Product Use Behaviour & Biomarkers Sub-Groups

Technical Report

Assessing Product Use Behaviour and Exposure: Definitions and Methods

October 2022

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1. INTRODUCTION

The process of smoking a cigarette comprises many complex behaviours, attributes like the volume and frequency of puffs taken, and number of cigarettes consumed per day are highly variable between individuals[1]. Together with many other parameters, such individual and group smoking characteristics are called 'smoking behaviour'. Thus, smoking behaviours are actions taken by a person that are associated with the burning of the tobacco rod and inhalation of the aerosol generated, and these have been researched and evaluated to complement assessing exposure using standard machine smoking.

During the last decade, smoking behaviour research has broadened and is referred to as 'product use behaviour' research with the emergence of new tobacco and/or nicotine containing products. Examples of these products and their associated descriptions are:

- **Heated tobacco product (HTP)[2,3,4] / Non-combusted cigarette[5] / Tobacco heating product (THP)[6] / Tobacco heating system (THS)[7] / (Novel) tobacco vapor product (NTV or TVP)[8] / Heat-not-Burn tobacco product (HnB)[9]:**

  Product containing a tobacco substrate that is designed to be heated and not combusted by a separate source (e.g. electrical, aerosol, carbon, etc.) and produces an inhalable aerosol. This product category has recently come onto the market in several countries, and regulatory agencies, researchers, and manufacturers use a variety of terms and acronyms to describe this product category.

- **E-vapor product[2] / Electronic cigarette (e-cigarette or e-cig)[4,10,11,12] / Vape[13] / Vapor product[14] / Electrical nicotine delivery system (ENDS)[15,16]:**

  Product not containing tobacco that is designed to electrically heat a liquid (may also be called an e-liquid), which usually contains nicotine and propylene glycol and/or glycerol, to produce an inhalable aerosol. Regulatory agencies, researchers, and manufacturers use a variety of terms and acronyms to describe this product category. Moreover, they are known by many different names and can be found in many shapes, sizes, and device types (e.g., cig-a-like, pen, tank, mod)[13].

- **Nicotine-containing, tobacco-free oral product[17] / Oral tobacco derived nicotine product (OTDN)[2] / Modern oral product (MOP)[18] / Tobacco-free nicotine pouch[19]:**

  Pre-portioned, non-pharmaceutical product containing nicotine, but does not contain tobacco. It is exclusively intended for oral use with nicotine uptake occurring via the oral mucosa. This innovative product category is popular in Sweden and Norway and is gaining in acceptance in other countries. Regulatory agencies, researchers, and manufacturers use a variety of terms to describe this product category.

In 2013, Gregg *et al.* published a set of terms and definitions to describe both smoking behaviour and tobacco smoke exposure in relation to conventional cigarettes[20]. Over the past number of years, the tobacco and/or nicotine containing product portfolio has expanded to include products as described above (e-vapor product, HTP and nicotine-containing tobacco-free oral product). Moreover, other alternative tobacco and nicotine products, such as nicotine-free e-vapor products and cannabis containing products, have also been introduced to the market, but this technical document focuses on non-pharmaceutical, tobacco and/or nicotine containing products.

Although scientific papers and regulatory requirements / recommendations related to product use behaviour and exposure have been published for these novel nicotine-containing/tobacco products, there is great diversity in the terminology used to describe these types of products. The aim of this paper is to define the terms and methods used for assessing product use behaviour and exposure, with the objective of generating a uniform application of the terms used by scientists working in this field of research.

2. TERMINOLOGY

A list of the terms included in this paper along with common synonyms, definitions etc. is shown in Table 1 (updating the Gregg's list[20]). The terms are loosely divided into two categories: 'Product use behaviour' and 'Biomarkers'. The terms are separated into related categories, and the terms related to 'Puffing topography' are listed as a subset of product use behaviour. For each term, units of measurement are provided typically with an example and a comment on the method or its application.

In Table 1, most examples are given in relation to inhalable products because they are the most widely researched and form the vast majority of scientific publications on product use behaviour and exposure.

3. DISCUSSION

3.1 Product user categories

The smoking status of conventional cigarettes is commonly described by three categories: current smoker, ex-smoker/former smoker and never smoker. There are several ways to survey research study participants about smoking status, and the operational definition of "current smoker", "ex-smoker/former smokers" and "never smokers" may vary depending on the objective of a given questionnaire. Although the definitions of active smoking status have not been fully standardized, the definition of "smoker" can be based on the total number of cigarettes smoked in the respondent's lifetime (e.g., "Have you ever smoked 100 cigarettes") or in a question about current use and/or frequency of use (e.g., some days or every day)[21,22,23,24]. Moreover, some different definitions of ex-smoker/former smokers can be also found based on the duration of their quit smoking period[21,2123,24].

In contrast, a common definition of e-vapor product current use prevalence does not exist. Several definitions can be found in the literature. For example, in the Population Assessment of Tobacco and Health (PATH) Study, the e-vapor product using status among the adult population is assessed by asking the following questions (Q1: Have you ever used e-cigarettes, even one or two puffs? [Yes / No], Q2: Have you used e-cigarettes fairly regularly? [Yes / No], and Q3: Do you now use e-cigarettes? [Every day / Some days / Not at all]), without the lifetime threshold question[24].

Additionally, no definitions of use prevalence for HTP and nicotine-containing tobacco-free oral products exist at this time.

Dual use is an interesting phenomenon and while there is no consensus on its definition, most evidence-based literature commonly refers to those who use both novel tobacco and nicotine products, and conventional cigarettes; whereas those who use more than two products are

referred to as poly users. Those who use only one product are referred to as exclusive or single/solo users. This term may also be extended to individuals who use one of the novel tobacco and nicotine products, but do not consume conventional cigarettes.

3.2 Product use behaviour

During a single usage of inhalable product, use behaviour primarily consists of repeating three steps[25,26,27]: (1) drawing out the smoke or aerosol from the product to the mouth (called 'puffing'), (2) inhaling the smoke or aerosol from the mouth into the trachea and respiratory space with air dilution (called 'inhalation') and (3) expelling inhaled and puffed constituents from the respiratory space and mouth or nasal passages (called 'exhalation'). In addition, there may be mouth-hold and breath-hold involved, depending on individual use behaviour. Measurement of puffing topography has been widely researched using specific puff recording instruments; however, these studies are technically difficult and smoking parameters are less well defined for e-vapor product and HTP compared to traditional cigarette consumption. For example, number of puffs and inter-puff-interval are well defined for one cigarette but less defined for e-vapor product use as consumers can puff continuously throughout a 24-hour period.

Oral nicotine containing products are generally placed between the gum and the lip (upper lip or cheek/jaw) for a certain period of time (referred to as mouth-hold time), product use instructions are given by each manufacturer[28].

3.3 Portable puff recording instruments

A number of recent research reports have detailed use of portable smoking instruments to record puffing topography measurements. Typically, these devices measure parameters of puffing behaviour such as puff number, puff interval, peak flow and mean flow. However, these devices are not capable of measuring other parameters such as mouth hold, mouth spill and any parameters associated with inhalation. Therefore, puffing topography instruments only give estimates of human smoking yield, but not an entire smoking cycle. As noted above, there is no direct relationship between puffing parameters and subsequent inhalation, and therefore, measurements of inhalation and exhalation are also required to complete the smoking cycle[29,30]. Nonetheless, despite these limitations, these portable puffing topography instruments are extremely useful in field studies (i.e., away from a laboratory environment) and they can be used easily in cross-sectional studies of puffing behaviour. Portable topography instruments measure fewer parameters of human smoking but produce data more rapidly than those obtained with more-labour intensive, laboratory-based, puffing duplication techniques[31]. Further, product use behaviour is affected by the setting of the investigation[31] and the use of a portable instrument in an ambulatory setting may allow a product user to interact with a consumable in a more typical manner than a laboratory environment.

Annotated graphics showing how some of these terms relate to a typical puffing topography profile and a typical product use topography profile are given in Figures Figure **1** and Figure **2**.

3.4 Intake, uptake and retention

Regarding inhalable products, the terms intake, uptake and retention and their definitions cover subtly different aspects of exposure to smoke or aerosol. For intake, the definition refers to the amount of a smoke or vapour constituent that is taken into the mouth. This usage is consistent with the approach of other researchers, in separate disciplines such as risk assessment[32,33,34],

where intake is defined as maximum exposure, prior to an absorption step. Even if smoke or aerosol is expelled from the mouth immediately after puffing, a portion of the amount of any smoke or vapour constituent's intake may be retained in the oral cavity. In a similar manner, if an inhalation step occurs, then, after exhalation, a portion of any smoke or vapour constituent may be retained in the respiratory tract. This constituent retention may occur by several processes; for example, particle deposition onto a mucosal surface or by absorption into the tissue or bloodstream[27]. Uptake is used to define the absorption of smoke or vapour constituents, which is distinct from retention. For some smoke or vapour constituents, such as nicotine, retention and uptake are practically identical[34]. The topic of retention has been considered in more detail in a review by Baker and Dixon[26].

Regarding oral products, the fraction of nicotine extracted has been assessed as uptake derived from the difference in nicotine amount before and after product use. Several studies have indicated that on average about 15 %–20 % of the total nicotine content is extracted and absorbed, with large inter-individual variation[35].

3.5 Biomarkers

Biomarkers could play an important role in assessing product use behaviour and exposure including an assessment of the exposure to smoke constituents (called "biomarkers of exposure") and investigation of the potential health risk (called "biomarkers of effect" or "biomarkers of potential harm")[36,37,38,39]. Biomarkers of exposure are defined as the *"chemical, or its metabolite, or the product of an interaction between a chemical and some target molecule or cell, that is measured in a compartment in an organism"*.[38, 40] The measurement of biomarkers of exposure has become one of the major approaches to quantify human exposure to smoke constituents within clinical studies for the evaluation of new tobacco and/or nicotine containing products[41,42,43,44].

The use of a biomarker for smoke and/or aerosol exposure assessment is not straightforward, for several reasons. First, the biomarker chosen should be the smoke or vapour constituent of interest itself or a well-characterised metabolite. When such a biomarker is available, the degree of polymorphism within the metabolic pathway should also be understood because fast- and slow-metabolisers of the same smoke or vapour constituent could give different biomarker measurements in body fluids following a similar exposure. Therefore, even in the case of relatively abundant smoke constituents such as nicotine, the parent molecule plus five metabolites or the parent plus up to nine metabolites are typically measured to estimate uptake[45,46]. Second, metabolism may vary depending on the route of exposure to the constituent of interest; for example, inhalation and respiratory absorption compared to buccal and gastrointestinal absorption following oral exposure. Third, the assays used for biomarker measurement should be fully validated in line with recognized international guidelines on bioanalytical method validation like the US FDA, EMA, or Japanese guidelines[47, 48, 49]. Moreover, the International Council for Harmonization (ICH) is developing a guideline on bioanalytical method validation that will harmonise existing guidelines used in the US, Europe and Japan (ICH M10). Well-characterized reference standards are crucial to ensure valid measurements. The characterization of the reference standard will be documented in a certificate of analysis providing information about the identity and the purity of the substance[50]. Moreover, to monitor the accuracy of the assay over time, inter-laboratory comparisons and participation in ring trials are encouraged. Fourth, the availability of a biomarker and an assay does not automatically qualify it for all uses.

A biomarker of exposure should be specific for the use of one product compared to smoking and/or non-use. This issue was discussed in detail in Gregg et al. for smoking conventional cigarettes. In recent clinical studies for new tobacco and/or nicotine containing products, a similar pattern of biomarkers of exposure to smoke constituents, defined as tobacco toxicants or "harmful and potentially harmful constituents" (HPHCs) as summarized in several guidance documents by regulatory agencies or health organizations, has been widely assessed[51,52,53,54]. In order to demonstrate that new tobacco and/or nicotine containing products reduce exposure to smoke constituents, the assessment focuses on measuring changes in the levels of biomarkers of exposure when switching from conventional cigarette to the new tobacco and/or nicotine containing products[41]. Additionally, the identification and measurement of biomarkers specific to the exposure to one product (or product category) allows for a more robust risk assessment of these products once available.

Dual or multiple use of different products including cigarettes adds to the complexity in the exposure assessment of new tobacco and/or nicotine containing products using biomarkers. While compliance in terms of product use can be controlled in short-term clinical trials, long-term evaluations are more challenging. Biomarkers specific to cigarette smoking were recently discussed as a tool for compliance monitoring over longer time periods in switching studies (switching from conventional cigarettes to a new tobacco and/or nicotine containing product). A biomarker of compliance must meet two criteria. First, the biomarker concentration should be specific for exposure to the investigated product. The biomarker may be present from other sources but at significantly lower (or different) levels so the measured concentration must unambiguously reveal product use. Secondly, to be suited for long-term studies, a long half-life of the biomarker is desirable. Haemoglobin adducts are promising candidates as biomarkers of compliance because their half-life depends on the washout cycle of the erythrocytes, such as *N*-2-cyanoethylvalin globin adduct deriving from acrylonitrile exposure, while the mercapturic acid of acrylonitrile, cyanoethylmercapturic acid (CEMA), is assessed as a short-term biomarker of compliance in urine[55,56]. Other adducts, such as DNA adducts, may also be of interest depending on the biological specimen. Moreover, urinary metabolites which are excreted over a longer time period could be useful, e.g. the tobacco-specific nitrosamine metabolite NNAL.

In this technical document, we have deliberately limited our description of biomarkers to those concerned with human exposure to smoke/aerosol constituents, including the possibility of compliance monitoring. Biomarkers of risk have been defined as *"biomarkers that indicate a risk factor for a disease"*.[57] Although clinical outcomes such as cancer, cardiovascular disease (CVD), and chronic obstructive pulmonary disease (COPD) are definitive endpoints of smoking-related disease[58,59,60], they take decades to develop and thus are not practical to assess in the regulatory setting. Therefore, assessing biomarkers related to inflammation, oxidative stress, and other conditions, has become one of the major approaches to investigate the biological changes that may indicate a change in long-term disease risk within clinical studies for the evaluation of new tobacco and/or nicotine containing products[39,61]. These biomarkers are commonly referred to in the literature as biomarkers of potential harm, biomarkers of effect, or biomarkers of biological effect. However, it would be premature to provide definitions for these categories of biomarker in this document due to currently unclear definitions.

Table 1. Definition of terms, units, and comments

No.	Term	Definition	Units and methods	Example and comment
	Product use behaviour			
1	Daily product consumption	The number or amount of consumables used per day	• Consumption per day (cigarettes/day [CPD], sticks/day, portions/day, pouches/day, and so on) can be assessed by interview, questionnaire, or diaries or number counted • Amount per day (mL/day, mg/day) can be calculated by measuring or weighting the consumables (e.g., e-liquid) before and after using	• 20 CPD • 5 to 25 mL/day • With regard to oral products, weekly consumption of cans is also assessed
2	Exposure	The concentration × time for a specified constituent present in the external medium (such as air, water, tobacco smoke, vapour, extraction, food)	• Mass (mg, µg, ng, pg) × time (min, h, d, and so on) • Also assessed by measuring biomarkers of exposure in body fluids or organs (biological monitoring)	• Daily exposure of smokers to nicotine from cigarettes = Human Smoking Yield $_{[nic]}$ × CPD • Exposure may be thought of as the maximum potential dose of the smoke constituent. Intake and uptake cannot exceed exposure
3	Exhalation	The act of expelling inhaled and puffed material from the respiratory space and mouth or nasal passages of an inhalable tobacco/nicotine product user	*See 'Exhalation duration' and 'Exhalation volume'*	
4	Exhalation duration	The time from end of inhalation until the end of the exhalation phase of the breathing cycle	• Time (s) typically measured by flow monitoring through the oral cavity; can be measured from start of expiratory flow until the end of this action by respiratory inductive plethysmography devices such as bands or vests, which record chest movements[26]	• 5 s

No.	Term	Definition	Units and methods	Example and comment
	Product use behaviour			
5	Exhalation volume	The volume of material expelled from the respiratory space after a puff and inhalation from an inhalable tobacco/nicotine product	• Volume (mL) typically measured by flow monitoring through the oral cavity; can be measured from start of expiratory flow until the end of this action by respiratory inductive plethysmography devices such as bands or vests, which record chest movements[26]	• 500 mL
6	Mouth level exposure (MLE) / Yield in-use (YIU) / Intake	• The amount of a given mainstream smoke or vapour constituent exiting the inhalable tobacco/nicotine product into the mouth, when a given person uses that product • The amount of a given constituent extracted from the oral product into the mouth, when a given person uses that product	• Mass (mg, µg, ng, pg) can be measured by duplication of the human puffing profile in a smoking/vaping machine; can be estimated by used cigarette filter analysis techniques in terms of conventional cigarette; measured by used oral product analysis techniques; or estimated from extract fraction from placement of product and length of exposure.	• mg NFDPM / cig • mg nicotine / cig • µg acrolein / cig MLE varies across individuals and is usually different from machine-delivered yield[30,62]
7	Inhalation	The act of moving puffed material from the mouth into the trachea and respiratory space of an inhalable tobacco/nicotine product user	*See 'Inhalation duration' and 'Inhalation volume'*	This is a separate action from puffing[63]
8	Inhalation duration	The time from start of inhalation until start of exhalation phase of the breathing cycle	• Time (s) can be measured from start of expiratory flow until the end of this action by respiratory inductive plethysmography devices such as bands or vests, which record chest movements[26]	• 5 s This time does not include the initial puff or any mouth-hold period[63]
9	Inhalation volume / Inhalation depth	The volume of inspiration into trachea and respiratory space	• Volume (mL) can be measured from start of expiratory flow until the end of this action by respiratory inductive plethysmography devices such as bands or vests, which record chest movements[26]	• 500 mL • 11% vital capacity[27,63]

No.	Term	Definition	Units and methods	Example and comment
	Product use behaviour			
10	Machine yield / Machine-derived yield	The amount of a given constituent exiting the inhalable tobacco/nicotine product under machine smoking conditions at a specified smoking regime, e.g., ISO, Health Canada[64]	• Mass (mg, µg, ng, pg) Trap on glass fiber filter (Cambridge filter) or suitable solid or liquid traps, and quantitate with common analytical techniques (e.g., gravimetric, photometric, GC, HPLC)[64]	• mg nicotine / cig • µg acrolein / cig MLE varies across individuals and usually is different from machine-delivered yield[30,62]
11	Pack years (PY)	The number of packs smoked in a day multiplied by the number of years spent smoking	• Index (PY, or no units) calculated based on information about smoking starting and current/ending ages and the recalled number of packs smoked in a day (i.e., number of packs smoked per day × number of years smoking).	• 20 PY (One pack per day × 20 years / 2 packs per day × 10 years [based on a pack of 20 cigarettes]) Used as a crude surrogate for lifetime exposure without allowing for constituent yield and smoking behaviour, often used in epidemiological studies[65] although this has been criticized.[66] • Brinkman index (BI; number of cigarettes smoked in a day multiplied by the number of years spent smoking) has also used as a crude surrogate for lifetime exposure.[67,68]
12	Retention / Pulmonary retention	The difference between the amount of smoke or vapour constituent inhaled and the amount exhaled over subsequent breathing cycles	• Mass (mg, µg, ng, pg) • % retained Volumes are measured as described for inhalation and exhalation parameters Mass is estimated by appropriate physical or chemical analysis	• µg solanesol / cig • mg nicotine / cig • n % nicotine retained / puff For many smoke constituents, e.g., nicotine, retention is practically identical with the amount absorbed but for others, e.g., some PAH, retention does not equal absorption[26]
13	Uptake / Amount absorbed	The amount of smoke, vapour or extracted constituent which is absorbed through the mucosa of the mouth, respiratory tract, and lung	• Mass (mg, µg, ng, pg) Cannot be directly measured in a human but is deduced indirectly from the level of the biomarkers of exposure in body fluids or organs (biological monitoring)	• mg nicotine / cig • mg nicotine / day After absorption, a local tissue accumulation or a systemic distribution may occur
14	Dropped puffs	The puffs not counted toward the puff number, if their volume is below a specified amount	• Number (puffs) Number of puffs below a pre-defined volume Measured on a puffing topography instrument	• 4 puffs less than 5 mL In some e-cigarettes and HTPs designed to activate the heating device by draw pressure, and the dropped puff may not be enough volume or pressure for activation

No.	Term	Definition	Units and methods	Example and comment
	Product use behaviour			
15	Mean draw pressure / Mean draft pressure	The average mouth-end pressure (vacuum) per puff for each puff or across all puffs from an inhalable tobacco/nicotine product	• Pressure (mm H_2O) Calculated from measurements made with a puffing topography instrument	• 225 mm H_2O
16	Mean flow / Average flow	The average volumetric flow rate for each puff or across all puffs from an inhalable tobacco/nicotine product	• Flow (mL/s) Calculated from measurements made with a puffing topography instrument	• 32.4 mL/s
17	Mean puff duration	The average time from start to end of a puff, across all puffs from an inhalable tobacco/nicotine product	• Time (s) per puff Calculated from measurements made with a puffing topography instrument	• 2.4 s/puff
18	Mean puff interval	The average time between the end of one puff and the start of the next puff, across all puffs from an inhalable tobacco/nicotine product	Time (s) Calculated from measurements made with a puffing topography instrument	• 41 s
19	Mean puff volume	The average volume across all puffs from an inhalable tobacco/nicotine product	• Volume (mL) per puff Calculated from measurements made with a puffing topography instrument	• 42.5 mL/puff
20	Mean resistance	The average of the ratio of mean draw to mean flow across all puffs from an inhalable tobacco/nicotine product	• Pressure (mm H_2O) per flow (mL/s) Calculated from measurements made with a puffing topography instrument	• 6.9 mm H_2O / (mL/s) Often used to indicate the amount of effort that a smoker must make to draw a puff from a smoking article
21	Mouth hold	The time over which a puff is held in the oral cavity before inhalation or exhalation	• Time (s) Measured by observation, typically with a puffing topography instrument or by difference between the end of a puff and the start of inhalation measured as described above	• 1 s
22	Mouth spill (MSp)	The amount of smoke, vapour, or constituent that is spilt from the mouth after puffing and not inhaled by an inhalable tobacco/nicotine product user	• Mass (mg, µg, ng, pg) Mass per inhalable tobacco product No established method	• MSp is observed in many smoking behaviour studies and it affects exposure, intake and uptake but no standard method for quantification has been devised[26]

No.	Term	Definition	Units and methods	Example and comment
	Product use behaviour			
23	Peak draw pressure / Peak draft pressure	The maximum mouth end pressure (vacuum) for each puff or across all puffs from an inhalable tobacco/nicotine product	• Pressure (mm H_2O) Measured with a puffing topography instrument	• 360 mm H_2O
24	Peak flow	The maximum volumetric flow rate for each puff or across all puffs from an inhalable tobacco/nicotine product	• Flow (mL/s) Measured with a puffing topography instrument	• 44.3 mL/s The highest peak flow for any puff from a smoking article is usually described as the maximum flow per article
25	Peak resistance / Peak draft resistance	The highest ratio of peak draw to peak flow across all puffs from an inhalable tobacco/nicotine product	• Pressure (mm H_2O) per flow (mL/s) Calculated from measurements made with a puffing topography instrument	• 7.1 mm H_2O / (mL/s)
26	Puff duration	The time from start to end of a specified puff	• Time (s) per puff Measured with a puffing topography instrument	• 1.65 s/puff Puff duration is fixed in standard machine smoking regimes (e.g., 2 s for the ISO method)[64]
27	Puff frequency	The number of puffs taken in a fixed amount of time	• Number per time interval (puffs/min) Measured with a puffing topography instrument	• 2 puffs/min Puff frequency is fixed in standard machine smoking regimes (e.g., 1 puff/min for the ISO method)[64]
28	Puff interval / Inter-puff interval / Puff to puff interval / Time between puffs	The time between the end of a specified puff and the start of the next puff	• Time (s) Measured with a puffing topography instrument	• 43 s Puff interval is fixed in standard machine smoking regimes (e.g., 58 s for the ISO method)[64]
29	Puff number / Puff count	The count of the total number of puffs per inhalable tobacco/nicotine product	• Number per inhalable tobacco/nicotine product (puffs/cig, puffs/stick, and so on) Measured with a puffing topography instrument	• 7 puffs/cig As defined in ISO 10185 (33)
30	Puff pressure / Individual puff draft pressure / Individual puff draw pressure	The pressure (vacuum) recorded per individual puff from an inhalable tobacco/nicotine product	• Pressure (mm H_2O) Measured with a puffing topography instrument	• 244 mm H_2O

No.	Term	Definition	Units and methods	Example and comment
	Product use behaviour			
31	Puff volume	The amount of smoke or vapour and air drawn into the mouth during an individual puff	• Volume (mL) per puff Measured with a puffing topography instrument	• 42.1 mL/puff Puff volume is fixed in standard machine smoking regimes (e.g., 35 mL/puff for the ISO method)[64]
32	Puffing topography / Puffing pattern	The profile of puff characteristics including puff frequency, duration, volume, interval, and regularity of these parameters for an inhalable tobacco/nicotine product	A complex set of measurements including puff frequency (per minute) with a comment on puff interval Other units are described in related definitions Puffing topography is assessed by specialised instruments with pressure and flow measurement capabilities	Puff pattern is fixed, with regular puff intervals in standard machine smoking regimes (e.g., 1 puff/min with a 58 s puff interval for the ISO method)[64] [See Figure 1]
33	Duration of product use	The total elapsed time from the start of the first puff to the end of the last puff of an inhalable tobacco/nicotine product	• Time (s) Calculated from measurements made with a puffing topography instrument	• 390 s
34	Product use topography	The complete pattern using an inhalable tobacco/nicotine product, including puffing, mouth hold, inhalation and exhalation	A complex set of measurement parameters based on definitions included herein. The product use topography is assessed by two or more specialised instruments. The puffing topography is supplemented with separate measurements of inhalation and exhalation	[See Figure 1]
35	Smoulder time / Total puff interval	The puffing duration minus the sum of individual puff durations during a single usage of inhalable tobacco/nicotine product. This also equals the sum of puff intervals for that inhalable tobacco/nicotine product	• Time (s) per inhalable tobacco/nicotine product (s/cig, s/stick, and so on) Calculated from measurements made with a puffing topography instrument	• 373 s/cig

No.	Term	Definition	Units and methods	Example and comment
	Product use behaviour			
36	Sub-puffs / Partial puffs	The count of contiguous puffs within a pre-specified time interval, prior to the combination of data for analysis as a single puff	• Number (puffs) Recorded on a puffing topography instrument	• 3 sub-puffs A concatenated sub-puff is one of a group of puffs for which the start of the following puff occurs within a specified time interval from the end of the previous puff (e.g., 0.8 s) [See Figure 1]
37	Total puff duration	The cumulative time for all puffs from an inhalable tobacco/nicotine product	• Time (s) per inhalable tobacco/nicotine product (s/cig, s/stick, and so on) Calculated from measurements made with a puffing topography instrument	• 16.5 s/cig
38	Total puff volume	The combined volumes for all puffs from an inhalable tobacco/nicotine product	• Volume (mL) per inhalable tobacco/ nicotine product (s/cig, s/stick, and so on) Calculated from measurements made with a puffing topography instrument	• 425 mL/cig
	Biomarker			
39	Biomarker of exposure (BOE)	A smoke constituent or its metabolite that is measured as a concentration in body fluids, excreta, or tissues (e.g., blood, urine, saliva, exhaled air, hair, sweat). BOE may also be measured as protein or DNA adducts	• Mass per unit volume (body fluid) • Mass per mg creatinine (urine sample) • Mass per 24h (total urinary collection) • Mass per mass other marker (e.g., tissues, urine, exhaled breath) • Measured with a validated method	• mg cotinine / mL (plasma) • mg nicotine equivalents / 24h (urine) • mg nicotine equivalents / mg creatinine (urine) • ppm CO (exhaled air) • % COHb (blood) Certain BOE can be used as biomarkers of compliance in order to check whether study subjects use assigned product: Compliance can be verified using highly specific markers especially with respect to long-term studies but also during study initiation / screening.[69]
40	Biologically effective dose (BED)	The amount of a smoke constituent or metabolite that is bound to a macromolecule (e.g., protein, DNA, RNA) of a specific tissue or organ	• Mass per mass (tissues) • Mass per mass (protein- or DNA-adducts) • Measured with a validated method	

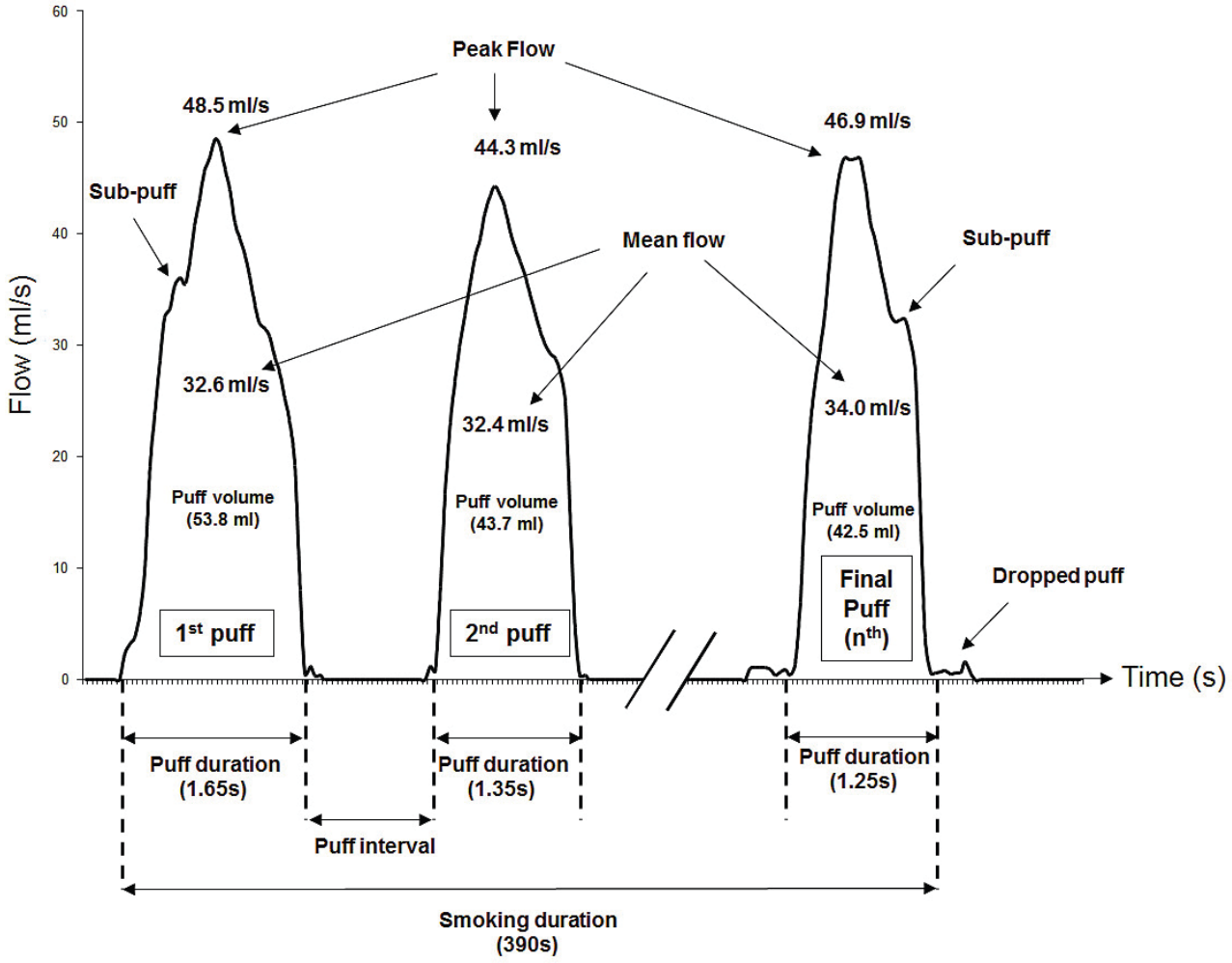

Figure 1. An example of a puffing topography trace. Reprinted from Gregg et al. 2013[20] above

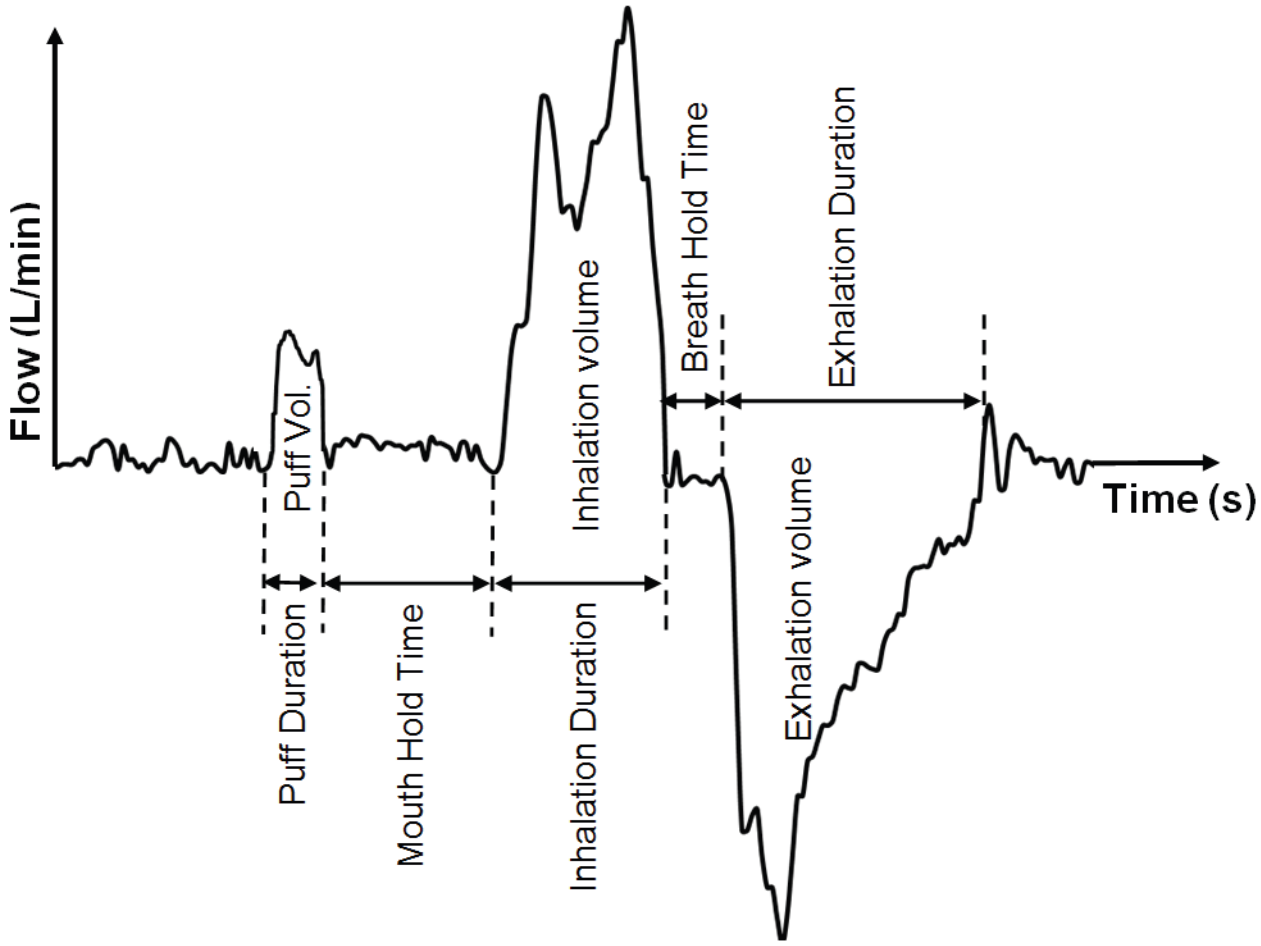

Figure 2. An example of a product use behaviour trace. Reprinted from Gregg et al. 2013[20]

This profile was generated with a BIBO (Breathe In, Breathe Out) instrument. A BIBO instrument works on similar principles to the puffing topography instruments but differs in that it has bi-directional pressure transducers which measure inhalation flow through the mouth and subsequent exhalation flow (with the nose clamped).

A BIBO instrument can be used to collect information on the volume of smoke taken into the mouth, the volume drawn into the airways and then exhaled. This enables researchers to measure the topography of the complete smoking cycle.

4. REFERENCES

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