50/50	EVIC	0.15	NA	51ml/s
	[52]	25	100/0	EVIC	0.15	NA	1.4
	[53]	25	50/50	NA	0.15	NA	1.27
	[54]	25	50/50	NA	0.15	NA	1.27
	[55]	12	50/50	EVIC	NA	8V	3s
Unknown	[56]	24	50/50	NA	NA	NA	NA
	[57]	6,14,18,24	50/50 80/20	NA	NA	NA	NA
	[58]	NA	30/70	NA	NA	NA	NA
	[59]	0	NA	NA	NA	NA	NA
	[60]	0	70/30	NA	NA	NA	70ml/s
	[61]	24	50/50	NA	NA	NA	NA
	[62]	20	50/50	NA	NA	NA	NA
	[63]	4% of liquid	Varied	NA	NA	NA	2.00
	[64]	0 and 4% mass	50/50	NA	NA	NA	1.10
	[65]	20	30/70	NA	NA	NA	1.40

Table 1. Aerosol generation parameters of the 40 revised studies using the InExpose. Power tested as declared by authors allegedly from display in the instrument screen of the box mod. 6 articles used nicotine concentrations above 30 mg/mL, 14 in]20 mg/mL;30 mg/mL], 9 between]10 mg/mL;20 mg/mL], 12 between]3 mg/mL;10 mg/mL] and 11 did not used nicotine. 20 articles were above Tobacco Product Directive requirement of 20 mg/mL.

In the 40 studies listed in Table 1, we can immediately identify the first top-down 14 studies ("Certain", "Almost Certain" and "Suspicious") that provided at least basic information on their aerosol generation procedures. The lack of information on one parameter (for example, power or coil resistance) can be reasonably inferred from the remaining parameters and the constraints from the usage of the InExpose (also from the similarity of the studies). Of the remaining 26 studies, the 16 ("Uncertain") provided even less information, but their mention of terms such as "E-VIC", or "0.15Ω coil", or "KangerTech" is sufficient to infer that they followed a similar aerosol methodology as the first 14 studies, which is consistent with the usage of the InExpose, as explained in the instruction video in[13]. The remaining 10 studies (marked "Unknown") lack even minimal information, and thus inference on their methodology becomes much more difficult. Consequently, these studies are completely unreproducible (a serious methodological flaw).

4. Critical reviews of specific individual studies using the InExpose.

The protocol described by Noël et al.[13] explains the experimental procedures to operate the InExpose, and it has likely been a methodological guideline in the studies using this equipment that we list in Table 1. Of the 40 studies, 9 provided sufficient information on their aerosol generation parameters. Of these 9 studies, only 5[26][28][30][33][34] conducted a chemical analysis of the aerosols injected into the exposure chamber. We regard the description of aerosol generation in these 5 studies as representative of (at least) the overwhelming majority of the 40 studies listed in Table 1.

4.1. Noël et al. 2020

The authors[34] considered three coil resistances (0.15, 0.5, and 1.5 Ω) and three nominal voltages (2.8, 3.8, and 4.8 V) in the quantification of nicotine and the main aldehydes (formaldehyde, acetaldehyde, acrolein). They reported a significant increase in aldehyde yields while puffing the 0.15Ω coil with various voltages. The airflow rate was 1.1 L/min. Aldehyde yields were low for 2.8V (comparable to those obtained with the 1.5 Ω coil). However, rising the voltage to 3.8V and then to 4.8V in the 0.15 Ω coil produced an abrupt increase of these yields, reaching respectively 136, 273, and 232 times the yields measured with respect to the 1.5Ω coil using the Butter flavor e-liquid.

The large increase in aldehyde yields for the voltages 3.8V and 4.8 V and the 0.15Ω coil bears evidence of an aerosol generated under an overheating regime. This fact follows from the results of our voltage calibration test displayed in Figures 3 (with Figures 1 and 2 as reference), showing that 3.8 V and 4.8 V correspond to values of measured power of 38W and 44W, which are clearly above the upper end (above 30 W) of the optimal regime (Figure 4), well into the overheating regime for 1.1 L/min airflow. As a contrast, the measured supplied power is in the optimal regime (below 30W) for 2.8V with 0.15Ω and for all tested voltages with the 1.5Ω coil.

Another problematic issue with Noël et al. is the excessively high nicotine concentration of 36 mg/mL in e-liquids puffed with the high-powered E-VIC Mini box (see Table 1). The authors justify this as an effort to "mimic nicotine exposure by heavy smokers," but this combination of high nicotine with high power strongly misrepresents consumer usage in which high nicotine levels are used with low-powered devices. This fact emerges from observations carried out by Soule et al.[66], who found a strong correlation between high-powered devices (even at 30W) and low nicotine concentrations below 6 mg/mL. Besides this point, such high nicotine levels also produce an unrealistic overexposure to nicotine in in vitro and in vivo systems. This problem is shared by other studies that we review further ahead[28][30].

4.2. Cahill et al. 2022

The study by Cahill[30] likely used the same cinnamon fireball-flavored e-liquid (reported as 50/50 PG/VG). The authors used the same device and puffing protocols as[34], but supplied only 4.2V, which corresponds (see Figure 3) to 40W-45W measured power. Therefore, Cahill et al. also generated aerosol under certain overheating conditions.

The authors report yields of 0.021 μg and 0.386 μg per puff, respectively, for formaldehyde and acetaldehyde. Although these levels are lower than those of[34], they signal very likely overheating conditions, since the formaldehyde mass yield is always higher than that of acetaldehyde under optimal conditions. Theoretically, the dehydration reaction leads to formaldehyde and to vinyl alcohol, which itself reacts into acetaldehyde[67][68], explaining why acetaldehyde cannot be higher than formaldehyde under optimal conditions (a situation mainly observed in the literature). However, acetaldehyde is also released by the pyrolysis of cellulose[69], suggesting that the reported extra acetaldehyde was released under overheating conditions leading to this pyrolysis of the wick.

The study exhibits other minor irregularities: the authors claim that 0.165 mg/puff of e-liquid was vaporized (but the "mg" unit they used is erroneous, since even a low-power device like the Juul vaporizes at least 1 mg of e-liquid per puff[70]). The e-liquids had a 3.3% nicotine concentration, which (as commented before on Nöel et al.[34]) is also unrealistic for usage in a high-powered device nicotine concentration[66].

4.3. Noël and Ghosh 2022

The authors[33] examined the impact of e-liquid composition in testing in-vitro systems, with the same aerosol generation parameters as Cahill et al.: the 0.15Ω coil with 4.2V (40W measured, see Figures 1, 2, and 3) and 1L/min airflow.

As a preliminary step in their investigation, the authors generated aerosols from non-commercial e-liquids composed only of PG and VG. The study quantified around 7 µg of formaldehyde (333 times higher) and above 2 µg of acetaldehyde (5 times higher). When commenting on these high yields, the authors claim that the aerosols "were not produced under 'dry puff' conditions," a statement that, without further explanation, must be regarded as merely an assumption, probably based on e-liquid not being depleted. However, having used the same experimental setup as[34] and having obtained similar aldehyde yields clearly points to overheating conditions, which can occur without e-liquid depletion.

The authors then used commercial e-liquids with their most common PG/VG ratio and two nicotine concentrations: a 70/30 strawberry-flavored e-liquid, a 50/50 Catalan cream-flavored e-liquid, and a 30/70 vanilla-flavored e-liquid with 12 or 18 mg/mL of nicotine concentration. Of the 6 tested conditions, two (Vanilla 12 and Catalan cream 18) produced significant quantities of formaldehyde (above 3 µg/puff). Two others produced significant quantities of acetaldehyde, higher than formaldehyde (strawberry 12 and strawberry 18), and the two last combinations (Vanilla 18 and Catalan cream with 12 mg/mL) are too low for comparison.

Finally, the authors also tested pod devices. While pods are out of the scope of our present review, the comparison between fourth-generation and third-generation devices is interesting, as the tested e-liquids have close solvent composition (at least the solvents themselves are the same). Removing the Puff Bar OMG 5% nicotine salt, which is suspicious regarding the other results, a rough approximation leads to 0.01 µg of formaldehyde released for 2 mg of e-liquid vaporized (MEV) per puff (5 ng of formaldehyde per mg of e-liquid vaporized). Without providing the MEV that results from the non-commercial e-liquid, the ratio of yields cannot be investigated. However, reaching 7 µg of formaldehyde per puff suggests that 1400 mg of e-liquid per puff was vaporized (assuming the same ratio and indirectly the same condition). This enormous ratio suggests possible conditions significantly hotter than usual for the tested pods. Since the vaporization occurs under the same conditions in high-powered devices and pods under an optimal vaping regime, this suggests a high likelihood that overheated aerosol was also generated by the fourth-generation pods.

4.4. Pinkston et al. 2023

The authors[28] also used an airflow rate of 1.1 L/min and the Joyetech E-VIC Mini VTC mod with a 0.15 Ω coil at 3V. No justification was provided for setting this voltage, which would correspond to 60W from Ohm's law, though our calibration tests (Figures 2, 3, and 4 in Section 2) determine that it corresponds to a measured 30W (the upper limit of the optimal regime). The tank was filled with a Crème-Brûlée-flavoured e-liquid (30/70 PG/VG and 30 mg/ml of nicotine) or a Menthol-flavoured e-liquid (40/60 PG/VG and 36 mg/ml of nicotine). Cells were exposed to both flavored e-liquids during 1 hour 1 day, but an additional experiment was done with Crème Brûlée, where the protocol was replicated during three consecutive days.

Both flavored aerosols were generated and chemically characterized. Nicotine yields were around 27.8 ng per puff with the Menthol-flavoured e-liquid (40PG/60VG) and 25.5 ng per puff for the Crème-Brûlée one (30PG/70VG). Formaldehyde, acetaldehyde, and acrolein were quantified respectively at around 2.6 µg/puff, 1 µg/puff, and 0.02 µg/puff for the menthol e-liquid, and very high levels of 13 µg/puff, 4 µg/puff, and 9 µg/puff of acrolein for the Crème-Brûlée-flavoured e-liquid.

High levels of aldehydes are not expected at 30W (calibrated power from 3V), a power level marking the onset of the overheating region (see figure 4 and[14]). However, as mentioned in our evaluation of Nöel et al[34] and Cahill et al[30]), the usage of e-liquids with free base nicotine concentrations of 30 mg/mL and 36 mg/mL is completely at odds with consumer usage (such high levels of nicotine are used with nicotine salts, not base nicotine). Besides being unrepresentative of consumer usage of sub-ohm devices, the in vitro system in this study was exposed to enormous nicotine yields (0.776mg and 0.5 mg by puff).

The authors used a module of exposure for 4x6 wells where a 24mm diameter cell insert is placed in each well. 4 over the 24 wells were dedicated to the aerosol generated with the vaping device. The e-liquid has an initial concentration of 30 or 36 mg/mL. In other words, 0.0018 m^2 of cells were directly exposed to the aerosol. By contrast, a user with this device and power would be vaping a liquid with a nicotine concentration around 6 mg/mL. An average lung surface of $70m^2$ is exposed. Assuming that the same amount of e-liquid is vaporized in both conditions, this means that the study concentrated the aerosol on a 39,000 times lower surface and that the quantity of nicotine vaporized is between 5 and 6 times higher, leading to a global overexposure of around 200,000 times (195,000-234,000).

It is not obvious that such excessive nicotine delivery could have contributed to rising aldehyde levels in conditions close to overheating, but this nicotine overexposure of macrophages renders the cytotoxicity results of[28] questionable.

4.5. Muthlalage and Rahman (2023)

Authors[26] used the 0.15Ω coil at 220°C or 80W, which also corresponds to overheating conditions characterized by measured values of 45W and close to 300 °C (Figures 1,2,3). They tested two commercial e-liquids containing menthol or tobacco flavor with 0 or 6 mg/mL of nicotine and one laboratory liquid with a 1:1 PG/VG ratio. Interestingly, each flavored liquid was chemically analyzed in both liquid and vapor phases. The resulting lists were compared for each phase. Of the 98 compounds found in the tobacco liquid, 33 were also available in the 91 ones detected in the menthol liquid. However, in the aerosol, 82 compounds are common, and only 2 and 7 are specific to the tobacco and menthol liquids.

The authors did not examine the comparison between the e-liquid and the aerosol. The tobacco flavor had only one common compound (Ethane, 1,1,1-trichloro-) in both analyses, whereas in the menthol case, there are four (Ethane, 1,1,1-trichloro-; α and β-pinene; Acetaldehyde). The high quantified levels of some compounds (ethanol) in the aerosol but not in the liquid suggest problems in the e-liquid analysis, as well as the formation of byproducts during the vaporization, such as acrolein resulting from glycerol degradation. It should be noted that the absence of formaldehyde and acetaldehyde, with the quantification of propionaldehyde, raises questions about the methodology used to sample or to analyze the aerosol.

Of the 82 molecules found in the aerosol from both liquids, 35 contain chlorine atoms, 4 contain bromine atoms (1,2-dibromoethane, bromofluorobenzene, bromomethane, and bromoform), and 1 contains sulfur (carbon disulfide). The list of compounds in both liquids does not show the presence of Br and S, while chlorine (Cl) is only present in one molecule (ethane, 1,1,1-trichloro-). Based on the points argued before, the quantification of chlorine molecules raises concern about the possible availability of sucralose in the e-liquids (a molecule that is difficult to measure using GC-MS due to its low volatility). This sweetener is documented for its pyrolysis and the releasing of chlorinated byproducts and for enhancing overheating conditions due to its caramelization on the coil. The lack of information on the specific commercial e-liquids that were analyzed prevents a deeper investigation of these inconsistencies.

Finally, the authors used passive sensors to evaluate the air quality in the mouse exposure chamber, measuring carbon monoxide (CO) and volatile organic compounds (VOCs). Interestingly, the concentrations of CO were higher than the sum of the concentrations of all VOCs detectable in three of the five experiments carried out (PG/VG, tobacco, tobacco+nic), whereas using the menthol and menthol+nic, the concentration of VOCs reached values at least two times higher under those conditions. These averaged values limit the interpretation of the measurements. Indeed, the concentrations are real-time values, and the curves would have probably shown abrupt changes in some of these concentrations (an assumption made based on the deviations equivalent to the average values in CO). Besides the presence of these quantified values, the presence of CO is a clear indication of cotton pyrolysis and supports the conclusion that the aerosol was generated under overheating conditions.

5. Discussions

We have conducted a review of studies focused on the effects of exposure of in vitro and in vivo systems using the EC aerosol generator additional module of the InExpose system with a 0.15 Ω coil and airflows around 1 L/min. An extensive literature search based on the device information produced 40 studies listed in Table 1, which highlight many experimental issues.

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5.1. Lack of information on aerosol generating procedures

While the 40 selected studies provide detailed information on the characteristics of cell lines and rodents and on the methodology of the biological and toxicological procedures, most of the studies failed to provide sufficient information on their aerosol generating procedures. As discussed in Sections 2 and 3 and depicted in Table 1, only 9 studies provide sufficient information to analyze their aerosol generation with full or almost full certainty (the ones denoted as "certain" and "almost certain"). Six studies (the "suspicious") provided bare minimal sufficient information. The remaining 26 articles failed at different levels to provide this information, with 10 of them providing no information besides having used the InExpose.

Since these studies are focused on the effects on biological systems from aerosol exposure, the generation of the aerosols constitutes an important technical issue that must be fully described in their Materials and Methods section. The vacuum of information on aerosol generation procedures renders these studies as unreproducible and/or impossible to replicate, a serious methodological flaw in experimental research that certainly hinders the quality of these studies, irrespective of the possible impeccable quality of the biological procedures.

Evidently, we cannot claim full certainty in our evaluation of aerosol generation in the 26 studies that provided insufficient information. Hence, we only reviewed (Section 4) in detail 5 studies that provided this information in full, also reporting aldehyde yields and nicotine concentrations. However, given the thematic similarities of all the studies listed in Table 1 and the common usage of the InExpose that requires training and a learning curve to operate, we can claim with high likelihood that the 16 studies that reported the 0.15 W coil and usage of SCIREQ equipment followed the methodology of the 14 studies that did provide at least a minimally acceptable degree of this information (which includes the 5 studies reviewed individually). Besides the 10 completely irreproducible studies with practically zero information, in at least 30 of the 40 selected studies, we have sufficient direct and indirect evaluation elements to assert that cell lines and rodents were exposed to overheated and aldehyde-loaded aerosols, generated by the combination of high power with low airflow and low resistance, a combination that is also at odds with consumer usage of these devices for the 'direct to lung' inhalation.

5.2. Excessive nicotine concentrations

Four studies[28][29][30] generated aerosol from e-liquids with high nicotine concentrations (30, 36, and 50 mg/mL). Given the large puff volumes produced by high-powered devices like the EVIC Mini, such high nicotine concentrations would involve the inhalation of an excessive and unpleasant and harsh nicotine dose. As an example, Pinkston et al[28] quantified around 0.8 mg and 0.4 mg of nicotine vaporized per puff (menthol liquid and Crème Brûlée liquids). This nicotine dose implies the delivery of the amount of nicotine from 10-15 puffs of a tobacco cigarette into one or two puffs of the generated aerosol. Obviously, vaping machines continue operating, but a human user (even a heavy smoker) will resent the throat harshness of such nicotine excess, which would very likely trigger pharmacological effects (C_{max} mainly) that could reach an order of magnitude higher than smoking. Evidently, this is an excessive overexposure for cell lines and 20 g mice.

Nöel et al[34] justify their experimental setup of high nicotine levels as a way of mimicking the nicotine delivery in heavy smokers. However, this justification has no basis in known evidence of consumer usage, as shown by an observational study[66], these high nicotine levels are not representative of the usage of devices like the EVIC Mini. In fact, observations[66] reveal that high nicotine levels (overwhelmingly in nicotine salts) are used with low-powered pods, while high-powered devices are normally used with low nicotine concentrations (between 0 and 6 mg/mL)[66]. Nicotine overexposure (to a milder degree) can also be ascribed to the 14 studies in Table 1 that reported nicotine concentrations between 20 and 30 mg/mL.

A more realistic nicotine dose could be obtained by dividing the nicotine delivery in Pinkston et al[28] by at least a factor of 5, leading to a nicotine dose closer to the delivery by a JUUL (their case of menthol liquid) (≈ 0.1 mg per puff with nicotine-salt designed to avoid harshness). The same reasoning applies to the other three studies[29][30][34] that puffed the EVIC Mini with free-nicotine concentrations above 30 mg/mL.

5.3. High levels of aldehydes

The high levels of quantified aldehydes found in[28][33][34] support the main criticism of the present literature review: overheating accompanied by an exponential production of aldehydes occurs when puffing a high-powered device like the EVIC mini with a 0.15Ω coil and an airflow of 1.1 L/min (see Figure 5). The authors of[28][33][34] reported yields that (at least) signal early stages of the overheating

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conditions (masses above 1 or 2 μg per puff), with higher levels (7-8 μg per puff) denoting more advanced stages of overheating that can be associated with the onset of highly energetic pyrolysis close to ignition, a highly likely development supported by levels of acetaldehyde comparable to or higher than formaldehyde. To highlight overheating conditions, it is useful to compare these high aldehyde yields with the formaldehyde yields from the Juul in[28]. The Juul released 5 ng of formaldehyde per mg of e-liquid vaporized; hence, the same ratio of mass per puff means that 7μg of formaldehyde would have to be generated by 1400 mg of e-liquid per puff (assuming indirectly the same condition), an enormous e-liquid quantity that questions the conditions used in these studies to generate the aerosol.

Reference	[34]	[30]	[28]	[26]							
Conditions	But[1] (2.8V)	But[1] (3.8V)	But[1] (4.8V)	Cin[2] (2.8V)	Cin[2] (3.8V)	Cin[2] (4.8V)	Cin[2] (4.2V)	Men[3] (3V)	CB[4] (3V)	Tob[5] (80W)	Men[3] (80W)
Acrolein	0.05	3.5	9.5	NA	NA	NA	NA	0.02	10	3.5	150
Acetaldehyde	0.05	15	16	0.17	0.33	0.39	0.386	0.1	4	3.1	150
Formaldehyde	0.08	3.5	8	0.10	0.18	0.02	0.021	2.5	13	NA	NA
Reference	[33]										
Conditions	30/70 (4.2V)	50/50 (4.2V)	70/30 (4.2V)	Str[6].12 (4.2V)	Str[6].18 (4.2V)	Van[7].12 (4.2V)	Van[7].18 (4.2V)	Cat[8].12 (4.2V)	Cat[8].18 (4.2V)		
Acrolein	0.8	0.15	0.9	0.01	0.01	1.3	0.1	0.2	0.5		
Acetaldehyde	0.7	3	2	0.6	0.7	0.8	0.1	0.2	2		
Formaldehyde	3	7	7	NA	NA	3.5	0.05	NA	7		

Table 2. Summary of the aldehydes quantified in the five articles reviewed.

[1] Butter liquid; [2] Cinnamon fireball flavor; [3] Menthol; [4] Crème Brulée; [5] Tobacco flavor; [6] Strawberry flavor; [7] Vanilla flavor; [8] Catalan cream.

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5.4. Presence of carbon monoxide

The quantification of CO by Pinkston et al[28] is an important outcome supporting the main criticism of the present review. Also, Muthumalage, T.; Rahman[26] quantified CO inside the exposure chamber at levels similar to those of the sum of the volatile organic compounds (VOCs). To assess the toxicological relevance of these outcomes on biological systems, we remark that they constitute a clear signal of advanced overheating through energetic pyrolysis in the onset of oxidizing processes acting on the cotton element of the wick (and its subsequent structures)[71]. The presence of CO also means that other cotton byproducts must have also been released, thus polluting the aerosol generated and increasing its toxicity. The literature on cotton pyrolysis shows that during various stages of pyrolysis, the cellulose (in the wick) releases highly toxic furans and polycyclic aromatic hydrocarbons (PAHs). In fact, the results of previously published articles that looked at CO in vaping aerosols highlight a high correlation with excessive supplied power and insufficient airflow (i.e., conditions above the power ranges of the optimal regime)[72][73][74].

5.5. Air dilution of the aerosol

The EC aerosol puffed by the box of the EVIC mini is transported by a pump at an airflow of 2 L/min to the exposure chamber through a tubular conduct. It is well known from filtration and sampling in aerosol physics that this transport involves, besides air dilution of the generated aerosol, modifications of its particle/gas partition, mostly affecting the particulates (liquid droplets) with larger diameters that either impact or settle on the conduct walls or (pending on environmental variables) might coagulate, condense, or evaporate. However, this air dilution and aerosol physics phenomena will not remove or decrease the toxicity levels of an aerosol generated under overheating conditions. Empirical proof of this is furnished by Muthumalage and Rahman[26], who found high levels of CO and VOCs in their chemical analysis of the aerosol in the exposure chamber (after its passage through the conducts).

5.6. Toxicological implications

Considering the results of our own laboratory tests summarized in Section 2 (published in[14]) and our review of 5 individual studies in Section 4, it is clear that overheating conditions increase the toxicity of the generated aerosol as specified by high levels of aldehyde yields and signals of energetic pyrolysis of the organic wick material (specially CO). Therefore, it is not surprising that deleterious effects in cellular physiology detected in cells and animal tissues result (at least in part) from the molecular presence of these toxic byproducts.

The study by Muthumalage and Rahman[26] that quantified concentrations of CO and COVs of similar magnitude provides an illustrative example of how inappropriate laboratory conditions that lead to overheating can induce misleading physiological effects. As recounted in their figure 9, summarizing the altered proteins in lung tissue, these authors report that a pure solvent PGVG mixture leads to the highest number of altered proteins (around 240 proteins). Adding tobacco flavor leads to 156 alterations, with 88 in common with those of the PGVG mixture. Adding nicotine leads to 118 alterations, with 52 in common with both PGVG only and tobacco flavor. PGVG exposure has the highest overheating condition observable with the maximal CO, followed by the tobacco+nicotine and the tobacco experiments.

Applying the same reasoning to menthol liquids leads to 174 alterations and 107 alterations without and with nicotine, where 49 and 51 are in common with PGVG alterations. The reduction in common alterations with PGVG compared to tobacco experiments can be due to lower overheating conditions, which is observable by the CO concentrations closer to 0ppm in menthol experiments, whereas it is above 15ppm in tobacco experiments. Despite it can be a coincidence, the absence of PGVG testing under normal conditions prevents investigating the contribution of abnormal testing and flavoring agent or nicotine adding in the alteration process.

5.7. Insufficient awareness of consumer patterns

The (JoyeTech® EVIC Mini) was released in 2015 as one of the early devices allowing for a temperature control mode. Currently, this device is difficult to find, and its usage is marginal. This follows from the evolution of consumer patterns. Between 2015 and 2019, usage of third-generation tank devices became very popular, including devices operating at high power (> 30 W) with sub-ohm resistances (< 1 Ω). The demographic study by Jiang et al[75] shows the market evolution of third-generation devices in the USA between 2015 and 2019. These devices are subdivided into "tanks" and "mods," with "mods" describing the bulky devices that are used at high power settings. While tanks became very popular, reaching over 70% of preferences in all ages in 2017, their usage declined to 30-35% in 2019. "Mods" have remained low in preference for all ages, decreasing in the period 2015 to 2019 to 6.3% (young users) and 9.5% (young adults). It is very likely that these percentages of "mod" usage are currently even smaller, with usage of low-powered devices, including cartridge-based pods and

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disposables, becoming dominant. Preference for low-powered devices is even more marked among adolescents and young adults[76][77].

Given these developments in consumer preferences, it becomes hard to justify the usage of a device like the EVIC Mini in current preclinical studies that aim to assess the toxicological profile of generic EC aerosols. At best, if such toxicological assessment is conducted under appropriate laboratory conditions, it will be only applicable to a reduced minority of consumers (which can still be useful). At worst, an assessment based on inappropriate laboratory conditions (high power with insufficient airflow) unrepresentative of consumer usage is necessarily misleading and has little utility to all consumers and stakeholders.

Usage in the laboratory of any arbitrary combination of e-liquids, coils, and devices does not necessarily qualify as testing under "normal" conditions simply because all these combinations are commercially available. The case in point that we have stressed in the present review is laboratory usage of high-powered devices (for example, operating well above 30 W). This testing cannot be justified as normal usage under any arbitrary airflow or nicotine levels simply because devices and e-liquids with these characteristics are available to consumers in the vaping market (by the same token, driving at 160 km/h or faster cannot be regarded as generic "normal driving", simply because such high speeds are accessible in many commercial automobiles). For laboratory testing of high-powered sub-ohm devices to be objective and useful, authors must acknowledge their representative and overwhelming majority usage with low nicotine concentrations and deep inhalation (i.e., large airflow) needed to cool and condense efficiently the air-diluted aerosol (up to 50 mg/puff) in large puff volumes (~ 500 mL) produced by such devices (whose design with wide mouthpieces to minimize air resistance is consistent with this usage).

It would be very useful and would enhance the quality of preclinical research to pay more attention to consumer behavior. Consumer patterns are not only reported in consumer forums and magazines[78] and in peer-reviewed publications sampling social network films[79], but are described by manuals elaborated by manufacturers, and they are also reported in published peer-reviewed literature[16][80][81], including observational studies[66]. The different ways different devices are used can also be understood as a result of the trial-and-error self-training guided by sensorial effects that vary from user to user, but practically all users conduct this self-training when they begin vaping. A naïve user may try inhaling a powerful device as if puffing a Juul and will receive a hot and unpleasant

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aerosol, since the low airflow appropriate for a Juul is insufficient to evacuate and cool the large amount of vaporized e-liquid. But users learn and adapt.

5.8. Misunderstanding on "dry puffs" and "normalizing" abnormal usage.

When reporting high aldehyde yields, Nöel and Ghosh[33] comment that the aerosols "were not produced under 'dry puff' conditions" presumably because the e-liquid in the InExpose tank was not depleted. Therefore, these authors mistakenly assumed that the aldehyde yields occurred under normal usage conditions without a dry puff. This comment reveals a misunderstanding in the assumption that the repellent "dry puff" phenomenon is only tied to a single event marked by the depletion of e-liquid in the atomizer, thus normal usage occurs as long as e-liquid is not depleted. The same misconception was expressed by Beard et al.[82], who also puffed a high-powered device with insufficient airflow to examine cytotoxicity from "dry hits". These authors classified as normal "standard vaping" all usage without e-liquid depletion.

These assessments by Nöel and Ghosh[33] and Beard et al[82] fail to understand that e-liquid depletion only marks the endpoint of an overheating process characterized by critical thermal phenomena such as film boiling, when the coil temperature goes above the boiling point of the liquid mixture[24]. This endpoint is the advanced manifestation of overheating and is unequivocally accompanied by energetic pyrolysis of the organic material of the wick and a rapid increase of thermal degradation byproducts.

Beard et al[82] argued that abnormal vaping conditions should also be studied. We fully agree; abnormal and critical conditions are researched in many issues, for example, simulating automobile accidents at high velocities in order to improve safety measures. However, this type of simulation or experiment should not convey (not even hint) the notion that the tests somehow describe "standard" driving conditions. The same applies to cytotoxicity tests on dry puff conditions. High toxicity levels from overheating conditions need to be investigated to improve our knowledge of this phenomenon and to achieve a comprehensive perspective of vaping products. However, studying these conditions is misleading without an explicit acknowledgement that they are abnormal and deviate from normal consumer usage.

Just as automobile drivers recognize an unpleasant risk of excessively high velocities, there is solid peer-reviewed published observational evidence that users perceive sensorially the onset of overheating well before e-liquid depletion. The organoleptic experiments by Visser et al.[25] proved that increasing pyrolysis is perceived by users in gradual progressive stages, increasing from 0 (no perception) to 1 (full dry hit), a perception that was correlated to results of laboratory testing (even with a filled tank). Another clear signal of overheating of aerosol generated by the InExpose is in the introductory video in Noël et al[13], showing the initially colorless e-liquid before the experiment became brownish at the end. This brownish color is a clear signal of compounds produced by cotton pyrolysis[83].

5.9. Comparison with effects from cigarette smoke

The overwhelming majority of users of ECs (vapers) are currently still adult smokers replacing cigarettes with the much safer nicotine delivered through the EC aerosol not generated by combustion. Therefore, the comparison with cigarette smoke is still highly desirable for toxicological and preclinical studies to best contribute to advancing public health goals.

A low priority, in comparison with tobacco smoke, can be based on the broad scientific consensus already existent that supports harm reduction for adult smokers who switch to vaping. An additional argument supporting this low priority follows from the surge of vaping among teenagers and young adults who have never smoked (or have smoked very infrequently). An assessment of these arguments is beyond the scope of the present review, but even if taking them at face value, given the overwhelming preference of adolescents and young adults for low-powered devices[76][84], there is no justification or public health benefit in assessing EC aerosol toxicity under inappropriate laboratory conditions and/or testing high-powered devices whose usage has become marginal.

While the authors can argue that using outdated devices in preclinical studies is inevitable, since these studies are expensive and require a long time frame, from assuring sufficient grant funding to preparing the materials and analyzing the outcomes, this claim is not consistent with the fact that the consumer shift to low-powered devices was already in full swing when most of the studies we reviewed were published: only 9 were published between 2016 and 2020, 6 in 2021, while 20 (50% of the studies) were published in 2022 and 2023 (10 each year). Therefore, this delay of publication really applies only (at most) to 15 studies published before 2022 (and to only one of the 5 studies reviewed individually).

5.10. Limitations

The present review is evidently limited by the various degrees of insufficiency in providing information on aerosol generating procedures in 26 of the 40 selected studies. As argued in Section

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5.2, this vacuum of information leads to various degrees of uncertainty in assessing the generation of EC aerosols under overheating conditions in these 26 studies. However, in 16 of these 26 studies, there is sufficient indirect evidence to infer these conditions. Another limitation (common to all research on ECs) is the difficulty of considering the full effect of the wide diversity and individual usage patterns of the devices, further complicated by the rapid changes in vaping technology and the regulatory landscape, as well as the capacity of users to modify behavior to adapt to all these developments. Nevertheless, in dealing with complexity, there is no better option than to resort to the best assessment from general tendencies supported by observation and by solid theoretical and experimental facts.

6. Guidelines for laboratory usage of high-powered devices

We believe that the InExpose and the SCIREQ equipment are valuable tools for the preclinical assessment of ECs. Hence, we would like this review to contribute to its improvement and better use by researchers, not only those using it or considering its future usage, but researchers in the preclinical assessment of ECs in general. Therefore, we provide in this section a series of guidelines that concretely aim at correcting the problems we have spotted in using the InExpose with high-powered vaping devices.

- The first recommendation for improving the InExpose is to perform preliminary calibration (or to recommend users to do it on their own) of the EVIC Mini. This calibration is necessary and useful, since aerosol is bound to be generated by the box mod of this commercial device equipped with low-cost commercial coils, all of which lack the required manufacturing quality for laboratory purposes.
- The next recommendation concerns the airflow rate. The same calibration tests conducted with the EVIC Mini box for the airflow rate of 1.1 L/min were also conducted in[14] with an airflow rate of 10 L/min. For this airflow, screen and measured values are well calibrated for much wider ranges of power up to 63 W and temperature up to 300°C (these values are consistent with the functional limits displayed in Figure 4). Comparing the calibration tests in the two airflow rates (1.1 L/min and 10 L/min) clearly highlights an important problematic issue in experiments using the InExpose: the EVIC Mini is much more efficient, and its screen values are much more reliable when it is puffed at 10 L/min. This increased efficiency and reliability is consistent with the design of this device for ‘Direct to Lung’ vaping (low air resistance and large puff volumes).

- Therefore, we recommend SCIREQ to instruct users of the InExpose to avoid generating aerosols with the EVIC Mini at high power levels (marked on the screen) with the low airflow limited by the maximal instantaneous peak flow of 1.675 L/min, which leads to airflow rates around 1-2 L/min for puff durations around 2-4 s. In case there is no other option but to puff the EVIC Mini with this reduced airflow, users should be instructed to avoid coils with low resistance and/or to puff the device with sub-ohm coils at low powers below screen values of 35 W (below 30 W measured) that keep the device operating within the optimal regime and with diminished aldehyde yields (see figures 4 and 5).
- To generate aerosols with the EVIC Mini or any other sub-ohm device with low resistance at high power settings, SCIREQ should modify the pump controller to allow for airflow rates of up to 10 L/min and inform the users of the equipment to puff the devices at this airflow. Otherwise, the generated aerosol will be in the overheating regime. Modifying the controller to allow for much higher airflow rates would make the experiments with the InExpose and EVIC Mini (or any other sub-ohm device) consistent with consumer usage of such devices for the "Direct to Lung" puffing style. It would also be able to generate an aerosol without overheating and with minute aldehyde and toxin production.
- However, the EVIC Mini is an outdated mod device released in 2015, so if SCIREQ continues the usage of sub-ohm devices, it might as well replace this device with a more updated one. However, the main question to ponder in the end is why consider high-powered sub-ohm devices in preclinical studies when their usage is not representative of consumer preferences that have currently shifted overwhelmingly to low-powered devices and disposables.

7. Conclusion

We have conducted a literature review focused on the aerosol generation procedures in 40 studies that used the InExpose, a modular part of computerized equipment manufactured by SCIREQ (Scireq®, Montreal, QC, Canada), to examine the effects in biological systems (cell lines and rodents) exposed to EC aerosol. The InExpose has the capacity to generate EC aerosol from any vaping device but uses as default configuration the box mod of a third-generation device (JoyeTech® EVIC Mini) equipped with a variety of coils and puffed at airflows limited by a maximal instantaneous peak flow of 1.675 L/min (1-2 L/min with 2-4 s puffs). The 40 reviewed articles listed in Table 1 were selected in Section 3 from an extensive literature search focused on the usage of the InExpose to generate EC aerosol with the EVIC Mini equipped with a 0.15 Ω coil.

To evaluate aerosol generation procedures, we consulted the publicly available information supplied by SCIREQ[12][13] and conducted electric calibration of the EVIC Mini and its parts, as well as functionality tests that replicate as best as possible the functioning of the InExpose (see[14] and a summary in Section 2). For the airflow rate of 1.1 L/min, consistent with the InExpose allowed airflows, our functionality test showed an optimal regime of efficient aerosol production in the range of 15-30 W, with overheating conditions and exponential aldehyde production for power levels above 30 W (figures 4 and 5). The calibration tests (Section 2) showed that above the 40-45 W threshold, significant differences arise between the values of power, voltage, and temperature displayed on the instrument screen of the EVIC Mini with respect to the values measured in the laboratory. We then compared the aerosol generation parameters reported (from the instrument screen) in the 14 studies that provided (at least) a minimal level of information (the "certain", "almost certain", and "suspicious" in Table 1). The 14 studies with sufficient information reported instrument screen values of 70-80 W, 3 to 4.8 V, and temperatures of 200-250 °C, which, once referred to the laboratory measurements in the calibration tests (Figures 1, 2, 3) and the functional curves (Figures 4 and 5), make it clear that these values correspond to aerosols generated under overheating conditions

Of the 14 studies that provided minimally acceptable information on their aerosol generation procedures, we selected 5 studies for individual review (Section 4)[26][28][30][33][34] that provided a full description of aerosol generation and quantified aldehydes, nicotine, and other byproducts. Of the remaining 26, 10 studies merely reported the usage of the InExpose or mentioned "SCIREQ" (these are the "unknown" in Table 1). Since aerosol generation is very relevant to assess studies of exposure to EC aerosols, this information vacuum on such a key technical point renders these 26 studies as basically unreproducible or impossible to replicate. This is a serious methodological flaw in studies conducting experimental research, which hinders their quality and relevance, even if the implementation of the biological procedures was impeccable. This is also a serious flaw in the peer-reviewing process in the journals that published this research, with reviewers and editors incompetent to evaluate (or simply disregarding or overlooking) this important technical issue.

Evidently, we cannot claim full certainty on the evaluation of aerosol-generating procedures of the 26 studies that failed to provide sufficient information. However, there is a high likelihood that the criticism of the 14 studies that provided information also applies to them. All 26 studies that lack information mention “SCIREQ” or “InExpose,” and 16 of them mention or hint at minimal details, such as the usage of a third-generation device or the 0.15 Ω coil. Also, the usage of the InExpose is not a trivial matter; it is not low-cost equipment, and it requires training and a learning curve to operate it. Therefore, it is extremely likely that authors had to resort to available information to operate the equipment, either following the methods of previous studies using it, requesting technical advice from SCIREQ, or learning from the introductory video by Nöel et al[13]. All these options should have led, at least in most cases, to the aerosol generation configuration that we have reviewed and criticized.

The problematic puffing with insufficient airflow of 1-2 L/min from a sub-ohm high-powered device, like the EVIC Mini with a 0.15 Ω coil, reveals that authors of the reviewed studies have been oblivious and unaware of the overwhelming consumer usage of sub-ohm devices for Direct to Lung vaping, which involves a deep inhalation of large aerosol volumes. This consumer usage is not only stressed in consumer magazines but also recommended by manufacturers and in peer-reviewed observational studies (see references in Section 5.8). The usage of these devices with high airflows is supported by their design and by physical arguments: high-powered devices have wide mouthpieces (low air resistance) to facilitate the deep inhalation (large airflow) necessary to achieve a sufficient forced convection to condense and cool the large amount of vaporized e-liquid produced by high-powered operation[16][80][81].

In the individual reviews of the 5 studies in Section 4, we also found other problematic issues that contradict consumer usage, such as using the EVIC Mini with e-liquids containing high nicotine concentrations (above 20 mg/mL and up to 50 mg/mL). However, such concentrations delivered through the large puff volumes of sub-ohm devices would involve such an excessive yield of inhaled nicotine (the equivalent of a full cigarette in one puff) that it would be intolerable even to heavy smokers (more so for 25 g mice). In fact, consumers only use these high levels with nicotine salts in low-powered devices (refillable, cartridge-based, or disposables)[66].

We also found the misconception that identifies the dry puff phenomenon as a single event marked by e-liquid depletion, thus claiming that “normal” vaping conditions occur as long as the e-liquid is not depleted. This is mistaken; the dry puff is the end point of an overheating regime that initiates when e-liquid is not depleted and is characterized by a critical thermal process: film boiling[17][24]. However, users recognize sensorially the development of this process before the e-liquid depletes[25].

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While research under abnormal, critical, or exceptional conditions like a dry puff is legitimate and necessary to achieve full knowledge of vaping, this does not justify the systematic usage of high-power devices puffed with a low airflow rate in toxicological studies (not only the ones we reviewed here), with most authors failing to stress the abnormality of this experimental set-up. It is unacceptable to justify testing under these conditions without stressing their abnormality simply because users have commercial access to devices and e-liquids that allow them. It is worrying that this failure to acknowledge abnormality might normalize overheating conditions as representative usage in toxicology studies, with a possible undesired consequence of producing a doubtful consensus based on unrealistic experimental results that overstate the risks of vaping but will have limited utility to assess its risks under normal consumption patterns[18][19].

Demographic trends since 2018 show a tendency evolving towards an overwhelming majority consumer usage of low-powered devices (starter kits, refillable and cartridge-based pods, disposables), with usage of high-powered sub-ohm “mods” increasingly becoming marginal, used only by dedicated minority niches of vaping hobbyists (more so with the JoyeTech EVIC Mini, which was released in 2015 and is now outdated and hard to find in vaping retailers). This shift in consumer preferences questions the utility of using sub-ohm devices to generate aerosol in tests and preclinical experiments to assess a generic EC risk profile.

Finally, we regard the InExpose and the SCIREQ equipment as valuable tools to examine the effects of ECs in biological systems; hence, we provide also (Section 6) a series of guidelines based on the results of the present review that we believe can contribute to enhance its operational quality and might serve also for all preclinical research on ECs.

Statements and Declarations

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used: “Conceptualization, S.S. and R.A.S.; methodology, S.S. and R.A.S.; software, S.S. and R.A.S.; validation, S.S. and R.A.S.; formal analysis, S.S. and R.A.S.; investigation, S.S. and R.A.S.; resources, S.S.; data curation, S.S. and R.A.S.; writing—original draft preparation, S.S. and R.A.S.; writing—review and editing, S.S. and R.A.S.; visualization, S.S. and R.A.S.; supervision, S.S. and R.A.S.; project administration, S.S. and R.A.S.; funding acquisition, Y.Y. All authors have read and agreed to the published version of the manuscript." Please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

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