DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Centers for Disease Control
and Prevention (CDC)
Atlanta GA 30333

May 18, 2017

Chris Fedeli
Judicial Watch, Inc.
425 Third Street SW., Suite 800
Washington, District of Columbia 20024
Via email: cfedeli@judicialwatch.org

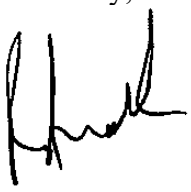
Dear Mr. Fedeli:

This letter is in response to your Centers for Disease Control and Prevention and Agency for Toxic Substances and Disease Registry (CDC/ATSDR) Freedom of Information Act (FOIA) request of March 21, 2017, regarding all internal CDC emails discussing the relative carcinogenicity of inhalation from Electronic Nicotine Delivery Systems compared to inhalation from traditional combustible cigarettes.

We located 74 pages of responsive records. After a careful review of these pages, no information was withheld from release.

If you need any further assistance or would like to discuss any aspect of the records provided please contact either our FOIA Requester Service Center at 770-488-6399 or our FOIA Public Liaison at 770-488-6277.

Sincerely,



Roger Andoh
CDC/ATSDR FOIA Officer
Office of the Chief Information Officer
(770) 488-6399
Fax: (404) 235-1852

17-00430-FOIA

Chris Fedeli

From: Viana, Bruno <foiarequests@cdc.gov>
Sent: Thursday, May 18, 2017 7:04 AM
To: Chris Fedeli
Subject: Your CDC FOIA Request #17-00430-FOIA
Attachments: Your CDC FOIA Request #17-00430-FOIA.pdf; 17-00430-Fedeli.pdf

May 18, 2017

Request Number: 17-00430-FOIA

Dear Mr. Fedeli:

This is regarding your Freedom of Information Act (FOIA) request of March 21, 2017, for all internal CDC emails discussing the relative carcinogenicity of inhalation from Electronic Nicotine Delivery Systems compared to inhalation from traditional combustible cigarettes.

Please see the attached letter.

Sincerely,
CDC/ATSDR FOIA Office

From: King, Brian a. (CDC/ONDIEH/NCCDPHP)
Sent: 3 Oct 2016 12:33:50 -0400
To: Marynak, Kristy (CDC/ONDIEH/NCCDPHP);Forrest, Maxine (CDC/ONDIEH/NCCDPHP) (CTR)
Cc: Tucker, Jennifer (CDC/ONDIEH/NCCDPHP)
Subject: FW: Study in question

FYI in case other inquiries come in.
Brian

From: King, Brian a. (CDC/ONDIEH/NCCDPHP)
Sent: Monday, October 03, 2016 12:32 PM
To: 'Cofer,Jennifer'
Subject: RE: Study in question

Hi Jennifer,
CDC doesn't officially comment on studies that are published by non-CDC authors. As such, we don't have a formal statement on this study.
However, I will say that it's important to consider that this is a single study of 20 e-cigarette users over a very short time period (2 weeks) – approximately half of whom continued smoking conventional cigarettes at follow-up. We know that e-cigarette products vary considerably in terms of constituents, as do patterns and frequency of use among individual users. As such, the extent of generalizability for these findings to the broader population, e-cigarette products other than those used by the participants, and longer trajectories of e-cigarette and conventional cigarette use, is quite limited.
It's also important to looks at the actual study, which could be misinterpreted based on the framing in the press release (e.g. "those who switch completely to e-cigarette may reduce their cancer risk"). The major conclusion of the actual study was: "this is the first study that demonstrates that substituting tobacco cigarettes with an e-cigarette may reduce user exposure to numerous toxicants and carcinogens otherwise present in tobacco cigarettes". In the end, this study conclusion is largely consistent with what the CDC and many others have been saying for a while now: "For adult smokers to benefit from e-cigarettes, they must completely quit combusted tobacco use. Smoking even a few cigarettes per day is dangerous to health." (http://www.cdc.gov/tobacco/stateandcommunity/pdfs/cdc-osh-information-on-e-cigarettes-november-2015.pdf) The important caveat here is that, currently, most users don't actually quit completely, and instead continue to use both products (i.e. dual use).
That being said, there's relatively broad consensus across the scientific community that transitioning exclusively to e-cigarettes would benefit adult conventional cigarette smokers. However, reduced risk is not the same as no risk. There are still harmful and potentially harmful constituents present in e-cigarettes, and the extent to which the use of these products impacts long-term health, particularly in the context of cancer outcomes, remains under-studied. For the most part, these current study findings reinforce that the harmful constituent profile of e-cigarettes is less than conventional cigarettes, which we've really been saying for some time now – however, the impact on actual disease risk is beyond the scope of what this study can tell us.
I hope this helps.
Best,
Brian

From: Cofer,Jennifer [mailto:JCofer@mdanderson.org]
Sent: Monday, October 03, 2016 10:28 AM
To: King, Brian a. (CDC/ONDIEH/NCCDPHP) <iyn3@cdc.gov>
Subject: Study in question

Hi Brian,

There's some controversial conversation happening over the weekend within our institution about this study.
Does your office have a statement or comment on it yet? Officially or unofficially?
https://www.roswellpark.org/media/news/study-smokers-who-switch-e-cigarettes-exposed-same-levels-nicotine-lower-carcinogen
Jennifer Cofer, MPH, CHES
Director, EndTobacco Program
Cancer Prevention and Control Platform
The University of Texas MD Anderson Cancer Center
Sent from my iPad

From: Wang, Lanqing (CDC/ONDIEH/NCEH)
Sent: 19 Sep 2016 10:14:31 -0400
To: Decastro, Rey (CDC/ONDIEH/NCEH);Blount, Benjamin (CDC/ONDIEH/NCEH);Chambers, David (CDC/ONDIEH/NCEH);De Jesus, Victor (CDC/ONDIEH/NCEH);Melstrom, Paul C. (CDC/ONDIEH/NCCDPHP);Richter, Patricia (CDC/ONDIEH/NCEH);Watson, Clifford (CDC/ONDIEH/NCEH)
Subject: RE: Benowitz's longitudinal e-cig study

Thanks Rey.

This also agrees what we found in two E-cig studies.

Lanqing

From: Decastro, Rey (CDC/ONDIEH/NCEH)
Sent: Monday, September 19, 2016 10:08 AM
To: Blount, Benjamin (CDC/ONDIEH/NCEH) <bkb3@cdc.gov>; Chambers, David (CDC/ONDIEH/NCEH) <mzz7@cdc.gov>; De Jesus, Victor (CDC/ONDIEH/NCEH) <foa5@cdc.gov>; Melstrom, Paul C. (CDC/ONDIEH/NCCDPHP) <gha8@cdc.gov>; Richter, Patricia (CDC/ONDIEH/NCEH) <pir1@cdc.gov>; Wang, Lanqing (CDC/ONDIEH/NCEH) <lfw3@cdc.gov>; Watson, Clifford (CDC/ONDIEH/NCEH) <cow1@cdc.gov>
Subject: Benowitz's longitudinal e-cig study

Nicotine Tob Res. 2016 Aug 17. pii: ntw160. [Epub ahead of print]

Exposure to Nicotine and Selected Toxicants in Cigarette Smokers Who Switched to Electronic Cigarettes: A Longitudinal Within-Subjects Observational Study.

Goniewicz ML(1), Gawron M(2), Smith DM(3), Peng M(4), Jacob P 3rd(4), Benowitz NL(4).

INTRODUCTION: Electronic cigarettes (e-cigarettes) are purported to deliver nicotine aerosol without any toxic combustion products present in tobacco smoke. In this longitudinal within-subjects observational study, we evaluated the effects of e-cigarettes on nicotine delivery and exposure to selected carcinogens and toxicants. METHODS: We measured seven nicotine metabolites and 17 tobacco smoke exposure biomarkers in the urine samples of 20 smokers collected before and after switching to pen-style M201 e-cigarettes for 2 weeks. Biomarkers were metabolites of 13 major carcinogens and toxicants in cigarette smoke: one tobacco-specific nitrosamine (NNK), eight volatile organic compounds (1,3-butadiene, crotonaldehyde, acrolein, benzene, acrylamide, acrylonitrile,

ethylene oxide, and propylene oxide), and four polycyclic aromatic hydrocarbons (naphthalene, fluorene, phenanthrene, and pyrene). Changes in urine biomarkers concentration were tested using repeated measures analysis of variance. RESULTS: In total, 45% of participants reported complete abstinence from cigarette smoking at 2 weeks, while 55% reported continued smoking. Levels of total nicotine and some polycyclic aromatic hydrocarbon metabolites did not change after switching from tobacco to e-cigarettes. All other biomarkers significantly decreased after 1 week of using e-cigarettes ($p < .05$). After 1 week, the greatest percentage reductions in biomarkers levels were observed for metabolites of 1,3-butadiene, benzene, and acrylonitrile. Total NNAL, a metabolite of NNK, declined by 57% and 64% after 1 and 2 weeks, respectively, while 3-hydroxyfluorene levels declined by 46% at week 1, and 34% at week 2. CONCLUSIONS: After switching from tobacco to e-cigarettes, nicotine exposure remains unchanged, while exposure to selected carcinogens and toxicants is substantially reduced. IMPLICATIONS: To our knowledge, this is the first study that demonstrates that substituting tobacco cigarettes with an e-cigarette may reduce user exposure to numerous toxicants and carcinogens otherwise present in tobacco cigarettes. Data on reduced exposure to harmful constituents that are present in tobacco cigarettes and e-cigarettes can aid in evaluating e-cigarettes as a potential harm reduction device.

DOI: 10.1093/ntr/ntw160 PMID: 27613896 [PubMed - as supplied by publisher]

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From: Sneegas, Karla S. (CDC/ONDIEH/NCCDPHP)
Sent: 17 Jun 2016 16:55:20 -0400
To: Melstrom, Paul C. (CDC/ONDIEH/NCCDPHP)
Subject: RE: Today's Tobacco News - 6-14

Thanks Paul. I always appreciate your help in digging in a little deeper for me. Cheers!

From: Melstrom, Paul C. (CDC/ONDIEH/NCCDPHP)
Sent: Wednesday, June 15, 2016 10:06 AM
To: Sneegas, Karla S. (CDC/ONDIEH/NCCDPHP) <hri0@cdc.gov>
Subject: RE: Today's Tobacco News - 6-14

Howd.

The solutions and devices differ so much that it's hard to make generalizations, but I could surely imagine that their measurements are accurate.

And the point about pathogens… there are a number of issues – such as the pathogenic bacteria, below – that maybe didn't get recognition with cigs, but were still dangerous. Plus, there are a number of new issues, too, that are probably what we in the public health field should be looking at. We're definitely looking at ecigs for the same types of bad stuff that we looked for in cigs, and that doesn't make a lot of sense. So, I'm glad to see this type of stud, because it highlights – for me, anyway – how hard it is to effectively analyze this incredibly diverse market of products.

Happy to expand someday. -paul

From: Sneegas, Karla S. (CDC/ONDIEH/NCCDPHP)
Sent: Tuesday, June 14, 2016 12:52 PM
To: Melstrom, Paul C. (CDC/ONDIEH/NCCDPHP) <gha8@cdc.gov>
Subject: FW: Today's Tobacco News - 6-14

Hi Paul, Can you put on your science hat? Is this first article on e-cigs on target? Accurate?

From: Lewis, Del (CDC/ONDIEH/NCCDPHP)
Sent: Tuesday, June 14, 2016 9:01 AM
Subject: Today's Tobacco News - 6-14

TODAY'S TOBACCO NEWS

Tobacco Products / Tobacco Industry (includes new products, product development, marketing, e-cigarettes)

E-Cigarettes have more cancer causing ingredients than tobacco
The Richmond Register, June 13, 2016

Global

Curb tobacco sale near schools: High Court
IndiaToday, June 14, 2016

Tobacco Products / Tobacco Industry (includes new products, product development, marketing, e-cigarettes)

E-Cigarettes have more cancer causing ingredients than tobacco
The Richmond Register, June 13, 2016

Many smokers have turned to e-cigarettes to help them quit, but recent research suggests they may be headed down the wrong path. An article published in the journal Nicotine and Tobacco Research found that e-cigarettes can produce formaldehyde, a toxic substance and potent carcinogen. They also found that e-cigs produce a nicotine-laced vapor that users inhale and which is also toxic.

Furthermore, a Japanese research team learned that e-cigarettes can contain much higher levels of carcinogens than regular cigarettes — as much as 10 times the amount of formaldehyde was found in the vapor produced by e-cigs. "When the…wire (which vaporizes the liquid) gets overheated, higher amounts of those harmful substances seemed to be produced," said researcher Naoki Kunugita, explaining to the press how formaldehyde is formed in e-cigs.

"One new brand of e-cigarette, whose name has not been made public, showed a more than 10-fold increase in formaldehyde levels in nine out of every 10 sets," said Justin McCurry of the Guardian. "The device produced 1,600 micrograms of formaldehyde per 15 puffs."

Another somewhat surprising finding was that e-cigs affected life-threatening drug-resistant pathogens. This was based on a study in which the e-cigs vapor was tested on live methicillin-resistant Staphylococcus aureus (MRSA) and human cells. The vapor increased the already drug-resistant bacteria's virulence. It also decreased the human cells' ability to overcome the

bacteria. MRSA often live in the human throat, which increases the negative effect of e-cig vapor. Regular cigarettes also increase the effect of MRSA.

The Food and Drug Administration has already banned the sale of e-cigarettes to minors and have announced that stricter policies on them are coming. The sale of e-cigarettes, particularly to young people, has exploded in recent years. According to the US Centers for Disease Control and Prevention (CDC), “more than a quarter of a million youth who had never smoked a cigarette used electronic cigarettes in 2013, according to a CDC study published in the journal Nicotine and Tobacco Research. This number reflects a three-fold increase, from about 79,000 in 2011, to more than 263,000 in 2013.”

Global

Curb tobacco sale near schools: High Court
IndiaToday, June 14, 2016

The Justice cited survey and research materials as evidence showing increase in tobacco consumption among adolescent children and directed the Government to ensure tobacco free area to be set within 100 yards of any school.

He advised the authorities to immediately constitute special teams to conduct surprise raids on shops near schools and if anyone is caught selling tobacco products to an underage kid, severe punishment should be imposed.

He even quoted King James' phrase, "Smoking is hateful to the nose, harmful to the brain and hurtful to the lungs" and said that he was shocked to know school going children are using tobacco products extensively.

Kirubakaran also noted that cigarettes and other tobacco products (Prohibition of Advertisement and Regulation of Trade and Commerce, Production, Supply and Distribution) Act, 2003 should be used to book people who smoke in public areas.

The issue got exposed when an NGO filed a petition on this matter. Though school going kids are not much into smoking cigarettes, they indulge in using other forms of tobacco products like tobacco leaves soaked in chlorine. The State has already brought in many laws banning sale of such things to minors but it is seldom implemented.

Justice Kirubakaran concluded the issue by saying that it was time to sensitize parents and children about the ill effects of tobacco.

From: Wouter Visser
Sent: 2 Apr 2016 02:13:18 +0200
To: Melstrom, Paul C. (CDC/ONDIEH/NCCDPHP)
Cc: Edwards, Sarah (CDC/ONDIEH/NCCDPHP);Agaku, Israel T. (CDC/ONDIEH/NCCDPHP);Richter, Patricia (CDC/ONDIEH/NCEH);Reinskje Talhout
Subject: Feedback on report health effects of e-cigarette to bystanders
Attachments: 160401E e-sigaretten omstanders.docx

Dear Paul,

Attached you will find a report on an assesment of the health effects that e-cigarette use presents to bystanders (ie. 'second-hand vaping'), which was commissioned by the Dutch ministry of health. Patricia Richter suggested that you would be willing to reviewit, which we appreciate very much. Any comments and/or critisism that youcan provide will be extremely helpful to us.
You will find that the first 5 chapters are in Dutch. They contain only a summary of the most important findings, andunless you happen to understand Dutch, ignore them. The other chapters are in English and contain much more detailed information.
Preferably, I would allow you a few weeks, but unfortunately I need to ask you if you can return your comments to us by april the 8th. Sorry for the deadline; even if you can only partially review the report in that time, it would be most valuable to us.

Please note that the report is still confidential at this time.

Thank you very much in advance for your time and efforts!

Kind regards,
Wouter

Wouter Visser, PhD
National Institute for Public Health and the Environment (RIVM),
Postbus 1
3720 BA Bilthoven
email: wouter.visser@rivm.nl
tel: +31-30-2743330

De gezondheidsrisico's van e-sigaretten voor omstanders

RIVM Briefrapport 2016-0036

Wouter Visser | Liesbeth Geraets
Peter Bos | Reinskje Talhout

Colofon

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Dit onderzoek werd verricht in opdracht van Ministerie van Volkgezondheid, Welzijn en Sport, in het kader van het kader

Dit is een uitgave van:
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Publiekssamenvatting

De gezondheidsrisico's van e-sigaretten voor omstanders

Hier komt de publiekssamenvatting

Synopsis

De gezondheidsrisico's van e-sigaretten voor omstanders

Hier komt een vertaling van de Nederlandse samenvatting

Keywords: Noteer hier de trefwoorden waarop het rapport door een zoekmachine gevonden moet worden (minimaal 3, maximaal 10 kernwoorden).

Inhoudsopgave

Samenvatting

De populariteit van e-sigaretten neemt snel toe, en daarmee ook aandacht voor de mogelijke gezondheidsrisico's voor omstanders die zelf geen e-sigaret gebruiken. In dit rapport is een schatting gemaakt van de toxicologische risico's voor omstanders. Er is niet gekeken naar andere mogelijke gezondheidseffecten, zoals bijvoorbeeld schade ontstaan door inslikken van e-liquid door kinderen, het ontploffen van een batterij of mogelijke effecten op populatie niveau door het renormalizeren van roken

In tegenstelling tot gewone tabakssigaretten produceren e-sigaretten geen aerosol als ze niet gebruikt worden. Dit is een belangrijk verschil, omdat bij gewone tabakssigaretten tot 85% van de schadelijke stoffen in de omgeving onstaat in de tijd dat er van een sigaret geen trekje wordt genomen, de zogenaamde zijstroomrook. De rest van de schadelijke stoffen wordt uitgeblazen door de roker. Bij e-sigaretten worden omstanders uitsluitend blootgesteld aan aerosol die eerst door de gebruiker is geïnhaleerd en vervolgens uitgeblazen.

Voor het onderzoek is experimenteel bepaald wat de samenstelling is van de damp die ervaren e-sigaret gebruikers uitademen. Daarbij zijn de hoeveelheden van alle stoffen gemeten waarvan is vastgesteld dat ze tot gezondheidseffecten voor de gebruikers zelf kunnen leiden. Het gaat om nicotine, propyleen glycol, glycerol, aldehydes, ketonen, tabak-specifieke nitrosamines en metalen.
Ook zijn metingen verricht aan het dampgedrag van ervaren e-sigaret gebruikers. Daarbij bleken er grote verschillen tussen individuen te zijn. De gevonden waarden voor duur, interval en volume van trekjes zijn in overeenstemming met gepubliceerde gegevens van andere onderzoekers.

Uit metingen van de samenstelling van de aerosol die ervaren e-sigaret gebruikers uitblazen blijkt een aanzienlijk deel van de schadelijke stoffen in de aerosol achter blijft in de gebruikers. In de eerste uitademing na het nemen van een trekje werd maximaal 5% van de ingeademde hoeveelheid nicotine uitgeblazen, en meestal veel minder. Na uitademing zal de aerosol zich verspreiden in de ruimte, en de risico's voor omstanders zijn daarbij dus sterk afhankelijk van de afmetingen en mate van ventilatie.van de ruimte.

In een ongunstig scenario (een kleine ruimte zonder ventilatie met meerdere dampers) kunnen gezondheidseffecten optreden: Bij een nicotine-houdende e-sigaret bestaat als gevolg van de blootstelling aan nicotine risico op een verhoogde hartfrequentie en verhoogde systolische bloedruk. Dit is uiteraard niet het geval bij gebruik van nicotine-vrije e-liquids.
Als gevolg van blootstelling aan propyleen glycol en glycerol (componenten van de dragervloeistof) kan een milde irritatie van de luchtwegen optreden.
Specifiek bij E-vloeistoffen die relatief hoge concentraties tabak-specifiek nitrosamines (TSNAs) bevatten kan niet worden uitgesloten dat deze een risico geven op een verhoogde incidentie van tumoren in de luchtwegen. Bij dit laatste moeten echter wel een paar belangrijke kantekeningen worden gemaakt. Allereerst is het zo dat de meeste e-vloeistoffen slechts lage concentraties TSNAs bevatten. Ze kunnen soms aanwezig zijn in e-liquids als verontreiniging in de door fabrikanten gebruikte ingredienten, zoals bijvoorbeeld in tabaksextracten die als smaakstof kunnen worden toegevoegd, of in nicotine. Verder berust de schatting op de aanname dat de carcinogeniteit van TSNAs gelijk is aan die van een andere nitrosamine, namelijk dimethylnitrosamine (NMDA). Het is echter mogelijk dat er verschillen zijn wat dit betreft tussen de vier verschillende TSNAs, en alleen van NNK is vastgesteld dat de mutageniteit vergelijkbaar is met die van NMDA.

In een minder extreem scenario (gebaseerd op een kantoorruimte met 1 damper) kunnen bovengenoemde effecten van nicotine en TSNAs voor omstanders niet worden uitgesloten, maar er worden in dat geval geen effecten van propyleen glycol en glycerol verwacht.

De risico's zijn echter sterk afhankelijk van het gedrag van de e-sigaret gebruiker en de omgeving waarin gedampt wordt. Metingen aan vrijwilligers die hun eigen e-sigaret en vloeistof gebruiken laten zien dat er grote individuele verschillen zijn in dampgedrag. Voor sommige stoffen, zoals veel smaakstoffen, is niet goed bekend of deze schadelijk zijn bij inhalatie, en het is daarom aan te raden om de nieuwe ontwikkelingen op dit gebied nauwgezet te blijven volgen.

1 Inleiding

Uit recent onderzoek van het RIVM (1) en anderen (2) is gebleken dat de damp van e-sigaretten schadelijke stoffen bevat in zodanig hoge concentraties dat ze tot gezondheidsrisico's voor e-sigaretgebruikers leiden. Het was tot op heden nog onduidelijk in hoeverre deze stoffen een gezondheidsrisico vormen voor omstanders na uitblazen door de gebruiker en verdunning in de omgevingslucht.

Dit rapport beschrijft de resultaten van een onderzoek van het RIVM naar de gezondheidsrisico's voor omstanders die zelf geen e-sigaret gebruiken. Hierbij is uitsluitend gekeken naar de toxicologische risico's van blootstelling aan stoffen in de uitgeblazen damp en is geen rekening gehouden met mogelijke andere effecten, zoals bijvoorbeeld renormalisatie van roken als sociale norm (3, 4), verwonding door het exploderen van een e-sigaret (5-7) of vergiftiging door het inslikken van nicotine-houdende e-liquid door kinderen (8-10).

De hoofdstukken 1 t/m 5 bevatten een Nederlandse samenvatting van de belangrijkste resultaten en conclusies. Vanaf hoofdstuk 6 wordt een meer gedetailleerde Engelstalige beschrijving van het onderzoek gegeven.

1.1 Aanleiding

Bij de marketing van e-sigaretten wordt vaak de nadruk gelegd op de vermeende gezondheidsvoordelen ervan. In 2013 was een reclame voor e-sigaretten waarin een vrouw e-sigaret damp uitblies in een kinderwagen aanleiding tot felle kritiek van onder andere KWF kankerbestrijding en leidde tot kamervragen (11).

Veel e-sigaretgebruikers gebruiken e-sigaretten om te kunnen dampen op momenten of plaatsen waar een gewone sigaret niet is toegestaan (1). Tegelijkertijd zijn ook veel mensen e-sigaretten gaan gebruiken omdat ze hun "omgeving niet tot last willen zijn of gezondheidsschade toebrengen", waaruit blijkt dat een aanzienlijk deel van de e-sigaretgebruikers verwacht dat e-sigaret damp minder schadelijk is voor hun omgeving en/of minder (geur)overlast geeft.

De Nederlandse wetgeving staat het gebruik van e-sigaretten in openbare gelegenheden momenteel toe. Sommige andere overheden hebben wel wetgeving geïmplementeerd die het gebruik van e-sigaretten in de openbare ruimte beperkt, zoals bijvoorbeeld het geval is in Frankrijk, sommige staten en steden van de USA, en Wales. Daarbij zijn eventuele toxicologische gezondheidseffecten niet altijd de belangrijkste overweging geweest. Zo is in Frankrijk is besloten (12) tot een verbod op gebruik in de openbare ruimte om het aanzetten tot roken van de jeugd te vermijden (het zogenaamde '*gateway effect*'), onduidelijkheid over de gezondheidsrisico's voor omstanders en onduidelijkheid over de effectiviteit van e-sigaretten als hulpmiddel bij pogingen om te stoppen met roken.

1.2 Definities: eerstehands aerosol en tweedehands aerosol

Voor de zichtbare 'rook' die door een e-sigaret wordt geproduceerd wordt de term 'damp' veel gebruikt, hoewel het strikt gezien niet juist is. Echte damp (zoals bijvoorbeeld stoom) is een homogeen mengsel van een gas met lucht. Omdat dit niet uit gesuspendeerde druppeltjes vloeistof bestaat verstrooit damp geen licht en is daarom niet zichtbaar. Een

formeel meer correcte term is 'aerosol', en deze raakt ook meer algemeen in gebruik in de internationale e-sigaret onderzoeksliteratuur. Ook in dit rapport wordt daarom de term aerosol gebruikt.
Voor de doeleinden van dit onderzoek is het verder van belang om onderscheid te maken tussen de aerosol die door gebruikers van e-sigaretten wordt geïnhaleerd (*first-hand aerosol, FHA*) en de damp die door de gebruikers vervolgens wordt uitgeblazen en die zich verspreidt in de omgeving (*second-hand aerosol, SHA*). Uit verschillende studies is gebleken dat er grote verschillen zijn in de samenstelling van SHA en FHA. Dat is niet verrassend, omdat een deel van de stoffen in FHA worden geabsorbeerd in de luchtwegen van de e-sigaret gebruiker. Hiermee moet rekening worden gehouden bij onderzoek naar de blootstelling van omstanders

Comment [RT]: Die beperking hebbben wij ook, want je rapporteert hierin toch niet over de box-experimenten?

1.3 Definities: main-stream smoke en side-stream smoke

Gewone tabakssigaretten produceren ook rook als geen trekje wordt genomen omdat ze in de tussentijd doorsmeulen. De rook die daarbij ontstaat wordt *side-stream smoke (SSS)* genoemd. De rook die rokers inhaleren bij het nemen van een trekje wordt *main-stream smoke (MSS)* genoemd. De rook waaraan omstanders worden blootgesteld kan voor wel 85% uit SSS bestaan (13).
E-sigaretten produceren geen SSS. Alleen als de gebruiker een trekje neemt wordt het verwarmingselement geactiveerd, en omstanders worden dus uitsluitend blootgesteld aan SHA.
Als hiermee geen rekening wordt gehouden zou dit tot een overschatting leiden van de blootstelling aan schadelijke stoffen, zoals nicotine (2).

1.4 Eerder onderzoek naar de schadelijkheid voor omstanders

Hoewel er wel eerder onderzoek is verricht naar de mate waarin omstanders worden blootgesteld aan schadelijke stoffen uit e-sigaretten, is er tot op heden in de wetenschappelijke literatuur geen toxicologische beoordeling beschikbaar van de gezondheidsrisico's die daarvan kunnen worden verwacht.
Bovendien is in een aanzienlijk deel van de wetenschappelijke artikelen gebruik gemaakt van (verdunde) FHA terwijl de samenstelling van SHA wezenlijk anders is om de eerde genoemde redenen (zie 1.3 en 1.2). In deze onderzoeken zal sprake zijn van een onrealistische overschatting van de blootstelling.
Er zijn enkele onderzoeken verschenen waarin wel gebruik gemaakt wordt van SHA. Fernandez *et al* (14) heeft recent een goede systematische review van deze literatuur gepubliceerd. De experimentele opzet van de meeste van deze onderzoeken is vergelijkbaar: één of meerdere proefpersonen nemen plaats in een testkamer en gebruiken gedurende enige tijd een e-sigaret. Vervolgens wordt dan door chemische analyse de concentratie van verschillende stoffen in de lucht van de testruimte gemeten. Omdat de studies echter op belangrijke punten verschillen zoals het aantal proefpersonen, hun dampgedrag, de duur van het experiment en het volume van de testkamer en de mate van ventilatie is het niet goed mogelijk om de resultaten onderling te vergelijken.
Ook is het niet goed mogelijk om deze resultaten te vertalen naar andere situaties. De gemeten luchtconcentraties zijn een momentopname van een dynamisch proces dat beïnvloed wordt door een aantal factoren waarvan het effect niet altijd goed voorspelbaar is, zoals absorptie aan meubels, verdunning, ventilatie, uitblazen van damp door proefpersonen, etc.

1.5 Opzet van het huidige onderzoek

E-sigaretten worden vrijwel overal gebruikt (1). De Nederlandse wetgeving staat gebruik in de openbare ruimte toe, hoewel bedrijven in hun huisregels wel beperkingen kunnen

opleggen. Om een risicoschatting te kunnen uitvoeren van verschillende scenario's (bijvoorbeeld een auto of een kantoorruimte) is besloten tot de volgend opzet. Allereerst is experimenteel vastgesteld:

- Welke hoeveelheden aerosol gebruikers uitblazen in de ruimte bij normaal e-sigaretgebruik.

- Welke hoeveelheden schadelijke stoffen e-gebruikers uitademen in de aerosol.

Aan de hand van deze meetgegevens kan vervolgens door modelering van verschillende scenario's worden uitgerekend wat de blootstelling wordt voor omstanders, waarbij rekening kan worden gehouden met verdunning in de ruimte, ventilatie, absorptie aan oppervlakten, etc. Daarbij is gekozen voor een gefaseerde aanpak: in eerste instantie wordt een scenario doorgerekend waarbij de blootstelling relatief hoog zal zijn, namelijk een niet-geventileerde auto met daarin twee e-sigaretgebruikers en een derde persoon die geen e-sigaret gebruikt. Als in dat scenario een risico blijkt te bestaan, dan worden vervolgens ook minder extreme scenario's beoordeeld, zoals een kantoor. Mocht echter blijken dat er in het eerste scenario al geen sprake is van risico's ten gevolge van blootstelling aan de gemeten stoffen, dan is het niet nodig om ook voor andere scenario's de berekening uit te voeren omdat daarbij altijd sprake zal zijn van een lagere blootstelling.

2 Experimentele bepaling van de hoeveelheid aerosol die gebruikers uitblazen bij normaal e-sigaret gebruik

Naar de topografie van het roken van gewone tabakssigaretten, d.w.z. parameters zoals de duur, interval en volume van trekjes is al veel onderzoek gedaan. Over de topografie van e-sigaretgebruik is veel minder bekend. Wel is uit verschillende onderzoeken gebleken dat dampgedrag verschilt van rookgedrag (15-17). De duur van een trekje is bijvoorbeeld over het algemeen langer bij dampers. Een mogelijk verklaring is dat e-sigaretgebruikers hun gedrag aanpassen omdat de hoeveelheid nicotine in de aerosol anders is (18). Gebruikers kunnen door hun dampgedrag te veranderen toch dezelfde hoeveelheid nicotine opnemen als bij het roken van tabakssigaretten.
Voor het huidige onderzoek hebben we daarom metingen verricht aan het dampgedrag van 18 ervaren e-sigaretgebruikers. Deze vrijwilligers hebben gedurende een kwartier hun eigen e-sigaret en e-vloeistof gebruikt, waarbij het dampgedrag (de zogenaamde 'topografie') is vastgelegd met behulp van een kleine, draagbare debietmeter.

2.1 Methoden

2.1.1 Werving proefpersonen

Het onderzoek is beoordeeld en goedgekeurd door de Medisch Ethische Commissie van de Wageningen Universiteit (METC reg.nr. NL53471.081.15). Uit een landelijke database van TNS-NIPO (http://www.tns-nipo.com) zijn 44439 respondenten gescreend op e-sigaretgebruikers. Inclusiecriteria voor deelname aan de studie waren als volgt: (1) tussen de 18-55 jaar oud. (2) dagelijks gebruik van een e-sigaret met minimaal 6 mg/ml nicotine. Vrouwen die borstvoeding geven, zwanger waren, of van plan dat te worden ten tijde van het onderzoek waren uitgesloten van deelname, evenals mensen die nadelige gezondheidseffecten hadden ervaren van hun e-sigaret gebruik.

2.1.2 Meting

Deelnemers werd gevraagd hun eigen e-sigaret en navulvloeistof mee te nemen. Bij aankomst werd merk en model van de e-sigaret en het merk, de smaak en nicotine concentratie van de meegebrachte e-vloeistof geregistreerd. De e-sigaret werd aan een kleine debietmeter gekoppeld (CReSS pocket, Borgwaldt, Hamburg, Duitsland) en vervolgens werd de deelnemers gevraagd om gedurende een kwartier de e-sigaret te gebruiken. Gedurende deze periode was de deelnemer in gesprek, waarbij het onderwerp 'damptopografie' of onderwerpen die daar direct mee gerelateerd zijn werden vermeden

2.2 Resultaten

2.2.1 Werving

Onder 44439 respondenten werden 623 (1.4%) dagelijkse e-sigaret gebruikers geïdentificeerd door middel van Computer Assisted Web Interviewing (CAWI). 485 (78%) hiervan gebruikte een vloeistof met nicotine. 273 (44%) rookte daarnaast ook tabakssigaretten (minimaal 1 sigaret per week), zogenaamd *dual use*. 63% van de dagelijkse, nicotine-gebruikende e-sigaret gebruikers was ouder dan 40 jaar. Mogelijk houdt dit verband met de leeftijd waarop mensen een poging ondernemen om te stoppen met roken.

Onder de 18 deelnemers aan het onderzoek waren 10 mannen (56%) en 8 vrouwen (44%). 40% procent van de mannen en 62% van de vrouwen waren *dual users*.

2.2.2 *Topografie*

Er bleken aanzienlijke individuele verschillen te zijn in de topografie van de verschillende deelnemers. Dit blijkt ook uit andere onderzoeken. Robinson *et al* hebben hier recentlijk een goed overzicht van gepubliceerd en vergeleken met hun eigen metingen (17). Tabel 2.1 geeft een overzicht van de gevonden waarden voor de duur, het interval en het volume van trekjes.

Tabel 2.1: overzicht parameters damptopografie

	min	**max**	**mediaan**
Puff duur (sec)	0.7	17.39	3.8
Puff interval (sec)	5.05	376	42
Puff volume (ml)	5.1	174	55

Ondanks de grote individuele verschillende zijn de mediaan van de duur (4.0 sec) en het volume (56 ml) in goede overeenstemming met de eerder door literatuuronderzoek (1) vastgestelde waarden (4 sec, 55 ml respectievelijk).

3 Experimentele bepaling van de concentraties schadelijke stoffen in SHA

In tegenstelling tot tabakssigaretten produceren e-sigaretten alleen aerosol op het moment dat de gebruiker een trekje neemt. Omstanders worden dus alleen blootgesteld aan de aerosol die de gebruiker daarna uitblaast. In dit hoofdstuk wordt het experimentele onderzoek naar de samenstelling van de uitgeblazen aerosol beschreven.

3.1 Methoden

Aan 18 proefpersonen werd gevraagd om een van tevoren bepaald aantal trekjes te nemen van een e-sigaret en de damp via een mondstuk uit te blazen op een filter waaraan de relevante stoffen binden. Deze stoffen werden daarvan vervolgens ge-extraheerd en door chemische analyse werd de hoeveelheid van de verschillende stoffen bepaald. Omdat sommige stoffen ook van nature kunnen voorkomen in gewone adem of in de lucht in het laboratorium werd aan deelnemers ook gevraagd om eerst gewone adem uit te blazen op een filter. Deze controlefilters werden gelijktijdig geanalyseerd en gebruikt ter correctie van de achtergrond. Om de concentratie in de uitgeblazen lucht te kunnen uitrekenen is ook het totale volume adem/aerosol dat de deelnemers op de verschillende filters uitbliezen gemeten met een debietmeter.
In eerder onderzoek waren een aantal stoffen geïdentificeerd die voorkomen in de damp van e-sigaretten die tot gezondheidsrisico's voor de gebruikers zelf kunnen leiden (1). Het gaat om nicotine, propyleen glycol, glycerol, formaldehyde, acetaldehyde, acroleïne, tabak-specifieke nitrosamines (TSNAs) en metalen. Voor het huidige onderzoek zijn daarom de concentraties van deze stoffen gemeten. Van stoffen waarvan eerder was vastgesteld dat de concentraties in e-sigaret aerosol zo laag zijn dat ze niet van belang zijn voor een schatting van de gezondheidsrisico's, zoals vluchtige organische stoffen (VOCs), is geen analyse uitgevoerd.
De hoeveelheid van verschillende stoffen in de aerosol hangt uiteraard ook af van de gebruikte e-sigaret en vloeistof. Om een schatting te verkrijgen van de hoeveelheden die soms voor kunnen komen in de uitgeademde damp is ervoor gekozen om combinaties van e-sigaretten en vloeistoffen te gebruiken waarbij de concentraties schadelijke stoffen relatief hoog was (*worst-case*). Daarbij is gebruik gemaakt van de metingen die we eerder hadden verricht (1) aan commercieel verkrijgbare e-liquids en e-sigaretten. Op basis daarvan is een populair type 1^{e}-generatie e-sigaret gebruikt, en een hervulbare (2^{e} generatie) e-sigaret in combinatie met twee verschillende vloeistoffen. Het betreft vloeistof 33 uit het eerdere onderzoek, die relatief hoge emissies gaf van aldehydes, en vloeistof 157, waarin de concentraties TSNAs relatief hoog waren. De 1^{e} generatie e-sigaret bevat relatief hoge concentraties metalen. Ook werd gekozen voor een relatief hoge nicotine concentratie (18 en 11 mg/ml). Door metingen werd geverifieerd dat de concentraties metalen en nitrosamines in de nieuw aangeschafte vloeistoffen overeenkwamen met die van de producten van vorig jaar.

3.2 Resultaten en discussie

De gemeten concentraties van de verschillende stoffen zijn samengevat in tabel 3.1. De volledige resultaten zijn te vinden in de appendix (http://www.rivm.nl/bibliotheek/rapporten/2016-0036bijlage.pdf)

		concentratie				hoeveelheid per trekje			
		bereik				bereik			
	n	min	max	mediaan		min	max	mediaan	
nicotine	18	<LOQ	12391	323	ng / L	<LOQ	2140	108	ng
dragervloeistof									
propyleen glycol	18	<LOQ	839	64	µg / L	<LOQ	25	<LOQ	µg
glycerol	18	<LOQ	<LOQ	<LOQ	µg / L	<LOQ	<LOQ	<LOQ	µg
nitrosamines									
NNN	9	<LOQ	961	84	pg / L	<LOQ	111	29	pg
NAT	9	<LOQ	172	47	pg / L	<LOQ	40	14	pg
NAB	9	<LOQ	16	9	pg / L	<LOQ	8	2	pg
NNK	9	<LOQ	403	39	pg / L	<LOQ	71	15	pg
aldehydes									
formaldehyde	4	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng
acetaldehyde	4	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng
acroleine	4	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng
metalen									
arseen	3	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng
molybdeen	3	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng
tin	3	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng
cadmium	3	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng
lood	3	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng
zink	3	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng
koper	3	<LOQ	28	<LOQ	ng / L	<LOQ	2.92	<LOQ	ng
nikkel	3	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng
cobalt	3	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng
mangaan	3	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng
chroom	3	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng
vanadium	3	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng
uranium	3	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng

Tabel 3.1: Overzicht van de gemeten stoffen in SHA. De 'hoeveelheid per trekje' is de gemiddelde hoeveelheid in de eerste ademhaling die wordt uitgeblazen na het nemen van een trekje. De concentratie is de concentratie in die eerste ademhaling na het nemen van een trekje. Voor het berekenen van de mediaan is alle data gebruikt, inclusief monsters waarvan de concentratie onder de kwantificeringslimiet was.

De concentraties van deze stoffen zijn zonder uitzondering lager dan in FHA (1). Hiervoor zijn verschillende oorzaken aan te wijzen. Allereerst inhaleren veel e-sigaret gebruikers bij het nemen van een trekje ook nog wat lucht zodat de e-sigaret aerosol verdund wordt. Verder zal een deel van de geïnhaleerde stoffen achterblijven in de luchtwegen van de e-sigaret gebruiker. Tenslotte is de concentratie van deze stoffen bepaald in de eerste uitademing nadat de proefpersoon een trekje van de e-sigaret had genomen. Een deel van de aerosol blijft daarbij achter in de longen en wordt later alsnog uitgeademd. Dit wordt niet gemeten omdat alleen de eerste uitademing op een filter is opgevangen.

3.2.1 Vergelijking met gepubliceerde gegevens

Ook in andere studies van de samenstelling van SHA zijn lage concentraties nicotine en de componenten van de dragervloeistof in de uitgeblazen damp gevonden.
Een mogelijke verklaring voor de waargenomen individuele verschillen tussen proefpersonen in de uitgeademde hoeveelheden stoffen wordt gegeven door een onderzoek van O'Connel

et al (19), waaruit blijkt dat >99% van de nicotine wordt geabsorbeerd als gebruikers diep inhaleren, maar slechts 72-92% als ze de aerosol alleen een paar seconden in de mond houden. Om de nicotine-opname te optimaliseren zullen de meeste e-sigaretgebruikers de damp niet alleen in de mond nemen maar ook dieper inhaleren.

4 Schatting van de gezondheidsrisico's voor omstanders

In dit hoofdstuk zijn de belangrijkste resultaten en conclusies van de toxicologische risicobeoordeling voor de omstander van e-sigaret gebruik samengevat. Een gedetailleerde beschrijving van de gebruikte methoden en van de resultaten van de evaluatie is opgenomen in hoofdstuk 10 (en de bijbehorende appendices A en B).

4.1 inleiding

De bron van blootstelling voor de omstander van e-sigaret gebruik is de uitademing van stoffen door de e-sigaret gebruiker. Daarom is de risicobeoordeling voor deze omstander gebaseerd op chemische analyse van de uitgeademde lucht (eerste uitademing na het nemen van een trekje) van vrijwilligers die e-sigaretten dampen (zie hoofdstuk 2 en 3). Op basis hiervan worden concentraties in de omgevingslucht berekend die na verloop van tijd kunnen ontstaan en waaraan omstanders kunnen worden blootgesteld. De keuze voor de stoffen die gemeten zijn in de uitgeademde lucht en waarvoor een risicobeoordeling is uitgevoerd is beschreven in 3.1. Gebaseerd op deze analyses zijn voor deze stoffen concentraties van de uitgeademde stoffen in omgevingslucht berekend voor twee vooraf gedefinieerde scenario's van e-sigaret gebruik. Het ene scenario komt overeen met een dagelijkse autorit waarbij een kind wordt blootgesteld aan stoffen uitgeademd door twee e-sigaretgebruikers, terwijl het tweede scenario overeenkomt met blootstelling van een volwassen persoon aan stoffen uitgeademd door een e-sigaretgebruiker gedurende een halve werkdag (zie voor meer details 4.2.1). Voor het beoordelen van mogelijke gezondheidsrisico's worden de berekende luchtconcentraties in de ruimtes (respectievelijk auto en kantoor) vergeleken met gezondheidskundige normen voor de algemene bevolking. De *Air Quality Guidelines*, gepubliceerd door de WHO, worden primair gebruikt voor de risicobeoordeling, voor zover beschikbaar (20). Deze normen gelden voor een continue blootstelling van 24 uur/dag. Indien de berekende luchtconcentratie lager dan deze norm is, kan worden aangenomen dat in het betreffende scenario geen risico is op nadelige gezondheidseffecten. Wanneer geschikte, gezondheidskundige normen ontbreken, wordt voor de risicobeoordeling gebruik gemaakt van een '*Margin of Exposure*' (MOE)-benadering (zie appendix A). Bij deze MOE-benadering wordt de blootstelling van de omstander vergeleken met informatie over gezondheidseffecten die waargenomen zijn bij een blootstellingspatroon dat zoveel mogelijk aansluit bij het te beoordelen blootstellingsscenario (de PoD: '*point of departure*', relevante parameter voor een effectbeschrijving). Afhankelijk van de gezondheidseffecten waarom het gaat kan de MOE berekend worden als de ratio van de blootstellingsconcentratie waarop de PoD is gebaseerd en de luchtconcentratie voor de omstander, of, als de ratio van de opgenomen hoeveelheid bij de PoD en de door de omstander in het lichaam opgenomen hoeveelheid. De MOE dient voldoende groot te zijn om te kunnen concluderen dat er geen gezondheidsrisico aanwezig is. Of een berekende MOE voldoende is, is afhankelijk van een aantal factoren. Ten eerste moet er rekening mee worden gehouden dat er verschil in gevoeligheid kan bestaan tussen proefdier en mens (als de PoD gebaseerd is op proefdieronderzoek) en tussen mensen onderling. Ten tweede, moeten verschillen in het blootstellingspatroon voor de omstander enerzijds en bij het PoD anderzijds meegewogen worden. Zo kan het aantal uren blootstelling per dag van de omstander aanmerkelijk korter zijn dan dat van de dagelijkse blootstelling waarop de PoD is gebaseerd. Ten slotte moet bij de beoordeling van de MOE ook rekening worden gehouden of bij de concentratie waarop de PoD is gebaseerd wel of geen gezondheidseffecten zijn waargenomen, en zo ja in welke mate. Indien er effecten zijn waargenomen zal de MOE groter moeten zijn om te kunnen concluderen dat er geen gezondheidsrisico's aanwezig zijn. Specifiek voor carcinogene stoffen zonder drempelwaarde wordt bij een MOE van 10.000 of groter geconcludeerd dat de stof '*of low concern'* is (21), dat wil zeggen dat het tumorrisico zeer laag is. Zie appendix A voor verdere overwegingen bij de MOE-benadering.
Voor het verzamelen en selecteren van deze relevante informatie is zoveel mogelijk gebruik gemaakt van rapporten en evaluaties van (inter)nationaal erkende organisaties (onder andere US EPA, AEGL committee, ATSDR, WHO, Gezondheidsraad).

Blootstelling via inademing is gerelateerd aan de hoeveelheid van een stof per m^3 geïnhaleerde lucht gedurende een specifieke tijdsperiode. Veel stoffen die in de damp van e-sigaretten of uitgeademde lucht van e-sigaret gebruikers worden aangetroffen, zoals polyolen, zijn bij inademing irriterend voor de luchtwegen en kunnen beschadigingen van de luchtwegen veroorzaken. Naast effecten op de luchtwegen kan een stof ook gezondheidsnadelige effecten veroorzaken na opname (absorptie) in het lichaam, de zogenoemde systemische effecten. Bij voorkeur worden de risico's op systemische effecten beoordeeld op basis van informatie verkregen door studies met inhalatoire blootstelling. Indien geen goede inhalatie studies beschikbaar zijn, kan onder bepaalde voorwaarden gebruik worden gemaakt van studies met een andere route van blootstelling, bijvoorbeeld inname via de orale route. In dat geval wordt bij de beoordeling van de MOE zo goed mogelijk rekening gehouden met de verschillen tussen de blootstellingsroutes, bijvoorbeeld in hoeveelheid en snelheid van opname van de stof in het lichaam.

4.2 Risicobeoordeling

4.2.1 Blootstellingsscenario's

Voor de risicobeoordeling zijn twee vooraf gedefinieerde blootstellingsscenario's gebruikt. De risicobeoordeling is voor beide scenario's uitgevoerd voor de niet-dampende persoon, de omstander. Voor scenario 1 betreft dit een kind, terwijl voor scenario 2 een risicobeoordeling voor een volwassen persoon is uitgevoerd. Voor scenario 1 wordt aangenomen dat er een dagelijkse blootstelling (7 dagen/week) voor de omstander plaatsvindt, terwijl voor scenario 2 blootstelling gedurende 5 dagen/week plaatsvindt.

Scenario 1 - auto: Dit scenario beschrijft dat twee personen in een auto dampen en dat een derde persoon (de omstander; voor dit scenario een kind) in dezelfde auto aanwezig is en blootgesteld wordt aan de stoffen in de uitgeademde lucht van de twee dampers. Vanwege de kleine ruimte en blootstelling van een kind, betreft dit scenario een realistische worst-case. De totale damptijd (en de blootstellingstijd van de omstander) is op één uur gesteld, overeenkomend met een dagelijkse rit met de auto. Het dampgedrag voor beide dampers is gesteld op één trekje per twee minuten, wat overeenkomt met een gemiddelde damper volgens onze voorgaande studie (22).
Scenario 2 - kantoor: Dit scenario beschrijft dat één persoon in een kantoorruimte dampt terwijl een tweede persoon in dezelfde kantoorruimte aanwezig is en blootgesteld wordt aan de stoffen in de uitgeademde lucht van de damper. De Nederlandse wetgeving staat dampgebruik in de openbare ruimte toe, waardoor met dit scenario een realistisch worst-case scenario voor een werknemer geschetst kan worden. De totale damptijd (en de blootstellingstijd van de omstander) is op vier uur gesteld. Het dampgedrag van de damper is gesteld op twee trekjes per één minuut, wat overeenkomt met een zware damper volgens onze voorgaande studie (22).

Voor een gedetailleerde beschrijving van de blootstellingsschatting en toegepaste blootstellingsberekeningen wordt verwezen naar 10.2.2 en 10.2.3. Voor de blootstellingsberekening is zoveel mogelijk gebruik gemaakt van realistisch worst-case aannames. Gemeten is de hoeveelheid van een stof die werd uitgeademd tijdens de eerste uitademing na het nemen van een trekje. De hoogst gemeten waarde is gebruikt in de berekeningen. Op basis hiervan is de totale hoeveelheid uitgeademde stof berekend in scenario 1 (1 uur) en scenario 2 (4 uur), rekening houdend met het feit dat de stof niet volledig wordt uitgeademd tijdens de eerste uitademing. Vervolgens is de eindconcentratie in de lucht berekend en deze is als basis voor de risicobeoordeling gebruikt. Bij de beoordeling van de MOE is rekening gehouden met het feit dat in werkelijkheid de luchtconcentratie gestadig toeneemt en dat gebruik van een eindconcentratie een overschatting van de gezondheidsrisico's tot gevolg heeft.

4.2.2 Resultaten risicobeoordeling

Dragervloeistof

Propyleenglycol was aanwezig boven de LOQ in de uitgeademde lucht van 4 van de 17 vrijwilligers. Op basis van de gemeten hoeveelheid in de uitgeademde lucht kan voor scenario 1 (auto) niet uitgesloten worden dat lokale effecten op de luchtwegen (irritatie luchtwegen) van de omstander optreden als gevolg van blootstelling aan propyleenglycol. Bij de beoordeling van de MOE is rekening gehouden met diverse factoren zoals het verschil in blootstellingsduur, verschil in gevoeligheid tussen proefdier en mens en tussen mensen onderling en waargenomen effecten bij de PoD. Daarnaast moet er rekening mee worden gehouden dat gebruik is gemaakt van worst-case aannames. Op basis hiervan wordt verwacht dat, indien effecten optreden, deze mild van aard zullen zijn. Voor scenario 2 (kantoor) worden voor de omstander geen lokale effecten op de luchtwegen verwacht als gevolg van blootstelling aan propyleenglycol.
Met betrekking tot mogelijke systemische effecten kan geconcludeerd worden dat deze niet te verwachten zijn voor een omstander als gevolg van blootstelling aan propyleenglycol voor scenario 1 en 2.

Glycerol kon niet gedetecteerd worden in de uitgeademde lucht, *i.e.* glycerol was aanwezig in de uitgeademde lucht in een hoeveelheid lager dan de LOQ. Op basis van de beschikbare toxicologische informatie en de LOQ voor glycerol kan worden geconcludeerd dat bij hoeveelheden lager dan de LOQ geen risico op schadelijke gezondheidseffecten te verwachten is.

Nicotine
Nicotine was aanwezig boven de LOQ in de uitgeademde lucht van 16 van de 17 vrijwilligers.
De beschikbare toxicologische (inhalatie)gegevens voor nicotine zijn erg beperkt. Een norm voor de algemene bevolking is niet beschikbaar. Tevens is een geschikte PoD om levenslange inhalatoire blootstelling te evalueren niet beschikbaar. Daarom kan de MOE-benadering niet worden toegepast en is een 'weight-of-evidence' beoordeling toegepast. Dit houdt in dat op basis van 'expert judgement' van alle beschikbare gegevens zo goed en evenwichtig mogelijk wordt beoordeeld of gezondheidsrisico's te verwachten zijn.
Op basis van de gemeten hoeveelheid in de uitgeademde lucht kan voor scenario 1 (auto) geconcludeerd worden dat systemische effecten (toename in hartslagfrequentie en systolische bloeddruk) mogelijk zijn als gevolg van blootstelling aan nicotine voor een omstander van e-sigaret gebruik. Voor scenario 2 (kantoor) kan niet uitgesloten worden dat deze systemische effecten kunnen optreden.

Aldehydes
De aldehydes formaldehyde, acrolein, aceetaldehyde konden niet gedetecteerd worden in de uitgeademde lucht, *i.e.* deze aldehydes waren aanwezig in de uitgeademde lucht in een hoeveelheid lager dan de LOQ. Op basis van de beschikbare toxicologische informatie en de LOQ voor deze stoffen kan worden geconcludeerd dat bij hoeveelheden lager dan de LOQ geen risico op schadelijke gezondheidseffecten te verwachten is.

Tabakspecifieke nitrosamines
Vier tabakspecifieke nitrosamines waren geanalyseerd; N'-nitrosonornicotine, NNN; 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, NNK; N'-nitrosoanabasine, NAB; N'-nitrosoanatabine, NAT). Deze waren alle vier boven de LOQ aanwezig in de uitgeademde lucht bij 8 van de negen vrijwilligers.
Op basis van de gemeten hoeveelheid in de uitgeademde lucht kan voor scenario 1 (auto) niet uitgesloten worden dat verhoogde incidenties van tumoren in de luchtwegen van een omstander kunnen optreden als gevolg van blootstelling aan deze nitrosamines. Voor scenario 2 (kantoor) is het niet mogelijk om een duidelijke conclusie te trekken. De MOE-waarden waren zodanig dat verfijning van de MOE-berekening zou kunnen leiden tot de conclusie 'of low concern', dat wil zeggen dat het tumorrisico zeer laag is, maar dit kan niet met voldoende zekerheid worden vastgesteld.

Metalen

Koper was aanwezig in de uitgeademde lucht boven de LOQ bij 1 van de drie vrijwilligers. Op basis van de gemeten hoeveelheid in de uitgeademde lucht kan voor zowel scenario 1 (auto) als scenario 2 (kantoor) geconcludeerd worden, dat schadelijke effecten op de gezondheid als gevolg van blootstelling van een omstander van e-sigaret gebruik aan koper niet verwacht worden.

Naast koper waren ook andere metalen geanalyseerd: vanadium, chroom, mangaan, kobalt, nikkel, zink, arseen, molybdeen, cadmium, tin, lood en uranium. Deze konden niet gedetecteerd worden in de uitgeademde lucht, *i.e.* deze stoffen waren aanwezig in de uitgeademde lucht in een hoeveelheid lager dan de LOQ. Specifieke vormen van chroom, nikkel en arseen zijn kankerverwekkend, maar omdat niet bekend is in welke vormen deze metalen in de uitademingslucht voorkomen, kunnen geen definitieve conclusies worden getrokken over mogelijke risico's op kanker. Voor nikkel en arseen kan worden gesteld dat, aangenomen dat de kankerverwekkende vormen aanwezig zijn, het risico op kanker bij hoeveelheden lager dan de LOQ waarschijnlijk verwaarloosbaar klein is. Voor chroom kan geen uitspraak worden gedaan.
Voor tin zijn geen geschikte toxicologische gegevens beschikbaar om een uitspraak te doen. Voor de overige metalen (vanadium, mangaan, kobalt, zink, molybdeen, cadmium, lood en uranium) kan op basis van de beschikbare toxicologische informatie en de LOQ voor deze stoffen worden geconcludeerd dat bij hoeveelheden lager dan de LOQ geen risico op schadelijke gezondheidseffecten te verwachten is.

4.3 Discussie en conclusies

De huidige risicobeoordeling voor de omstander van e-sigaret gebruik is gebaseerd op chemische analyse van de uitgeademde lucht van vrijwilligers die geselecteerde e-liquids dampen. Lucht van de eerste uitademing volgend op een trekje was verzameld en geanalyseerd. Afhankelijk van de geanalyseerde stof werden per vrijwilliger in totaal 5 of 25 'eerste' uitademingen verzameld en geanalyseerd. Dit resulteerde voor elke vrijwilliger in een gemiddelde concentratie van een specifieke stof in de uitgeademde lucht van één eerste uitademing. Als gevolg van het poolen van meerdere uitademingsmonsters per vrijwilliger ontbrak inzicht in de intra-individuele variatie. Analyses werden uitgevoerd in de uitgeademde lucht van meerdere vrijwilligers, variërend van monsters van 3 vrijwilligers voor de metaalanalyses, 4 vrijwilligers voor de aldehydes, 9 vrijwilligers voor de nitrosamine-analyses en 17 vrijwilligers voor de nicotine en dragervloeistof analyses. De hoogste hoeveelheid van een specifieke stof als gemeten in de uitgeademde lucht werd gebruikt voor de risicobeoordeling. De metingen lieten een inter-individuele variatie zien in hoeveelheid stof aanwezig in de uitgeademde lucht. Het kan dan ook als worst-case gezien worden om de hoogste concentratie te gebruiken voor de risicobeoordeling. Dit geldt met name voor propyleenglycol dat niet kon worden gedetecteerd in uitademingslucht van 13 van de 17 vrijwilligers.
De risicobeoordeling is uitgevoerd voor twee vooraf gedefinieerde scenario's. De blootstelling van de omstander van e-sigaretgebruik kan zo gerelateerd worden aan het ontstaan van schadelijke effecten op de gezondheid. Scenario 1 komt overeen met een dagelijkse autorit waarbij een kind wordt blootgesteld aan stoffen uitgeademd door twee e-sigaretgebruikers. Voor dit scenario kan niet uitgesloten worden dat lokale effecten op de luchtwegen als gevolg van blootstelling aan propyleenglycol kunnen treden voor de omstander van e-sigaret gebruik. Echter, verwacht wordt dat indien effecten optreden, deze mild van aard zullen zijn. Bovendien wordt opgemerkt dat propyleenglycol niet kon worden aangetoond in de uitademingslucht bij 13 van de 17 vrijwilligers. Blootstelling van een kind aan nicotine in dit scenario zou kunnen resulteren in nadelige gezondheidseffecten zoals een verhoogd hartritme of een verhoogde systolische bloeddruk. Dampen van e-sigaretten kan ook resulteren in verhoogde luchtconcentraties van de tabakspecifieke nitrosamines. Op basis hiervan kan een verhoging van de incidenties van tumoren in de luchtwegen als gevolg van blootstelling aan deze nitrosamines niet uitgesloten worden voor het kind in scenario 1. Scenario 2 komt overeen met blootstelling van een volwassen persoon aan stoffen uitgeademd door een e-sigaretgebruiker gedurende een halve werkdag. Dampen van e-sigaretten kan resulteren in verhoogde luchtconcentraties van nicotine in dit scenario. Op basis hiervan kan niet uitgesloten worden dat schadelijke gezondheidseffecten zoals een verhoogd hartritme of een verhoogde systolische

bloeddruk kunnen optreden bij de omstander. Met betrekking tot de tabakspecifieke nitrosamines is het niet mogelijk om duidelijke conclusies te trekken voor dit scenario. De MOE-waarden waren zodanig dat verfijning van de MOE-berekening zou kunnen leiden tot de conclusie 'of low concern', dat wil zeggen dat het tumorrisico zeer laag is, maar dit kan niet met voldoende zekerheid worden vastgesteld.

Deze evaluatie geeft aan wat de mogelijke risico's voor de omstander van e-sigaret gebruik kunnen zijn. De risicobeoordeling is, zoals aangegeven, uitgevoerd voor twee vooraf gedefinieerde scenario's. De hoogte van de luchtconcentraties, en de daaraan gerelateerde gezondheidsrisico's, is sterk afhankelijk van het aantal personen dat dampt, de frequentie waarmee gedampt wordt (aantal trekjes/min), de totale dampduur, het volume van de ruimte waarin gedampt wordt en de mate van ventilatie. De absolute hoeveelheid van een stof die gemeten is in de eerste uitademing na het nemen van een trekje is gebruikt om luchtconcentraties te berekenen als gevolg van uitademing van geïnhaleerde damp van een e-sigaret. Vanwege de variabele samenstelling van e-liquids is de hoeveelheid van een specifieke stof in de eerste uitademing afhankelijk van de geselecteerde e-liquids. Opgemerkt dient te worden dat alle, in de huidige studie, geselecteerde e-liquids nicotine bevatten. Nicotine zal vanzelfsprekend geen probleem vormen bij het dampen van nicotine-vrije e-liquids. Ook de aanwezigheid van tabaksspecifieke nitrosamines in de damp is sterk afhankelijk van het type e-liquid. Tabakspecifieke nitrosamines zijn gekoppeld aan de aanwezigheid van nicotine en/of tabaksextract in de e-liquid. Tabakspecifieke nitrosamines zullen geen probleem vormen bij het dampen van nicotine-vrije e-liquids zonder tabaksmaak.

Tot slot, het dampgedrag van de vrijwilligers (*i.e.* volume van één trekje, tijdsduur tussen twee opeenvolgende trekjes, het oppervlakkig of diep ('over de longen') inademen en het volume van de uitgeademde lucht), welke inter- en intra-individuele variatie laat zien, zal van invloed zijn geweest op de gemeten hoeveelheid in de uitademingslucht en die is gebruikt in de risicobeoordeling.

5 Discussie

Hier komt een vertaling van de Engelstalige discussie (hoofdstuk 11)

6 Conclusies

Hier komt een vertaling van de Engelstalige conclusies (hoofdstuk 12)

7 Introduction

Previous research, by us (1) and others (2), has shown that the aerosol generated by e-cigarettes contains several harmful components at concentrations that constitute a risk to the health of users. However, it is not yet clear to what extent these components may be harmful to bystanders after exhalation by the user into the surrounding air and resulting dilution.

The following chapters describe an assessment of the health risks associated with exposure to second-hand e-cigarette aerosol performed by the Dutch National Institute for Public Health and the Environment (RIVM). The preceding chapters contain a summary of this information in Dutch.

7.1 Definitions: first-hand aerosol (FHA) and second-hand aerosol (SHA)

It is important to recognize that the composition of the aerosol that is produced by an e-cigarette and subsequently inhaled by the user (*first-hand aerosol, FHA*) is different from that of the aerosol that users exhale and that is dispersed in the surrounding space (*second-hand aerosol, SHA*). This is not surprising, because components of the aerosol are partially deposited or absorbed in the respiratory tract of the e-cigarette user. Several studies have used machine-generated FHA as a substitute for SHA to estimate the levels of harmful emissions that bystanders are exposed to. However, this will lead to an overestimate of the exposure of bystanders and the health risks associated with it. The magnitude of the differences can be considerable, as shown by several recent studies in which the composition of FHA and SHA was compared . The concentration of some components was lower in SHA by several orders of magnitude (14, 19, 23).

7.2 Main-stream and side-stream smoke

Tobacco cigarettes continue to burn and produce smoke even when no puff is being taken. The smoke emitted during this phase is called side-stream smoke (SSS). The smoke that is inhaled by smokers is known as main-stream smoke (MSS). Side stream smoke may contribute as much as 85% of the tobacco cigarette emissions that bystanders are exposed to (13).
In contrast, E-cigarettes do not produce the equivalent of side-stream smoke. Users activate the heating element by pressing a button only when taking a puff or the device is activated automatically by an integrated airflow sensor. Accordingly, bystanders are only exposed to SHA. If the lack of side-stream smoke in the case of e-cigarettes is not accounted for, this may lead to an overestimation of the exposure of bystanders. For instance, the assumption that bystanders exposure to nicotine is comparable for e-cigarettes and tobacco cigarettes (2) seems unfounded. However, this will result in an unrealistic overestimation of nicotine exposure to bystanders.

7.3 Bystander exposure to SHA

Others have studied the exposure of bystanders to harmful components resulting from e-cigarette use. To the best of our knowledge, there are no studies currently available in which a toxicological analysis of the health risks associated with that exposure is performed.
A large number of papers have been published in the past in which an attempt was made to estimate bystander exposure using machine-generated FHA. As discussed above (section 7.1 and 7.2), this is likely to lead to an unrealistic overestimation.
However, more recently, several papers have appeared in which human-generated SHA was used. Fernandez *et* al have recently published a systematic review on this topic (14). In

most studies, test subjects vaped for some time in a test chamber. The air in the chamber was sampled and analysed. An overview of such studies is shown in table 7.1.

Table 7.1: overview of publications on bystander exposure to harmful components in SHA based on vaping volunteers in a test chamber. *) One or more of the authors declared receiving financial support or being employed by the tobacco or e-cigarette industry.

reference	test chamber volume	number of volun-teers	vaping pattern	measurements
Bertholon (24)	60 m^3	3	20 puffs	particles
Schripp (25)	8 m^3	1	6 puffs in 6 minutes	VOCs particles aldehydes PG nicotine
Czogala* (26)	39 m^3	5	*ad lib.* twice for 5 minutes with 30 min. interval	nicotine particles CO (<LOD) VOCs (<LOD)
Ruprecht (27)	50 m^3	1	Two 7 minute sessions with 1 puff/min with 3 min. intervals	particles
Saffari (28)	48 m^3	1	7 minute sessions with 1 puff/min with 3 min. intervals	black carbon (<LOD) CO (<LOD) metals organics (<LOD) PAHs (<LOD) nicotine
Schober (29)	45 m^3	3	2 hours	particles VOCs PAHs carbonyls metals humectants
O'Connel* (30)	12.8 m^3	3	3.2 puffs/min	total VOC nicotine (<LOD) PG, aldehydes, isoprene, acetone, pentansiloxane PAHs (<LOD) metals (<LOD) TSNAs (<LOD)

In all studies, an effort was made to approximate natural conditions. As a result, however, the setup used differs considerably between studies, for instance in terms of the number of subjects, duration of the experiment, topography, level of ventilation and timing of the measurements. This makes it difficult to compare the results from different studies or apply them to different scenarios.

Ballbe *et al* (31) took a different approach, attempting to obtain a more real-world assessment of nicotine exposure of bystanders. They measured nicotine levels in air at homes of e-cigarette users, and levels of nicotine and cotinine in plasma, saliva and urine of members of the residence that did not themselves smoke or use e-cigarettes. Surprisingly, even though nicotine levels in the air of the e-cigarette users were 10-fold lower compared to the air levels in the house of smokers, the nicotine and cotinine levels in plasma, urine and saliva from non-smoking residents were comparable. The authors suggest that this discrepancy may be caused by the fact that the plasma samples reflect exposure over several days prior to the measurement, while air sampling in the house was only done at a specific time and place. Furthermore, they cannot completely exclude exposure of the subjects to cigarette smoke outside the context of the study.

Several authors performed chemical analysis of SHA directly, using similar methods as were used in the current study.
Long *et al (23)*, found no detectable levels of carbonyls and phenolic compounds in SHA over the background levels observed in control breath or room air. They were able to detect glycerin and nicotine, which amounted to 0.1% and 0.06% respectively of the mass of SHA collected, the rest being water.
Interestingly, O'Connel et al (16) found that while >99% of nicotine is retained by test subjects when they inhale the aerosol, only 77-92% is retained when test subjects only hold the aerosol in their mouth for several seconds, suggesting that differences between users in this respect may result from differences in the depth of inhalation. However, available data on topology suggest that most users do not exhibit this behavior in normal use, but typically enhance their absorption of nicotine by taking relatively long, slow puffs. In agreement with these findings, a technique known among users as 'stealth-vaping' consists of holding the inhaled vapour in the lungs for a few seconds to reduce the amount of visible vapour.

Comment [RT]: Dat is na aftrek blanco?

7.4 Exposure scenarios and setup of current study

E-cigarettes are used in a variety of situations (1). As mentioned (section 7.3), several studies have been published in which the levels of harmful components of aerosol in specific settings have been measured, for instance resembling a typical office. Fernandez *et al* has recently published a systematic review on this topic (14). However, it is difficult to translate the data obtained by this method to other scenarios. In the current study, we addressed this issue by calculating the exposure of bystanders in different scenarios by modeling the dispersion of components in the aerosol in a space after exhalation, taking into account dilution due to room volume, ventilation, etc.
The data required for this calculation includes the concentrations of harmful components in SHA and data regarding the vaping behavior of users. This was obtained experimentally.
To measure vaping behavior, we recruited experienced e-cigarette users and recorded their behavior while they were using their own e-cigarette and preferred e-liquid *ad libitum*.
The composition of SHA was measured close to its source, i.e. in the aerosol exhaled by the users. The chemical analysis of SHA focused on components that we previously found to contribute to the health risks of users (1): nicotine, propylene glycol (PG), glycerol, aldehydes, tobacco-specific nitrosamines (TSNAs) and metals. Since we did not observe significant levels of volatile organic compounds (VOCs) in e-liquids or FHA, this class of components was not included in the analysis.
The experimental data is then used to model the exposure of bystanders to the selected harmful components in different scenarios. A tiered approach was taken, in which an evaluation was first made of a scenario expected to present a very high level of exposure. Less extreme scenario's then only need to be evaluated if significant risks are found.

8 Vaping Topography

8.1 Introduction

The topography of tobacco cigarette smoking has been well studied, but much less is known regarding the topography of e-cigarette use. Several studies indicate that the topography of vaping is highly variable between users, but notably different from that of smoking (15, 17, 32). In order to explain the observed differences in topography between vaping and smoking, it has been suggested that e-cigarette users adjust their behavior to regulate the uptake of nicotine from the aerosol (33). This is supported by the observation that the topography of experienced e-cigarette users is different from that of users that have only recently started using e-cigarettes (15)and with the fact that plasma and urine levels of nicotine and cotinine of experienced vapers are comparable to that of smokers regardless of the nicotine levels in the aerosol of their e-cigarette (33).
We collected topography data of 18 experienced vapers (>3 months of daily use) with a CReSS pocket flowmeter (Borgwaldt, Hamburg, Germany). Subjects used their own e-cigarette and e-liquid of choice for 15 minutes *ad libitum*.

8.2 Methods

8.2.1 Recruitment of test subjects

The study was reviewed and approved by the Medical Ethical Committee of the Wageningen Universiteit (METC regnr. NL53471.081.15). E-cigarette users were identified in a screen by Computer Assisted Web Interviewing (CAWI) of 44439 respondents from a national database maintained by TNS-NIPO (http://www.tns-nipo.com). Inclusion criteria for test subjects were as follows: (1) between 18-55 years of age, (2) daily e-cigarette use for at least 3 months using an e-liquid containing at least 6 mg/ml of nicotine. Subjects that had experienced adverse health effects from vaping and women that were pregnant, lactating or had plans to become pregnant at the time of the experiment were excluded from participating. E-cigarette users that smoked tobacco cigarettes in addition to daily e-cigarette use were allowed to participate.

8.2.2 Topography measurements

Users were free to vape and/or smoke on the day of the experiment prior to arrival at the test location to avoid abnormal nicotine craving. Upon arrival, the brand and type of e-cigarette and the brand, flavour and nicotine content of the e-liquid brought by the subject were recorded. The use of the CReSS pocket flowmeter was demonstrated and subjects were asked to vape *ad libitum* for approximately 15 minutes. Subjects were engaged in conversation during the experiment, during which topics relating to vaping topography were avoided.

8.2.3 Data processing

In a few cases, the data recorded by the CReSS pocket exhibited brief gaps, revealed by the occurrence of two puffs with a <1 sec puff interval. These interruptions were corrected by combining volume and duration data from puffs that occurred with a <1 second inter-puff interval.

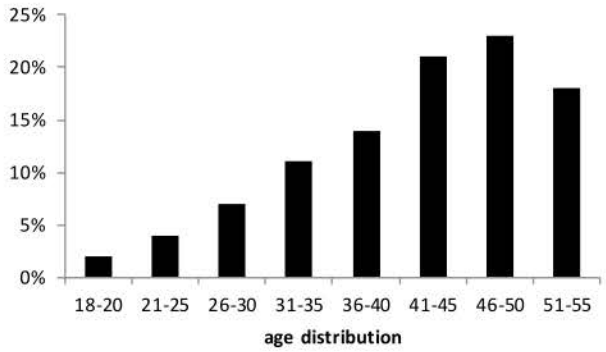
8.3 Results and discussion

8.3.1 *Screening and recruitment*

44439 respondents were screened to identify potential test subjects. 1940 (4.4%) of those indicated that they used e-cigarettes occasionally, and 623 (1.4%) indicated that they used e-cigarettes daily. 485 of daily users (78%) used an e-liquid containing nicotine, and 273 (44%) smoked tobacco cigarettes at least once a week in addition to daily e-cigarette use. The demographics of the nicotine-using daily e-cigarette users are summarized in Table 8.1. The subjects that actually participated in the study consisted of 10 males (56%) and 8 females (44%). Fourty percent of the males and sixty-two percent of the females were dual users (at least 1 tobacco cigarette a week in addition to e-cigarette use)

Table 8.1: demographic parameters of daily e-cigarette users using e-liquid containing 6 mg/ml nicotine or more

gender	n	%
male	232	48%
female	253	52%
age		
18-20	9	2%
21-25	17	4%
26-30	36	7%
31-35	55	11%
36-40	69	14%
41-45	101	21%
46-50	113	23%
51-55	85	18%
dual use		
yes	273	56%
no	208	43%
don't know	4	1%



The observed age distribution may be related to smoking cessation attempts (34).

8.3.2 *E-cigarettes*

Table 8.2 describes the e-cigarettes and e-liquids brought by participants for the experiment

Table 8.2: E-cigarettes and e-liquids used by participants

	e-cigarette		e-liquid	
subject	brand	model	flavour	nicotine (mg/ml)
1	Ego	Ego-C	tobacco	12
2	kangertech	EVOD2	Danish cookies	12
3	kangertech	EVOD	menthol	6
4			tobacco + caramel + vanille	11
5	Aspire	CF G-F	tobacco	18
6	Eleaf	iStick	lemon meringue pie	10
7	Eleaf	mini iStick	tobacco	6
8	seego	G-hit	strawberry	18
9	seego	G-hit	cherry	18
10	Vision	Spinner	caramel dolche leche	14
11	ego-T	Evolt	tobacco passionfruit	18
12	GS	GS eGo II	vanilla	6
13	Just Fog	C14	tobacco menthol	12
14	vapestick	vapestick	tobacco	12
15	joyetech	ego 1	cherry	16
16	Eleaf	iStick	banana	6
17	Eleaf	iStick	tobacco	12
18	Kangertech	EVOD 2	chocolate	12

All subjects used refillable tank-type e-cigarettes. 6 subjects (33%) used homemade e-liquids. Five (27%) subjects preferred a plain tobacco-flavoured liquid, while 10 subjects (56%) preferred a sweet flavour

8.3.3 Topography

18 test subjects participated in the experiment. An overview of the observed topography for the individual subjects is shown in table 8.3. There is large variation between subjects and between the individual puffs taken by each subject. Median values for all subjects combined were as follows: puff duration 3.8 seconds, puff volume 56 ml, and puff interval 43 seconds. In light of the large variation observed in these parameters, the median values for the puff duration and volume calculated from all subjects are remarkably similar to the values we previously inferred from available literature (4 sec puff duration, 55 ml volume) (1). The median puff interval was 42 sec, which is longer than we previously used, but is within the range of published values (17)

Table 8.3: Summary of vaping topography

	puff	puff interval (sec)			puff interval (sec)			Puff volume (ml)		
subject	**count**	min	max	median	min	max	median	min	max	median
1	19	5.05	86.37	40.07	2.35	17.39	2.84	47.0	174.1	106.3
2	10	18.37	164.66	68.14	3.33	6.92	5.02	15.9	96.4	66.1
3	5	10.31	375.50	75.57	1.91	4.09	3.13	44.3	103.4	88.3
4	24	6.10	118.78	45.80	3.30	5.55	4.65	48.2	116.5	95.7
5	28	4.61	202.45	15.37	0.80	6.93	4.11	6.3	77.0	41.9
6	14	7.35	153.46	42.73	3.62	10.04	5.82	23.2	152.5	121.8
7	17	8.21	200.24	39.70	2.22	8.45	3.78	20.6	85.2	36.6
8	13	18.71	112.89	49.35	4.67	11.76	7.20	44.5	123.4	64.5
9	15	7.70	150.64	45.99	1.20	7.78	2.94	17.2	100.8	30.3
10	9	23.51	168.61	90.02	4.42	7.32	5.02	30.9	86.7	56.2
11	18	6.57	145.84	34.73	0.72	15.34	6.40	5.1	113.8	37.6
12	30	5.17	97.96	18.02	0.75	9.52	3.48	5.5	74.7	25.9
13	22	6.66	134.51	36.07	2.04	5.02	2.80	11.9	125.0	68.6
14	18	7.54	121.02	43.08	0.81	8.36	2.73	7.4	68.9	20.1
15	24	7.60	82.33	19.00	1.85	11.99	2.55	32.2	162.0	47.0

8.4 Conclusions

Prevalence for daily e-cigarette use in the Dutch population amounts to approximately 1.4%. Of daily users, approximately 78% use a liquid containing 6 mg/ml of nicotine or more. Without exception, the experienced users that participated in the experiment preferred refillable, tank-style e-cigarettes.
Vaping topography data was collected for 15 subjects using their own e-cigarette and e-liquid. The observed topology was highly variable between subjects but comparable to published values (15, 17, 32, 33).

9 Chemical composition of second-hand e-cigarette aerosol

9.1 Introduction

In contrast to normal tobacco cigarettes, e-cigarettes do not produce side-stream smoke. As a result, the aerosol that bystanders are exposed to consists exclusively of second-hand aerosol, i.e. aerosol that has first been inhaled by a user and then exhaled. This chapter describes an analysis of the concentrations of harmful components in exhaled e-cigarette aerosol.

9.2 Methods

9.2.1 Generation and sampling of exhaled aerosol

The same 18 test subjects recruited for the experiment in chapter 6 also participated in this experiment. Each participant was provided with an e-cigarette containing a mild tobacco-flavoured liquid (section 7.2.2 and 7.2.3 describe details of the e-cigarettes and liquids used). The e-cigarette was connected to a CReSS pocket flowlogger (as described in chapter 6). A newly purchased clearomizer or cartomizer was used for every subject. The clearomizers were filled with approximately 1 ml of e-liquid at least 1 hour prior to the start of the experiment, or, in the case of the 1st generation cig-a-like devices, fitted with a new, unused cartomizer. The battery was fully charged for each subject. The clearomizers and cartridges were weighed immediately before and after the experiment to evaluate e-liquid consumption.
Subjects were explained the use of the aerosol collection devices, which consisted of a mouthpiece inserted into a Cambridge Filter Pad holder containing a clean preconditioned filter and (for the analysis of aldehydes) a cartridge of absorptive material, as described below. The aerosol collection devices were connected to a calibrated TSI4000 flowmeter (TSI Inc, Shoreview, MN, USA) connected to a computer running data logging software to establish the total volume exhaled onto each filter. The setup for SHA sample collection is shown in figure 9.1.

Figure 9.1: sample collection setup. A) schematic overview of the setup B) photograph of the aerosol sample collection device C) disassembled sample collection device showing the components.

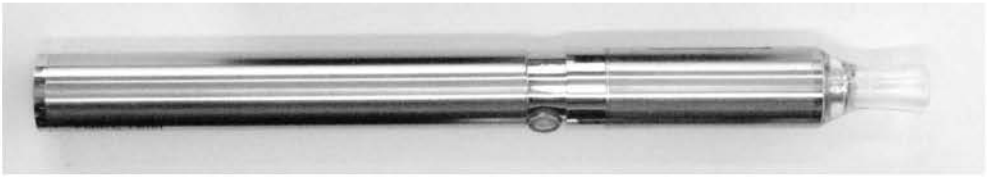
To account for components that normally occur in breath when the subjects were not vaping, samples of normal breath were first collected on control filters/cartridges immediately prior to collecting the SHA samples. After taking the control breath samples, subjects were asked to take a specified number of puffs from the provided e-cigarette and exhale immediately thereafter onto the trapping device. No other restrictions were made. Immediately after collecting the last sample, the trapping devices were transferred to the lab, weighed and the components of interest were extracted and analysed. The amounts trapped by the collection devices were normalized with respect to the volume of breath or SHA exhaled onto each filter. The concentration of the components found in the control breath samples were substracted from those in the SHA sample.

9.2.2 Selection of e-cigarettes

We previously conducted a market survey to establish which e-cigarettes and e-liquids are commonly used in the Netherlands (1). Based on the results from this market survey, we selected a popular 1st generation device ("cig-a-like") and a refillable 2nd generation

clearomizer (figure 9.2) for the current experiment. The refillable clearomizer was equipped with a 1.8 ohm dual coil atomizer and used with a 3.7V constant-voltage, non-adjustable 1000 mAh battery from the same manufacturer.

Figure 9.2: selected e-cigarettes



9.2.3 Selection of e-liquids

Based on the flavour preference users indicated in the market survey we conducted earlier (1), we selected mildly tobacco-flavoured liquids for the current experiments. In order to evaluate the maximum levels of metals, aldehydes, nicotine and TSNAs that may occur in SHA, a selection of liquids was made that yielded relatively high levels of these components in the FHA. Earlier, we measured the levels of these components in a relatively large sample of e-liquids and smoking machine-generated FHA (1). This data was used to select e-liquids for the current experiment.

First generation e-cigarettes often contain remarkably high levels of many different metals, some of which also occur in FHA (1, 35). Therefore, the 1st generation e-cigarette selected (section 7.2.2) represents the sample with high metal content. Tobacco-flavoured 18 mg/ml nicotine cartomizers were purchased for this device, which is near the maximum allowed level in the Netherlands (20 mg/ml) Analysis of the metal content of the e-liquid in the newly purchased cartridges confirmed that levels of metals was again comparatively high, characteristic of liquids in 1st generation devices (data can be found in supplementary data at http://www.rivm.nl/bibliotheek/rapporten/2016-0036bijlage.pdf)

The two liquids that we previously found (1) to yield the highest levels of aldehydes ("liquid 33") and TSNAs ("liquid 157") were selected for the refillable device. These had nicotine concentrations of 18 mg/ml and 11 mg/ml respectively. Analysis of the newly purchased bottles of e-liquids confirmed that the TSNA levels in liquid 157 were comparable to the levels found earlier.

9.2.4 Humectants and nicotine

Humectants and nicotine were trapped on 4mm glass fiber pads (commonly known as Cambridge Filter Pads (CFP)). The aerosol from 5 puffs was collected on each filter. Analytes were extracted from the filters with 15ml of methanol containing 1,3-butanediol and heptadecane as internal standards for humectants and nicotine respectively. The concentration of the humectants was analysed using GC-FID in accordance with WHO TobLabNet SOP6. The concentration of nicotine was measured using LC-MSMS.

9.2.5 Tobacco-specific nitrosamines (TSNA's)

Cambridge Filter Pads were used for collecting TSNA's. 25 puffs of aerosol were collected. After sample collection, stable-isotope labelled TSNAs were added as internal standard, and the TSNAs were extracted by the addition of 5 ml of 10 mM NaOH solution and 10 ml of

methyl-tert-butylether (MTBE). After 30 minutes of gentle shaking at room temperature the MTBE extract was removed and analysed using LC-MSMS.

9.2.6 Aldehydes

Aldehydes were trapped using a filter holder containing a cartridge with carboxen-572 beads followed by a Cambridge Filter Pad. To reduce the air flow restriction presented by the carboxen cartridge, the plastic fritted discs at each end were replaced by disks of fine gauze stainless steel. 25 puffs were collected onto each trapping assembly. After collection of the samples, the carboxen-572 from the cartridge and the filter were transferred to a stoppered flask and 10 ml of a mixture of methanol and carbon disulfide (80:20 v/v) was added to extract the aldehydes. After 20 minutes of shaking at room temperature, a sample of the extract was derivatized with 2,4-di-nitrophenylhydrazine (DNPH) and analysed with HPLC-DAD. For all samples, a blank sample of the methanol/CS2 solvent mixture was analysed in parallel and subtracted from the samples.

9.2.7 Metals

Cambridge Filter Pads were found to contain considerable and variable amounts of metals and not suitable for the analysis of metals in the aerosol samples. Pilot experiments indicated that the metal content of Whatman 47 mm QMA grade filters (Whatman, Maidstone, UK) was much lower and more consistent, and these were used to collect aerosol samples for the analysis of the metal content of exhaled aerosol. The samples were digested using a mixture of 12 ml concentrated nitric acid and 1 ml of 30% hydrogen peroxide. After digestion, the volume was adjusted to 30ml and samples were analysed using ICP-MS. Blank filters were analysed in parallel to correct for metal content of the filters.

9.3 Results and discussion

9.3.1 Concentration of harmful components in SHA

The concentrations of propylene glycol, glycerol and nicotine were measured in the SHA produced by all subjects. The other components were measured in samples from a subset of subjects. The results are summarized in table 9.1. The complete results are available as supplementary data (http://www.rivm.nl/bibliotheek/rapporten/2016-0036bijlage.pdf)

		concentration				amount per puff			
		range				range			
	n	min	max	median		min	max	median	
nicotine	18	<LOQ	12391	323	ng / L	<LOQ	2140	108	ng
humectants									
propylene glycol	18	<LOQ	839	64	µg / L	<LOQ	25	<LOQ	µg
glycerol	18	<LOQ	<LOQ	<LOQ	µg / L	<LOQ	<LOQ	<LOQ	µg
nitrosamines									
NNN	9	<LOQ	961	84	pg / L	<LOQ	111	29	pg
NAT	9	<LOQ	172	47	pg / L	<LOQ	40	14	pg
NAB	9	<LOQ	16	9	pg / L	<LOQ	8	2	pg
NNK	9	<LOQ	403	39	pg / L	<LOQ	71	15	pg
aldehydes									
formaldehyde	4	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng
acetaldehyde	4	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng
acroleine	4	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng
metals									
arsenic	3	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng
molybdenum	3	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng
tin	3	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng
cadmium	3	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng
lead	3	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng
zinc	3	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng
copper	3	<LOQ	28	<LOQ	ng / L	<LOQ	2.92	<LOQ	ng
nickel	3	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng
cobalt	3	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng
manganese	3	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng
chromium	3	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng
vanadium	3	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng
uranium	3	<LOQ	<LOQ	<LOQ	ng / L	<LOQ	<LOQ	<LOQ	ng

Table 9.1: Summary of SHA compositional analysis. The 'amount per puff' is the average amount recovered in the first exhaled breath after inhaling a puff, the concentration is the concentration in the first exhaled breath after inhaling a puff 'Range' lists the lowest and highest values measured. The median was calculated over all data, including samples with a value below the level of quantification.

Nicotine was detected in all samples except one. The control breath samples also contained small amounts of nicotine, possibly from nicotine consumption prior to the experiment. These were subtracted from the amounts observed in the SHA samples.
While propylene glycol was observed in the SHA from 4 out of 18 subjects, the amounts of glycerol remained below the level of detection. The e-liquids used do contain glycerol, but its concentration is 2 to 4-fold lower. Furthermore, the sensitivity of the analytical method used is somewhat lower for glycerol, mainly because the chromatographic peak is wider.
Although it cannot be excluded that glycerol is more readily deposited or absorbed by the

users, this does not appear very likely given the similar physical-chemical properties of these components.
The levels of aldehydes and ketones in SHA were below the limit of quantification. E-cigarettes are known to produce small amounts of different aldehydes and ketones during normal use (36), but these components are very reactive and water-soluble. They are therefore readily absorbed in the humid environment of the human respiratory tract. Certain aldehydes and ketones, including formaldehyde, acetaldehyde and acetone also occur naturally in exhaled breath as a result of normal metabolism. Small amounts of these compounds were indeed detected in all samples but the observed amounts in the SHA samples did not exceed the levels observed in control breath samples.
The volume of SHA exhaled by the test subjects onto the aerosol trapping assembly was measured with a flow meter, to allow conversion between concentration and absolute amounts. There is considerable variation between subjects in this respect (ranging from 40 ml to 1414 ml per exhalation) and these values are not representative for normal exhalation or breathing volumes, presumably because subjects were required to exhale via a mouth piece into the trapping assembly.

9.3.2 *Comparing the composition of FHA and SHA*

From the amount of e-liquid consumed during the experiments, a rough estimate was made of the amount of PG and nicotine recovered in the first exhalation of SHA as a percentage of the total amount vaporized (assuming the amount of e-liquid consumed in each puff was roughly constant throughout the experiment). A shown in table 9.2, the absolute amount of nicotine and PG recovered in SHA exhaled in the first exhalation was only a very small fraction of the vaporized amount. For 15 out of 17 subjects, this amounted to less than 1%. It should be noted that not all SHA is exhaled during the first puff. Due to lungs functional residual capacity, additional quantities of SHA are exhaled in later exhalations. Typically, the amount exhaled in the first exhalation is approximately 33% of the total amount if no absorption or deposition in the lungs takes place. However, the exact amount can differ considerably between subjects, for instance due to differences in lung capacities and breathing behavior.
Taken together, this strongly suggests that most of the humectants and nicotine are retained in the respiratory tract of the e-cigarette user and only a small fraction is exhaled in the SHA.

	% recovered in SHA	
subject	PG	nicotine
1	<LOQ	0.050
2	<LOQ	0.115
3	<LOQ	4.741
4	**0.17**	0.183
5	<LOQ	0.087
6	<LOQ	0.342
7	<LOQ	0.009
8	**2.35**	1.936
9	<LOQ	0.054
10	<LOQ	0.280
11	<LOQ	0.134
12	<LOQ	0.086
13	**0.63**	1.032
14	**0.54**	0.641
15	<LOQ	0.213
16	<LOQ	<LOQ
17	<LOQ	0.002

min	0.17	0.002
max	2.35	4.741
median	<LOQ	0.158

Table 9.2: Recovery of components in the SHA exhaled during the first exhalation after taking a puff, expressed as a percentage of the absolute amount that was vaporized and inhaled.

Similarly, the concentrations of glycerol and aldehydes in SHA were very low (<LOQ), suggesting these compounds are also predominantly retained by the e-cigarette user.

9.3.3 Comparison to literature values

A few recent studies have been published regarding the composition of SHA that has been sampled immediately upon exhalation in a similar fashion as in our investigation (19, 23, 37). In line with our findings, it was observed that only a small fraction of nicotine, propylene glycol and glycerol present in FHA remained in SHA.
Interestingly, O'Connel *et al* (19) found that while >99% of nicotine is retained by users when they inhale the aerosol, only 77-92% is retained when test subjects only hold the aerosol in their mouth for a few seconds, suggesting that differences between users in this respect may result from differences in the depth of inhalation. To enhance their absorption of nicotine, experienced users inhale and take relatively long, slow puffs and it is therefore likely that users typically absorb a large fraction of the inhaled aerosol components. Several studies have examined the composition of SHA after exhalation into a test chamber, but differences in the experimental setup makes it difficult to compare those with our results. Fernandez *et al.* (14) has recently conducted a systematic review of the literature on this topic.

9.4 Conclusions

The amounts of nicotine, PG, glycerol, aldehydes and ketones, metals and TSNA's were measured in the first breath of exhaled SHA produced by test subjects after taking a puff. The amount of the different components recovered in the first exhalation of SHA after taking a puff were much lower than present in FHA, suggesting that a large fraction is retained in the respiratory tract of the e-cigarette users. This underscores the necessity of using SHA (and not FHA) to allow an evaluation of the exposure to bystanders.

10 Asssesment of health risks associated with exposure to exhaled e-cigarette aerosol

10.1 Introduction

The source of exposure for the bystander is the amount of chemicals exhaled by the e-cigarette user(s) present in the immediate surrounding. The risk assessment for the bystander of e-cigarette vaping as described in this chapter is therefore based on the chemical analyses of the exhaled air (first exhalation after drawing a puff) of the volunteers vaping selected e-liquids (see chapter 9). The selection of chemicals included in the analyses of the expired air is described in section 8.4.

The e-cigarette in itself does not emit chemicals when not used in between puffs. This makes an adequate exposure assessment a challenging task. On the one hand chemicals are irregularly exhaled by the vaper and will cause a steady increase of the concentration in environmental air. The rate of increase will among others, depend on the vaping behaviour of the vaper and the alveolar absorption of the chemical. On the other hand, the concentration will decrease e.g. as a result of inhalation and absorption by the bystanders and/or ventilation of the room. Considering the many factors involved which are unknown, a pragmatic approach is chosen as a first worst-case estimation of potential health risks. If this approach does not indicate a health risk, no further refinement is needed. If a health risk is indicated a more detailed evaluation of the available data will be made for further refinement, if possible.

The actual source of exposure is the subsequent exhalation of chemicals by the vaper that were first inhaled from taking a puff from an e-cigarette. Therefore, special effort has been made to estimate the total amount exhaled. Following a puff, the inhaled chemical will enter the alveoli (depending on the depth of breathing) and will be exhaled in the next few exhalations. The total amount exhaled will, among others depend on the alveolar absorption of the chemical. Since only the first exhaled breath following a puff was captured and analysed, assumptions on breathing physiology had to be made to estimate the total amount exhaled from the amount in the first exhalation.

From the analyses of the first exhaled breath, the concentrations of the chemicals in indoor air are calculated for two specific predefined scenarios of e-cigarette use. The first scenario corresponds to an everyday car trip during which a child is exposed to chemicals exhaled by two e-cigarette users. The second scenario resembles exposure of an adult to chemicals exhaled by an e-cigarette user during part of a working day in an office room (see for more details 10.2). These air concentrations for these scenarios are compared with human limit values (air concentrations) for chronic exposure for the general population for the purpose of risk assessment. These limit values are in general applicable to continuous exposure of 24h/d. *Air Quality Guidelines* as derived by the WHO are examples of such limit values and these will be used as first choice in the current evaluation (20).

An exposure scenario resulting in an air concentration of a chemical which is below its limit value is considered not to result in adverse health effects. In cases where appropriate human health-based limit values are lacking, the risk assessment will be performed based on a Margin of Exposure (MOE)-approach (see for more details appendix A). Following this approach, the estimated human exposure to a chemical will be compared with relevant information on the chemical's potency of inducing adverse health effects (*i.e.,* an adequate Point of Departure, PoD). A PoD may be a No-Observed-Adverse-Effect Level (NOAEL), a Lowest-Observed-Adverse-Effect Level (LOAEL) or a Benchmark dose (BMD). Depending on the type of health effect under consideration the MOE can be calculated as the ratio of the exposure concentration at the PoD and the air concentration the bystander is exposed to, or, as the ratio of the dose taken up at the PoD and the dose taken up by the bystander. The MOE needs to be sufficiently large to reach the conclusion that no adverse health effects are to be expected. Whether a MOE is sufficient depends on several factors. First, differences in sensitivity between experimental animals and humans (if the PoD is obtained from animal experiments) and between human individuals need to be accounted for. Second, differences in exposure pattern between that for the PoD and for the bystander should be considered, and finally it should be considered whether effects are observed at the level of the PoD. As to carcinogens for which no safe threshold can be derived, the MOE should

be at least 10,000 to conclude that the exposure scenario for that chemical is of 'low concern', *i.e.* the risk for tumours is considered to be very low following the EFSA approach (21).
In order to obtain and select relevant information on possible adverse human health effects of the analysed chemicals, reports and evaluations of (inter)nationally recognized organizations (among others WHO, US EPA, ATSDR, AEGL committee, the Health Council of the Netherlands) were used as primary sources.
Exposure via the inhalation route is related to the amount per m^3 air inhaled during a specific time period. Many chemicals cause irritation to the respiratory tract upon inhalation. Exposure to these chemicals might result in clear damage to the respiratory tract. This is also applicable to many chemicals present in the vapour of e-cigarettes or exhaled air of e-cigarette users, such as polyols. Inhalation exposure to a chemical can also result in adverse effects after absorption into the body, the so-called systemic effects. The potency for systemic effects will preferably be assessed using information obtained from studies with inhalation exposure. If inhalation studies of sufficient quality are not available, it is possible to use data from studies with another exposure route, for example studies with oral exposure. In that case, differences between the exposure routes, for example differences in amount and rate of uptake of the chemical in the body, have to be accounted for.

Outline of this chapter
In section 10.2 the human exposure estimation will be presented. Section 10.3 will focus on the toxicological risk assessment in which the individual exhaled chemicals will be evaluated. Finally, the discussion and conclusion will be presented in section 10.4.

10.2 Human exposure

10.2.1 Description of exposure scenarios

Two predefined exposure scenarios were used for the risk assessment; these were considered to be relevant, realistic worst-case scenarios. The scenarios are described below; table 10-1 presents the parameter settings of these two scenarios.
For both scenarios, the risk assessment is performed for the non-vaping person. For scenario 1 this is a child, while for scenario 2 a risk assessment for an adult bystander is performed. A daily exposure (7d/week) of the bystander is assumed for scenario 1, while an exposure of 5d/week is assumed for scenario 2.

Scenario 1 – car
This scenario describes that two persons are vaping in a car while a third person (the bystander, in this scenario a child) is sitting in the same car being exposed to the chemicals exhaled by the two vapers. Given the relatively small volume of the indoor space and considering exposure of a child, this scenario is considered a realistic worst-case. The total vaping time (and thus exposure duration of the bystander) is set at one hour resembling an everyday car trip. The puff frequency for both vapers is set at 0.5 min^{-1}, which equals average vapers according to our previous study (22).

Scenario 2 – office
This scenario describes that one person is vaping in an office space while a second person is sharing the same office space and is exposed to the chemicals exhaled by the vaper. Currently, vaping is not prohibited in public spaces in The Netherlands, therefore this scenario is considered a realistic worst-case scenario. The total vaping time (and thus the exposure duration of the bystander) is considered to be four hours. The puffing frequency is set at 2 min^{-1}. Both the vaping period and the puffing frequency equal an intensive ("heavy") vaper according to our previous study (22).

Table 10-1 Parameter settings of the two predefined scenarios used for risk assessment.

Default parameter settings	
Scenario 1 - car	
Number of persons vaping	2
Puffing frequency	0.5 min^{-1}
Total vaping time *	1 h
Volume car	2 m^3
Ventilation	No
Scenario 2 - office	
Number of persons vaping	1
Puffing frequency	2 min^{-1}
Total vaping time *	4 h
Volume office space	30 m^3
Ventilation	Yes; 0.5 h^{-1}

* exposure duration of the bystander is considered similar to total vaping time

Starting point for the exposure estimation in the two scenarios is the amount exhaled by the vaper(s) in the first exhalation following a puff. The highest amount measured in the breath exhaled by the volunteers is used in the calculations. From this amount, the total amount exhaled in the two scenarios is calculated taking into account that exhalation of the chemical may not have been complete in the first exhalation but may continue with subsequent exhalations. From the total amount exhaled the end concentration in the regarding room is calculated and used for risk assessment as a first approximation. In the evaluation step, when judging whether the MOE is sufficiently large, it is considered that in reality the concentration in air will steadily increase and the use of an end concentration will result in an overestimation of potential health risks.
Since the total amount exhaled is the source of exposure, special effort has been made for its estimation. Section 10.2.2 describes the estimation of the total amount exhaled from the amount exhaled during the first exhalation following a puff, using physiological breathing characteristics. Section 10.2.3 then describes the calculation of the bystander exposure in the two scenarios.

10.2.2 Estimation of the total amount of a chemical exhaled by a vaper following one puff

As described in chapter 8, volunteers were asked to repeatedly draw a puff and after each puff the exhaled air of the first exhalation was collected and analyzed for the presence of selected chemicals. The absolute amount of a chemical present in the first exhalation was measured and used to calculate air concentrations resulting from exhalation of inhaled e-cigarette vapour. This calculation requires additional information (*e.g.*, on absorption and vaping pattern) and assumptions were made when information is not available. Initially, straightforward worst-case estimations were made. If no health risks were anticipated under these conditions no further refinement was needed. If risks could not be excluded, a further refinement towards a more realistic exposure estimation was performed. Exposure estimation included the following steps.

The amount of a chemical exhaled by a vaper in the first exhalation after drawing a puff is, to a large extent, dependent on the fraction absorbed in the respiratory tract. The higher the absorption fraction, the lower the amount of a chemical to be exhaled and thus the lower the bystander exposure. It should be mentioned that complete absorption via the inhalation route will in practice not occur since approximately 70% of the inspired volume reaches the alveoli where gas exchange takes place. The pulmonary absorption fraction is therefore maximally 0.7. For the present chemicals, no reliable quantitative estimations for the pulmonary absorption fraction were available and a worst-case assumption had to be made.
A distinction should be made between chemicals having local effects on the respiratory tract and chemicals having systemic effects upon pulmonary absorption. The exposure concentration (in a room)

is the most important dose metric in case of local effects on the respiratory tract, whereas the internal systemic exposure (expressed as mg/kg bw) would be the dose metric of choice for evaluating systemic effects in the present evaluation.

The source of exposure for the bystander is the total amount of the chemical exhaled by the vaper which can be calculated from the total amount inhaled in one puff. However, only the amount exhaled in the first exhalation is measured and although the main fraction will be exhaled during the first exhalation, additional amounts of the inhaled chemical will be exhaled during the subsequent breathing cycles until the next puff. The first step then is to estimate the fraction of the amount inhaled that will be exhaled in the first exhalation. Next, the fraction of the inhaled amount that will be exhaled during the subsequent exhalations until the next puff, needs to be estimated. From these two estimates, the total amount of chemical exhaled following one puff can be estimated.
The amount exhaled will to a large extent depend on the extent of absorption. In the present scenarios, a low absorption fraction would result in high air concentrations. Preferably, a chemical-specific value for absorption is to be used but often not available. Therefore, a pulmonary absorption fraction of zero (which will seldom be the case in reality) is used as a first worst-case estimate for evaluation of local effects on the respiratory tract of the bystander since this would result in the largest amount exhaled. However, for systemic exposure the situation is different. Although assuming no pulmonary absorption for the vaper will result in highest air concentrations for the bystander, at the same time this would result in a low internal systemic exposure for the bystander. Obviously the same absorption estimate should be used both for the vaper as for the bystander. This implies that assessing systemic exposure for the bystander requires a different worst-case assumption for the pulmonary absorption fraction. Table 10-2 shows the systemic exposure of the bystander as fraction of the inhaled amount of one puff by the vaper. It shows that the systemic exposure is potentially highest at an absorption fraction of 0.5; this leads to an estimated fraction of 0.25 of the amount inhaled in one puff by the vaper that might become systemically available in the bystander. A pulmonary absorption fraction of 0.5 is therefore used as a first, worst-case estimate for evaluation of systemic effects of the bystander. It should be noted that a *pulmonary* absorption fraction of 0.5 corresponds to an *alveolar* absorption fraction of 0.71 (*i.e.,* 0.5/0.7).

Table 10-2 Potential systemic exposure for the bystander (as fraction of the inhaled amount in one puff) as a function of the pulmonary absorption fraction.

Pulmonary absorption fraction for vaper and bystander	**Fraction of amount in one puff exhaled by vaper**	**Potential systemic exposure for the bystander (as fraction of the inhaled amount in one puff)**
0.1	0.9	0.09
0.2	0.8	0.16
0.3	0.7	0.21
0.4	0.6	0.24
0.5	***0.5***	***0.25***
0.6	0.4	0.24
0.7	0.3	0.21

The basis for the calculations is the measured amount of chemical exhaled in the first exhalation after drawing a puff (as analysed and presented in chapter 9). The fraction of the inhaled amount that is exhaled in the first exhalation can be estimated as follows, based on the method for smoking of tobacco cigarettes of Bos *et al*. (38) with slight adaptations for vaping of e-cigarettes. Estimations are made separately for local effects on the respiratory tract and systemic effects as different pulmonary absorption estimates are required to obtain worst-case exposure estimates for the two types of effects.

With a default tidal volume at rest of 500 ml, 350 ml (*i.e.,* a fraction of 0.7) reaches the alveoli and mixes with 2000 ml functional residual capacity (FRC) leading to a total volume of 2350 ml (see table 10-3 for default parameter values). The remaining fraction of 0.3 (*i.e.,* corresponding to the dead space) does not reach the alveoli upon inhalation and the chemical present in this fraction will be completely exhaled.

Evaluation of local effects on the respiratory tract: For the fraction that reaches the alveoli (*i.e.*, 0.7), a worst-case default of no absorption is assumed, meaning that this fraction of 0.7 will be completely exhaled. However, during the first exhalation only 350 ml (similar as the inhaled volume) of the total volume of 2350 ml is exhaled, *i.e.*, a fraction of 0.15 (*i.e.*, 350/2350). Combined with the exhaled dead space volume, a total fraction of 0.4 (*i.e.*, 0.3 + (0.15 x 0.7)) of the amount of a chemical present in one puff will be exhaled during the first exhalation after drawing that puff. Thus, for chemicals inducing local pulmonary effects upon inhalation, the absolute amount of a chemical inhaled in one puff is calculated by dividing the amount present in the first exhalation following a puff (as analysed and presented in chapter 9) by 0.4.

Evaluation of systemic effects: For the fraction that reaches the alveoli (*i.e.*, 0.7), the alveolar absorption fraction of 0.71 (*i.e.*, equivalent to a pulmonary absorption fraction of 0.5) applies, meaning that 0.29 of this fraction will be exhaled. However, during the first exhalation only 350 ml (similar as the inhaled volume) of the total volume of 2350 ml is exhaled, *i.e.*, a fraction of 0.15 (*i.e.*, 350/2350). Thus from the amount that has reached the alveoli, a fraction of 0.044 (0.15 × 0.29) is exhaled with the first exhalation. Combined with the exhaled dead space volume, a total fraction of 0.33 (*i.e.*, 0.3 + (0.044 x 0.7)) of the amount of a chemical present in one puff will be exhaled during the first exhalation after drawing that puff. Thus, for chemicals inducing systemic effects upon inhalation, the absolute amount of a chemical inhaled in one puff is calculated by dividing the amount present in the first exhalation following a puff (as analysed and presented in chapter 9) by 0.33, which equals multiplication by 3.

Table 10-3 Default parameter values for the exposure estimation

Parameter	**Default values**
Tidal volume (rest)	500 ml
Puff volume	70 ml
Functional Residual Capacity (FRC)	2000 ml
Breathing rate	12 min^{-1}
Dead space	30%
Pulmonary absorption fraction*	0.5
Alveolar absorption fraction*	0.71

* values used for evaluation of systemic effects; for evaluation of local effects on respiratory tract an absorption fraction of zero is assumed

However, exhalation of the chemical will continue in subsequent exhalations until the next puff is drawn. During each subsequent breathing cycle, clean air is inhaled and a fraction of 0.15 of the amount present in the alveoli will be exhaled as explained above. For scenario 2 with a puff frequency of 2 min^{-1} (highest frequency of the two scenarios), each puff is followed by 5 breathing cycles with clean air, assuming a breathing frequency of 12 min^{-1}. Figure 10-1 depicts a simulation of the alveolar concentration, relative to the concentration immediately after puff drawing, in a scenario with a puff frequency of 2 min^{-1} and four different alveolar absorption fractions: no absorption, low absorption (0.3), high absorption (0.71) and full absorption. As indicated in figure 10-1, in case of high absorption (red curve) and full absorption (green curve), the alveolar concentration has decreased to zero before drawing a new puff. Thus, starting from a pulmonary absorption fraction of 0.5 (equivalent to an alveolar absorption fraction of 0.71 (red curve in figure 10-1)) the total amount of a chemical that is inhaled in one puff, minus the fraction absorbed, will be exhaled completely before a new puff will be drawn. Thus the amount exhaled can be calculated from the total amount inhaled by adjustment for

the fraction absorbed, *i.e.*, by multiplication with 0.5. Since the puff frequency in scenario 1 (*i.e.*, 0.5 min^{-1}) is lower than in scenario 2, the same holds for scenario 1.

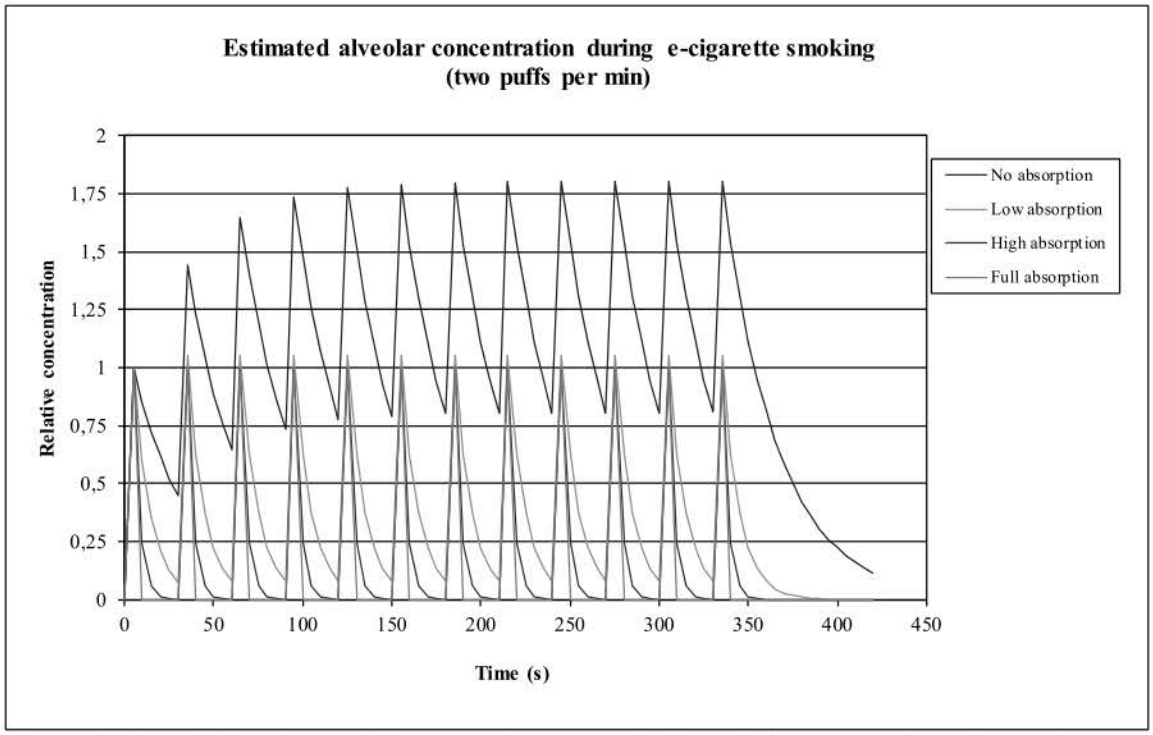


Figure 10.1 Estimated relative alveolar concentration during an e-cigarette vaping session of 6 minute duration with a puff frequency of 2 min^{-1}. Dark blue line ('no absorption') corresponds to an alveolar absorption fraction of zero; light blue corresponds to an alveolar absorption fraction of 0.3; red line ('high absorption') corresponds to an alveolar absorption fraction of 0.71; green blue ('full absorption') corresponds to an alveolar absorption fraction of 1.

10.2.3 Calculating bystander exposure in the selected scenarios

Based on the reasoning and assumptions as detailed in section 10.2.2, the exposure of the bystander of e-cigarette vaping is calculated.

The maximal final concentration in ambient air (*i.e.*, car or office space without ventilation) at the end of a vaping session by a specified number of persons during a given period of time can be calculated as:

$$conc = (1-F_{pulm,abs}) \times A_{puff} \times f \times t \times n / V$$

conc = concentration of a chemical in room upon vaping (mg/m^3)
$F_{pulm,abs}$ = pulmonary absorption fraction (zero or 0.5 for local and systemic effects, respectively)
A_{puff} = amount of a chemical inhaled with one puff (mg)
f = puff frequency (min^{-1})
t = vaping period (min)
n = number of persons vaping *
V = volume of room (m^3)
** it is assumed that all persons vaping in the room use the same constant puff frequency*

The amount of a chemical inhaled with one puff (A_{puff}) was calculated as:

$$A_{puff} = A_{exh-1} / F_{exh-1}$$

A_{puff} = amount of a chemical inhaled with one puff (mg)

A_{exh-1} = amount of a chemical present in the first exhalation after drawing one puff (mg)
F_{exh-1} = fraction of the amount of a chemical present in one puff that will be exhaled during the first exhalation after drawing that puff (*i.e.*, for evaluating local pulmonary effects a fraction of 0.4 is assumed; for evaluating systemic effects a fraction of 0.33 is assumed)

The systemic (internal) exposure of the bystander is calculated as:

$$D_{syst} = conc \times F_{pulm,abs} \times t \times RV$$

D_{syst} = systemic (absorbed) dose for the bystander (mg/kg bw/d)
conc = maximal final concentration in ambient air (*i.e.*, car or office space without ventilation) at the end of a vaping session by a specified number of persons during a given period of time (mg/m^3)
t = vaping period (min) *
$F_{pulm,abs}$ = pulmonary absorption fraction
RV = respiratory volume (default: 0.2 l/min/kg bw for a 70 kg person according to ECHA (2012) (39), corresponding to 2 $x10^{-4}$ m^3/min/kg bw. As default for a child an RV of 0.5 L/min/kg bw (corresponding to 5 $x10^{-4}$ m^3/min/kg bw) was used (calculated as a worst-case default from a RV of 11 m^3/24 h for a 4-6y old child based on (40))
** the exposure period is assumed to be similar to the vaping period for the selected exposure scenarios*

Ventilation was not included in scenario 1 (car; 1 h exposure). Scenario 2 did include ventilation, given that ventilation is obliged for office spaces. Table 10-4 shows the end concentrations upon vaping (assuming a constant puffing frequency) for specific time periods up to 4 h, in- and excluding ventilation. End concentrations are expressed relative to the end concentration upon 1 h without ventilation. As a realistic minimum, a ventilation rate corresponding to a replacement of the air of 0.5 office space per hour is assumed for scenario 2.

Table 10-4 End air concentrations upon vaping for a specific time period with or without ventilation. The concentrations are expressed relative to the concentration present after one hour vaping without ventilation (i.e., corresponding to y). End air concentrations were determined with ConsExpo (41) (read from a plot) in a preset scenario where only the duration and total amount was adjusted. This value was compared with the end air concentration of the same scenario without ventilation to obtain the relative end air concentration at the given end time.

vaping period	**1h**	**2h**	**3h**	**4h**
without ventilation	y	2y	3y	4y
*with ventilation**	0.75y	1.2y	1.5y	1.7y

* a ventilation rate corresponding to replacement of the air of 0.5 office space per hour is assumed as realistic minimum

Risk assessment of bystanders of e-cigarette vaping is complex, mainly due to the complexity of the exposure assessment. Therefore, a first risk assessment using worst-case assumptions was performed. If no risks were then anticipated for a chemical, no further analysis was needed. Refinement was only made in case of probable health risks. For the calculation of the exposure of the bystander of e-cigarette vaping, realistic estimates were used as much as possible. However, still some worst-case assumptions were needed which are described below and which should be taken into account for the final risk assessment.

The exposure concentration of a chemical present in the room at the end of the vaping period was used for risk assessment for the bystander, *i.e.*, it was assumed that the bystander was exposed to this end concentration for the entire exposure period. This is a worst-case estimate, as the concentration of a chemical in the room where vapers are vaping e-cigarettes will gradually build up during the vaping period. Therefore, the bystander will be exposed to an increasing concentration rather than to the end concentration. A time weighted average concentration or inhaled dose would be a factor of 2 lower than the end concentration.
No information on absorption after inhalation is available for the chemicals evaluated. Therefore default values were set. As a first worst-case estimate for evaluation of local pulmonary effects of the bystander, a pulmonary absorption fraction of zero is used. A higher pulmonary absorption fraction will reduce the exposure concentration, *e.g.*, an absorption factor of 0.5 will lead to a twofold lower exposure concentration.
The concentration of a chemical in the room will also change depending on the total number of persons present in the room and being exposed via inhalation to the chemical. For example for scenario 1, in total three persons are present in the car of which two persons are vaping. The risk assessment is in the first worst-case step performed for the third person sitting in the car, assuming that only the bystander inhales the exhaled chemicals. However, also the vapers will be inhaling and take up the chemical present in the exhaled air and will thus become re-exposed to the chemical. Thus the exposure of the bystander will be overestimated, especially for risk assessment of systemic effects.

10.3 Risk assessment bystander

For each individual chemical, a comparison with the human health-based limit value is made or, in case no human health-based limit value is available for a specific chemical, a MOE is calculated and evaluated for potential human health effects for the bystander of e-cigarette vaping. The risk assessment is based on the highest amount of that specific chemical measured in the exhaled air of the human volunteers (see chapter 9). As mentioned in section 10.1, it depends on several factors whether an MOE is sufficient. See also appendix A for further considerations.
In the next sections, evaluations of the individual chemicals will be presented.

10.3.1 Risk assessment humectants

Propylene glycol

Exposure

The amount of propylene glycol present in the exhaled air of the first exhalation after drawing a puff was measured to be 127 µg. No information on absorption after inhalation is available.
Scenario 1 - car: For risk assessment of local effects on the respiratory tract, a worst-case exposure concentration of 9.5 mg/m^3 is estimated to be present in a car upon 1h vaping. A worst-case exposure concentration of 5.8 mg/m^3 is estimated to be present in a car upon 1h vaping for risk assessment of systemic effects. The resulting systemic exposure is 0.087 mg/kg bw/d.
Scenario 2 - office: For risk assessment of local effects on the respiratory tract, a worst-case exposure concentration of 2.16 mg/m^3 is estimated to be present in an office space upon 4h vaping. A worst-case exposure concentration of 1.31 mg/m^3 is estimated to be present in an office space upon 4h vaping for risk assessment of systemic effects. The resulting systemic exposure is 0.032 mg/kg bw/d.

Point of departure

A subchronic inhalation study with rats showed that repeated exposure to propylene glycol in concentrations of 0, 160, 1000 and 2200 mg/m^3 for 6h/d, 5d/week for 13 weeks resulted in effects on the respiratory tract (increased number of goblet cells) with a NOAEL of 160 mg/m^3 and nasal haemorrhage with a LOAEL of 160 mg/m^3 ((42) as described in (43)).
A human study was described in which a one minute inhalation exposure of healthy human volunteers (n=27) to 176 – 851 mg/m^3 propylene glycol (geometric mean: 309 mg/m^3) resulted in (subjectively reported) irritation of eyes and the upper respiratory tract. The lowest concentration of this exposure

concentration range can be considered a LOAEL ((44) as described in (45)). Given the one-minute exposure period, this study was used as supportive.
Repeated inhalation exposure to propylene glycol resulted in the same rat study also in a reduced number of lymphocytes with a NOAEL of 160 mg/m^3 ((42) as described in (43)); this is equivalent to 46.4 mg/kg bw/d[1]. This value is used as PoD for the risk assessment of systemic effects.
In addition, a recommendation for maximum exposure levels of actors to propylene glycol via theatrical fog is also available. It was recommended that exposures to propylene glycol by actors should not exceed peak or ceiling concentrations of 40 mg/m^3 (46).

Risk for local effects on the respiratory tract
Scenario 1 - car: The MOE for irritation of the upper respiratory tract is 17 (based on the subchronic rat study) or 18 (based on a human LOAEL). The exposure concentration estimated for evaluating local effects on the respiratory tract (9.5 mg/m^3) is below the recommended maximum exposure level for actors.
Scenario 2 - office: The MOE for irritation of the upper respiratory tract is 74 (based on the subchronic rat study) or 81 (based on a human LOAEL). The exposure concentration estimated for evaluating local effects on the respiratory tract (2.16 mg/m^3) is below the recommended maximum exposure level for actors.

For the evaluation of the MOE the following factors are applicable:
less-than-lifetime exposure for the PoD,
the use of a LOAEL instead of a NOAEL as PoD,
interspecies extrapolation (rat to human, in case of animal experiment as PoD)
interindividual variability in sensitivity among bystanders,
differences in exposure profile between animal or human study on the one hand and (daily) exposure of the bystander on the other hand.

Additionally, the fact that the risk assessment was based on worst-case assumptions such as zero pulmonary absorption and using the end concentration of a chemical should be accounted for. This applies also to the fact that the concentration of a chemical in the room will also change (*i.e.*, decrease) depending on the total number of persons present in the room and being exposed via inhalation to the exhaled chemical.

Based on the calculated MOEs and considering the factors included, it is concluded that local effects on the respiratory tract upon exposure to propylene glycol for a bystander of e-cigarette vaping cannot be excluded for scenario 1. However, it is expected that effects, are expected to be mild, if they occur.
As to scenario 2, no local effects on the respiratory tract are expected from exposure to propylene glycol for the bystander as the MOE is sufficiently high also taking the abovementioned reasons of overestimating the risk into account.

Risk for systemic effects
Scenario 1 - car: The MOE for systemic effects is 535.
Scenario 2 - office: The MOE for systemic effects is 1475.

For the evaluation of the MOE the following factors are applicable:
less-than-lifetime exposure, interspecies extrapolation (rat to human)
interindividual variability in sensitivity among bystanders,
differences in exposure profile between animal or human study on the one hand and (daily) exposure of the bystander on the other hand.

[1] The inhalation exposure is converted to an equivalent systemic dose, based on a respiratory volume of 0.29 m^3/kg bw for the rat for a 6-h exposure (ECHA, 2012)

Additionally the use of a default absorption fraction leading to a worst-case systemic exposure estimate, and using the end concentration of a chemical should be accounted for. This applies also to the fact that the concentration of a chemical in the room will also change (*i.e.*, decrease) depending on the total number of persons present in the room and being exposed via inhalation to the exhaled chemical.

Based on the calculated MOEs, it can be concluded that systemic effects upon exposure to propylene glycol for a bystander of e-cigarette vaping are not expected for scenarios 1 and 2.

Glycerol
Analyses showed that glycerol could not be detected in the expired air, *i.e.,* glycerol was present in the expired air below the LOQ. Based on the available toxicological information and the LOQ for glycerol it was concluded that amounts below the LOQ are not expected to induce adverse health effects.

10.3.2 Risk assessment nicotine

Exposure
The amount of nicotine present in the exhaled air of the first exhalation after drawing a puff was measured to be 2.14 µg. No information on absorption after inhalation is available.
Scenario 1 - car: For risk assessment of local effects on the respiratory tract, a worst-case exposure concentration of 0.16 mg/m^3 is estimated to be present in a car upon 1h vaping. A worst-case exposure concentration of 0.097 mg/m^3 is estimated to be present in a car upon 1h vaping for risk assessment of systemic effects. The resulting systemic exposure is 0.00146 mg/kg bw/d.
Scenario 2 - office: For risk assessment of local effects on the respiratory tract, a worst-case exposure concentration of 0.036 mg/m^3 is estimated to be present in an office space upon 4h vaping. A worst-case exposure concentration of 0.022 mg/m^3 is estimated to be present in an office space upon 4h vaping for risk assessment of systemic effects. The resulting systemic exposure is 0.00053 mg/kg bw/d.

Available data and evaluation
The available toxicological (inhalation) data for nicotine are very limited. An appropriate PoD for evaluating of a lifetime inhalation exposure is not available. A MOE-approach can therefore not be applied and a weight-of-evidence evaluation is applied.

Appendix B provides a brief overview of animal and human studies, based on evaluations of the Health Council of the Netherlands (2005) and ACGIH (1994) (47, 48).
Some of these studies will be described below.

Results of a two-year rat inhalation study show that repeated inhalation exposure of 0.5 mg/m^3 nicotine during 103 weeks (20 h/d, 5d/wk) resulted in a small decrease of body weight. Macro- and microscopic evaluation did not show any treatment-related effect ((49) as decribed in (47)). The concentration of 0.5 mg/m^3 was considered a NOAEL. Based on this study, it can however not be determined at which concentration effects are to be expected. Nevertheless, the exposure concentration (assuming worst-case no absorption) of scenarios 1 and 2 are a factor 3-14 below this NOAEL.

Human volunteer studies showed that a single-breath inhalation exposure to nicotine induces effects on the respiratory tract. Exposure of non-smoking persons to nicotine (0 – 0.01 – 0.02 – 0.04 – 0.08 – 0.16 – 0.32 – 0.64 mg nicotine[2]) resulted in a concentration-dependent cough response and airway constriction ((50) as described in (47)). In the same study, volunteers were also repeatedly exposed to nicotine (a single breath was taken every 15 seconds up to 5 minutes (total 21 inhalations), resulting

[2] Inhalation of 0.01 ml nebulized nicotine-solution of 0 – 1 – 2 – 4 – 8 – 16 – 32 - 64 mg/ml

in an exposure of 0 – 0.42 – 0.84 – 1.68 mg nicotine[3]). This resulted in a dose-dependent increase in heart rate and systolic blood pressure ((50) as described in (47)).
A comparison with the effect level for local effects on the respiratory tract derived from this study (27 mg/m^3 as an *alveolar* concentration[4]) shows that the exposure concentrations for scenario 1 and scenario 2 are approximately a factor of 170 and a factor of 750, respectively, below this effect level. For the evaluation of potential local effects on the respiratory tract of nicotine, the interindividual variability in sensitivity among bystanders needs to be taken into account. Additionally, 1) the differences in exposure profile between the human study on the one hand and the (daily) exposure of the bystander on the other hand, 2) the fact that the risk assessment was based on assuming (worst-case) zero pulmonary absorption and 3) using worst-case the concentration of a chemical present in the room at the *end* of the vaping period and 4) the fact that the concentration of a chemical in the room will also change (*i.e.*, decrease) depending on the total number of persons present in the room and being exposed via inhalation to the exhaled chemical, also need to be considered. From this evaluation, local effect on the respiratory tract upon exposure to nicotine for a bystander of e-cigarette vaping is not expected for scenario 1 and scenario 2.
A comparison with the effect level for systemic effects derived from this human study ((50) as described in (47)) (0.42 mg nicotine, corresponding to an internal systemic dose of 0.003 mg/kg bw)[5] shows that the systemic exposure for scenario 1 and scenario 2 are a factor 2.1 and 6 lower, respectively, than this effect level. The margins between the bystander exposure and the effect level for systemic effects are rather low, especially considering the limited exposure duration in the human study. For the evaluation of potential systemic effects of nicotine the interindividual variability in sensitivity among bystanders and the fact that effects were observed at the PoD need to be taken into account. Additonally, 1) the differences in exposure profile between the human study on the one hand and the (daily) exposure of the bystander on the other hand, 2) the fact that the risk assessment was based on the use of a default absorption fraction leading to a worst-case systemic exposure estimate and 3) using worst-case the concentration of a chemical present in the room at the *end* of the vaping period and 4) the fact that the concentration of a chemical in the room will also change (*i.e.*, decrease) depending on the total number of persons present in the room and being exposed via inhalation to the exhaled chemical, are to be considered.

Overall, it is concluded for scenario 1 that systemic effects (increased heart rate and increased systolic blood pressure) upon exposure to nicotine for a bystander of e-cigarette vaping are expected. For scenario 2, it cannot be excluded that systemic effects upon exposure to nicotine for a bystander of e-cigarette vaping might occur.

The potential effects of nicotine on the development of the fetus are investigated in some studies. These studies show that exposure to nicotine may result in a delayed development of the fetus (characterized by a reduced body weight, but also reduced fetal organ weights of various tissues such as brain, heart, lung). In addition, studies are available which show a reduced gestational period and abortions, and effects on male reproductive organs. However, some of these studies had some limitations as for example no data on maternal toxicity were presented in the developmental toxicity studies. Moreover, it should be noted that reproductive toxicity studies with exposure via the inhalation route are not described.
A study with rhesus monkeys shows that subcutaneous exposure (via a mini-osmotic pump) to nicotine in a dose of 1 mg/kg bw/d during gestation days 26-134 resulted in detectable nicotine levels in amniotic fluid, a 8% lowered fetal body weight, reduced body length and biparietal, and reduced fetal

[3] Total 21 inhalations of 0.01 ml nebulized nicotine-solution of 0 – 2 – 4 – 8 mg/ml

[4] Assuming an effect level of 0.04 mg inhaled nicotine, converted to an alveolar concentration of 27 mg/m^3 taking into account a tidal volume of 500 ml, a correction factor for dead space of 0.7 and a dilution in a total volume (FRC: functional residual capacity) of 2L.

[5] Assuming an effect level of 0.42 mg inhaled nicotine, converted to a systemic dose of 0.003 mg/kg bw nicotine, taking into account a correction factor for dead space of 0.7, pulmonary absorption fraction of 0.5 and a bw of 70 kg.

organ weights of heart, pancreas, adrenals, kidney and brain. Fetal lung weight and volume were reduced by 13% and 12%, respectively (not significant). Further, there were some indications that fetal lung development was changed in response to prenatal nicotine exposure. The lungs of offspring had hypoplasia and a reduced surface complexity of developing alveoli. Maternal body weight and food consumption was unchanged ((51) as described in (47)).
Developmental effects are not a relevant endpoint for a child and thus this endpoint is not considered for scenario 1. A comparison with the effect level for reproductive toxicity derived from this latter study (1 mg/kg bw/d) shows that the systemic exposure for scenario 2 is approximately a factor of 1900 lower than the effect level in monkeys. Based on this study, it can be concluded that a risk for potential developmental effects are not expected upon exposure to nicotine for a bystander of e-cigarette vaping.

10.3.3 Risk assessment aldehydes

Analyses of formaldehyde, acrolein and acetaldehyde showed that these chemicals could not be detected in the expired air, *i.e.,* these chemicals were present in the expired air below the LOQ. Based on the available toxicological information and the LOQ for these chemicals it was concluded that amounts below the LOQ are not expected to induce adverse health effects.

10.3.4 Risk assessment tobacco-specific nitrosamines (TSNAs)

Exposure

Four tobacco-specific nitrosamines were analysed: N'-nitrosonornicotine, NNN; 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, NNK; N'-nitrosoanabasine, NAB; N'-nitrosoanatabine, NAT).
The amount of NNN, NNK, NAB and NAT present in the exhaled air of the first exhalation after drawing a puff was measured to be 111, 32.6, 7.78, 40.2 pg. No information on absorption after inhalation is available.
Scenario 1 - car: For risk assessment of local effects on the respiratory tract, a worst-case exposure concentration of 8.3, 2.4, 0.58 and 3.0 ng/m^3 for NNN, NNK, NAB and NAT, respectively, is estimated to be present in a car upon 1h vaping.
Scenario 2 - office: For risk assessment of local effects on the respiratory tract, a worst-case exposure concentration of 1.89, 0.56, 0.13, 0.68 ng/m^3 for NNN, NNK, NAB and NAT, respectively, is estimated to be present in an office space upon 4h vaping.

Point of departure

The tobacco-specific nitrosamines NNK, NNN and NAB induce tumours in experimental animals; in general this considers lung tumours independent of the exposure route (52). NNK and NNN are considered as genotoxic carcinogens and are classified by IARC as group 1 ('carcinogenic to humans') carcinogenic chemicals. NAB and NAT are classified by IARC as group 3 ('not classifiable as to its carcinogenicity to humans') carcinogenic chemicals (53). In vitro genotoxicity studies showed that NNK has a similar mutagenic potency when compared to N-nitrosodimethylamine (NDMA), and a higher mutagenic potency when compared to NNN. Of these four mentioned tobacco specific nitrosamines, NNK has the highest carcinogenic potency, followed by NNN. NAB is considered a carcinogen with a moderate potency while hardly any evidence is available for NAT pointing towards potential carcinogenicity (52).
The available adequate toxicological (inhalation) data for the mentioned four tobacco-specific nitrosamines are quite limited. Therefore, inhalation data from the nitrosamine N-nitrosodimethylamine (NDMA) were used for the risk assessment of the tobacco-specific nitrosamines.
A rat inhalation study (n=36/group) with exposure to NDMA was selected to derive the PoD for risk assessment. Exposure to 0, 120, 600 and 3000 µg/m^3 NDMA (4-5 h/d, 4 d/wk) during 207 days resulted primarily in tumours in the nasal cavity. These effects are considered relevant for the potency to induce respiratory tract tumours in humans. It is assumed that the 207 days refer to the number of exposure days, the total study period will therefore be one year. The tumour incidences for the nasal

tumours were 0/36, 13/36, 31/36, 19/36, respectively ((54) as described in (55)). The results of this study were analyzed with a BMD-analysis and a BMDL10 was derived. A detailed description of the BMD-analysis and an overview of the results can be found in section 11.4 of our previous report (22). The analysis resulted in a BMDL10 of 3 $\mu g/m^3$.

Risk for carcinogenic effects
Nitrosamines are considered carcinogenic chemicals for which no threshold can be derived. The lung is the primary target organ for tumour development upon exposure to tobacco-specific nitrosamines, independent of the exposure route (52). Whether these tumours upon inhalation exposure are related to the exposure concentration or the total inhaled dose is not known. An inhalation study with NDMA in which nasal tumours were observed is used for risk assessment. It is assumed that these tumours are related to the exposure concentration. In order to calculate the MOE, the concentrations of the four tobacco-specific nitrosamines are summed up (on molar basis) and converted to an equivalent concentration NDMA ($\mu g/m^3$) to be able to compare this with the PoD. This is done with the assumption that the four tobacco-specific nitrosamines have the same carcinogenic potency as NDMA. This resulted in an exposure concentration (on NDMA-basis) of 5.8 ng/m^3 for scenario 1 and 1.31 ng/m^3 for scenario 2.

Scenario 1 - car: The MOE for carcinogenic effects is 521.
Scenario 2 - office: The MOE for carcinogenic effects is 2297.
If the MOE is minimally 10,000 (when compared to a BMDL10) for genotoxic carcinogenic chemicals, it can be concluded that the chemical is 'of low concern', which means that the risk for tumours is very low ((21); see also appendix A).
As indicated before, current risk assessment is based on the highest amount of that specific chemical measured in the exhaled air of the human volunteers. Of all volunteers, the highest amounts of NNN, NAT and NAB were detected in the exhaled air of subject 6. The highest amount of NNK was detected in the exhaled air of subject 2, with a factor two higher amount of NNK than as measured for subject 6 (see chapter 9). The highest amount total nitrosamines (expressed as NDMA) was observed for subject 6. The nitrosamine amounts as measured in the exhaled air of this subject was therefore used for current risk assessment.
On molar basis, the exhaled air consisted of 60% NNN, 15% NNK, 4% NAB and 21% NAT. The carcinogenic potency of NNK is considered to be higher than for NNN, which in turn has a higher carcinogenic potency than NAB (52). However, these differences in carcinogenic potency cannot be quantified. For NAT, it has not been demonstrated that this nitrosamine has carcinogenic effects. For current risk assessment, it is assumed that the carcinogenic potency of NNN, NNK, NAB and NAT are not significantly lower than the carcinogenic potency of NDMA. However, it is noted that a risk assessment excluding NAT would not result in different conclusions.
When evaluating the MOE, it should be taken into account that the risk assessment was based on assuming (worst-case) zero pulmonary absorption and using worst-case the concentration of a chemical present in the room at the *end* of the vaping period and the fact that the concentration of a chemical in the room will also change (*i.e.*, decrease) depending on the total number of persons present in the room and being exposed via inhalation to the exhaled chemical. In addition, differences in exposure profile between animal on the one hand and (daily) exposure of the bystander on the other hand should be accounted for. For scenario 1, a one hour daily exposure was assumed, while for scenario 2 an exposure duration of 4 hour per working day was assumed. In contrast, the exposure in the animal study was 4-5 hour/day for only four days per week for a period of one year. The consequences of this difference in exposure profile on the expected tumour incidences cannot be estimated as data concerning this issue are lacking. For the time being, it is assumed that increased incidences of tumours in the respiratory tract upon exposure to tobacco-specific nitrosamines for a bystander of e-cigarette vaping cannot be excluded for scenario 1. For scenario 2 the end concentration is used for the 4-hour exposure and no absorption is assumed for the calculation of this concentration. The exposure concentration is therefore probably overestimated but data are lacking to verify the extent of overestimation. Furthermore, it is noted that the potency of the individual TSNA's are all considered to be equal to NDMA, meaning that the carcinogenic potential of the mixture is

overestimated to an unknown extent. It may be that refinement of the MOE calculation leads to the conclusion of 'of low concern', which means that the risk for tumours is very low, but this cannot be stated with sufficient certainty.

10.4 Risk assessment metals

Copper

Exposure

The amount of copper present in the exhaled air of the first exhalation after drawing a puff was measured to be 2.92 ng. No information on absorption after inhalation is available.

Scenario 1 - car: For risk assessment, a worst-case exposure concentration (assuming no pulmonary absorption) of 219 ng/m^3 is estimated to be present in a car upon 1h vaping.

Scenario 2 - office: For risk assessment, a worst-case exposure concentration (assuming no pulmonary absorption) of 50 ng/m^3 is estimated to be present in an office space upon 4h vaping.

Risk assessment

RIVM derived a tolerable concentration in air (TCA) for copper which was set at 1 µg/m^3 (56). The exposure concentrations for scenarios 1 and 2 are below this limit value. It can be concluded that a risk for adverse health effects upon exposure to copper is therefore not expected for the bystander of e-cigarette vaping.

Other metals

Analyses of vanadium, chromium, manganese, cobalt, nickel, zinc, arsenic, molybdene, cadmium, tin, lead and uranium showed that these chemicals could not be detected in the expired air, *i.e.,* these chemicals were present in the expired air below the LOQ. Specific species of chromium, nickel and arsenic are carcinogenic, but it is unknown whether these forms are present in the exhaled air since only total chromium, nickel and arsenic are measured. Therefore, no definite conclusions can be drawn on carcinogenic risks. As to nickel and arsenic, assuming that the carcinogenic species of these metals are present, it can be stated that the risk for cancer will be negligible for amounts below the LOQ. No conclusions can be drawn for chromium.

For tin, no adequate data are available. For the remaining metals (vanadium, manganese, cobalt, zinc, molybdene, cadmium, lead and uranium), it was concluded that amounts of these metals below the LOQ are not expected to induce adverse health effects based on the available toxicological information and the LOQ for the respective metals.

10.5 Discussion and conclusion

Current risk assessment for the bystander of e-cigarette vaping was based on the chemical analyses of the exhaled air of volunteers vaping selected e-liquids. Air from the first exhalation following a puff was collected for analyses. Depending on the chemical to be analyzed, 5 or 25 'first' exhalations were sampled per individual and analysis of the pooled sample was performed for each chemical. This resulted in an average amount in the exhaled air of one exhalation for each individual. However, due to the collection of multiple exhalations and pooling of these samples, insight in the intra-individual variation was not available. For each chemical, analyses were performed in the exhaled air of a number of volunteers, ranging from samples of 3 volunteers for metal analyses, 4 volunteers for aldehyde analyses, 9 volunteers for TSNA analyses and 17 volunteers for nicotine and humectants analyses. For each chemical, the highest amount measured was used for risk assessment. Inter-individual variation in amounts of a chemical present in the exhaled air was observed and it might therefore be worst-case to select the highest amount for risk

assessment. This applies in particular to propylene glycol that could not be detected in the exhaled air of 13 of the 17 volunteers.

The risk assessment was performed for two predefined relevant, realistic worst-case scenarios as presented in section 10.2.1. The evaluation of the individual chemicals (section 10.3) showed that exposure of the bystander of e-cigarette vaping might be related to adverse effects on health. Scenario 1 resembled an everyday car trip during which a child is exposed to chemicals exhaled by two e-cigarette vapers. For this scenario, it cannot be excluded that local effects on the respiratory tract upon exposure to propylene glycol will occur for the bystander of e-cigarette vaping. However, it is expected that effects, if they occur, are expected to be mild. Furthermore, it is noted that propylene glycol could not be detected in the exhaled air of 13 of the 17 volunteers. Further, bystander exposure to nicotine might result in adverse health effects such as increased heart rate and increased systolic blood pressure for the child in scenario 1. In addition, vaping of e-cigarettes might result in increased indoor air concentrations of tobacco-specific nitrosamines. Therefore, increased incidences of tumours in the respiratory tract upon exposure to tobacco-specific nitrosamines cannot be excluded for the child in scenario 1.

Scenario 2 resembled part of a working day at an office during which an adult was exposed to exhaled chemicals of an e-cigarette vaper. For this scenario, adverse health effects such as increased heart rate and increased systolic blood pressure upon nicotine exposure cannot be excluded for the bystander. With respect to the tobacco-specific nitrosamines, no firm conclusion was possible. The MOE value for the nitrosamines for scenario 2 was such that refinement of the MOE calculation may lead to the conclusion of 'of low concern' but this cannot be stated with sufficient certainty.

It should be noted that current worst-case risk assessment points towards the potential risks for the bystander of e-cigarette vaping, though no firm conclusions can be drawn. The level of the indoor air concentrations, and subsequently the resultant health risk, are highly dependent on the number of persons vaping, the puff-frequency, the total vaping time, the volume of a room, and the extent of ventilation. Further, the absolute amount of a chemical as measured in the first exhaled air of the volunteers was used to calculate air concentrations resulting from exhalation of inhaled e-cigarette vapour. Due to the variable composition of the e-liquids, the amount of a chemical in the first exhalation is dependent on the selected e-liquids (see section 9.2). It should be noted that all selected e-liquids in the current study were nicotine-containing e-liquids. Obviously, vaping of nicotine-free e-liquids does not pose a risk for the bystander with respect to nicotine. The presence of tobacco-specific nitrosamines in the vapour is also strongly dependent on the type of e-liquid. Tobacco-specific nitrosamines are related to the presence of nicotine and/or tobacco-extract in the e-liquids. Vaping of nicotine-free e-liquids without tobacco flavor will not pose a risk for the bystander with respect to tobacco-specific nitrosamines

Finally, the vaping pattern of the volunteers (*i.e.,* puff volume, puff interval, shallow or deep inhaling, volume of exhaled air), showing inter- and intraindividual variation (see chapter 8), affected this measured amount in the exhaled air and that was used for risk assessment.

11 Discussion

In light of the pronounced differences in the composition of FHA and SHA, observed by us and others (23) it is clear that any evaluation of the exposure of bystanders should be based on SHA. It is currently still unclear to what degree individual differences in vaping and breathing behavior affect the extent to which different components of the aerosol will be retained in the respiratory tract of the user, and consequently how much remains in the exhaled aerosol.
However, in the subjects tested in this study, which exhibited a large degree of variability in their vaping and exhalation behavior, the amount of nicotine and PG recovered in the first exhalation after taking a puff was never more than 5% of the inhaled amount with median values of 0.16% and <LOQ respectively. Although additional quantities will be exhaled during subsequent breathing due to the residual volume of the lung, these results strongly suggest that a large fraction of SHA components are retained in the respiratory tract of the e-cigarette users, reducing the exposure of bystanders. This is particularly relevant in the case of e-cigarettes, because in contrast to normal cigarettes they are only active when users take a puff (i.e. there is no side stream smoke). The concentrations to which bystanders are exposed are further reduced due to dilution of the aerosol into the surrounding air.
To account for the variation in the above and other parameters, the risk assessment was based on the most extreme (worst-case) values that were experimentally observed. This ensures that the chosen parameters are realistic and reduces the likelyhood of underestimating the health risks. A few limitations to the approach followed in this report should be noted. Firstly, the sample size was not identical for all measurements, and it is more likely that a more extreme value is observed in larger datasets. This is not a concern when the variability in the data is small, such as for aldehydes which were not observed in SHA in amounts that exceed naturally occurring amounts in control breath, but it should be considered for data in which the variability is high, for instance in amounts of exhaled PG which only occurred in detectable amounts in the SHA produced by 4 out of 17 subjects, with the amount exhaled by one subject being more than 4-fold higher than the second-highest value.

The risk assessment was performed for two predefined relevant, realistic worst-case scenarios (outlined in section 10.2.1) Briefly, scenario 1 resembled an daily car trip during which a child is exposed to aeorosol exhaled by two e-cigarette users. For this scenario, it cannot be excluded that local effects on the respiratory tract upon exposure to propylene glycol will occur for the bystander of e-cigarette vaping. However, it is expected that effects, if they occur, are mild. Furthermore, it is noted that propylene glycol could not be detected in the exhaled air of 13 of the 17 volunteers . Further, bystander exposure to nicotine might result in adverse health effects such as increased heart rate and increased systolic blood pressure for the child in scenario 1. In addition, vaping of e-cigarettes might result in increased indoor air concentrations of tobacco-specific nitrosamines. Therefore, increased incidences of tumours in the respiratory tract upon exposure to tobacco-specific nitrosamines cannot be excluded for the child in scenario 1.
Scenario 2 resembled part of a working day at an office during which an adult was exposed to exhaled chemicals of an e-cigarette vaper. For this scenario, adverse health effects such as increased heart rate and increased systolic blood pressure upon nicotine exposure cannot be excluded for the bystander. With respect to the tobacco-specific nitrosamines, no firm conclusion was possible. The MOE value for the nitrosamines for scenario 2 was such that refinement of the MOE calculation may lead to the conclusion of ‘of low concern’ but this cannot be stated with sufficient certainty.

It should be noted that current worst-case risk assessment points towards the potential risks for the bystander of e-cigarette vaping, though no firm conclusions can be drawn. The level of the indoor air concentrations, and subsequently the resultant health risk, are highly dependent on the number of persons vaping, the puff-frequency, the total vaping time, the volume of a room, and the extent of ventilation. Further, the absolute amount of a chemical as measured in the first exhaled air of the volunteers was used to calculate air concentrations resulting from exhalation of inhaled e-cigarette vapour. Due to the variable composition of the e-liquids, the amount of a chemical in the first exhalation is dependent on the selected e-liquids (see section 9.2). It should be noted that all selected e-liquids in the current study were nicotine-containing e-liquids. Obviously, vaping of nicotine-free e-liquids does not pose a risk for the bystander with respect to nicotine. The presence of tobacco-specific nitrosamines in the vapour is also strongly dependent on the type of e-liquid. A previously conducted analysis of 183 e-liquids indicated that only a small fraction of e-liquids contain significant levels of TSNAs, all of which were tobacco-flavoured. No correlation with their nicotine content was found. Vaping of TSNA-free e-liquids without tobacco flavor will not pose a risk for the bystander with respect to tobacco-specific nitrosamines
Finally, the vaping pattern of the volunteers (*i.e.,* puff volume, puff interval, shallow or deep inhaling, volume of exhaled air), showing inter- and intraindividual variation (see chapter 8), affected this measured amount in the exhaled air and that was used for risk assessment.
In addition to the components of the aerosol considered in this study, e-cigarettes typically contain flavorants, preservatives and may contain contaminants that have not been identified. For these components, there is currently not enough data available to permit a risk analysis, and future work in this area seems warranted given the continued popularity of e-cigarettes.

12 Conclusions

Prevalence for daily e-cigarette use in the Dutch population amounts to approximately 1.4%. Of daily users, approximately 78% use a liquid containing 6 mg/ml of nicotine or more.
Our results confirm that considerable individual variation in vaping behavior exists even between experienced users (15, 17, 32, 33).
Our results indicate that a large fraction of the harmful components that occur in e-cigarette aerosol is retained in the respiratory tract of the user. This is of importance for evaluating the exposure of bystanders to harmful components that occur in e-cigarette aerosol, because in contrast to normal tobacco cigarettes, e-cigarettes do not produce side-stream smoke. Bystanders are therefore only exposed to aerosol that is first inhaled by the user and subsequently exhaled into the surrounding air (SHA).
The risk assessment was performed for two predefined relevant, realistic worst-case scenarios (outlined in section 10.2.1) Briefly, scenario 1 resembled an daily car trip during which a child is exposed to aeorosol exhaled by two e-cigarette users. For this scenario, it cannot be excluded that local effects on the respiratory tract upon exposure to propylene glycol will occur for the bystander of e-cigarette vaping. However, it is expected that effects, if they occur, are mild. Furthermore, it is noted that propylene glycol could not be detected in the exhaled air of 13 of the 17 volunteers . Further, bystander exposure to nicotine might result in adverse health effects such as increased heart rate and increased systolic blood pressure for the child in scenario 1. In addition, vaping of e-cigarettes might result in increased indoor air concentrations of tobacco-specific nitrosamines. Therefore, increased incidences of tumours in the respiratory tract upon exposure to tobacco-specific nitrosamines cannot be excluded for the child in scenario 1.
Scenario 2 resembled part of a working day at an office during which an adult was exposed to exhaled chemicals of an e-cigarette vaper. For this scenario, adverse health effects such as increased heart rate and increased systolic blood pressure upon nicotine exposure cannot be excluded for the bystander. With respect to the tobacco-specific nitrosamines, no firm conclusion was possible. The MOE value for the nitrosamines for scenario 2 was such that refinement of the MOE calculation may lead to the conclusion of ‘of low concern’ but this cannot be stated with sufficient certainty. Furthermore, most e-liquids do not contain only negligible quantites of TSNAs.
However, the risks are strongly dependent the exposure conditions, such as the vaping pattern, room dimensions, ventilation, duration of exposure, etc, which are highly variable.
In addition to the components of the aerosol considered in this study, e-cigarettes typically contain flavorants, preservatives and may contain contaminants that have not been identified. For these components, there is currently not enough data available to permit a risk analysis, and future work in this area seems warranted given the continued popularity of e-cigarettes.

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14 Risk assessment according to the Margin of Exposure (MOE) approach

Following the MOE-approach, the estimated human exposure to a chemical will be compared with relevant information on the chemical's potency of inducing adverse health effect (*i.e.,* the Point of Departure, PoD). The MOE is calculated as follows:

$$MOE = \frac{PoD}{\text{Human exposure estimate}}$$

A PoD is preferably derived from results of a human study (for example an epidemiologic study), though this is in most cases not available. Therefore, a PoD is in most cases derived from results from experimental animal studies. A PoD may be a No-Observed-Adverse-Effect Level (NOAEL), a Lowest-Observed-Adverse-Effect Level (LOAEL) or a Benchmark dose (BMD). In order to obtain and select relevant information on possible adverse human health effects of the analyzed chemicals, reports and evaluations of (inter)nationally recognized organizations (among others WHO, US EPA, ATSDR, AEGL, the Health Council of the Netherlands) were used as primary sources. It is important that the exposure profile in the study that serves as basis for the PoD corresponds as much as possible with the human exposure. In case large differences are present, this should be accounted for when evaluating the MOE.

Evaluation of the MOE for non-carcinogenic chemicals

The minimal value of the MOE is mainly based on factors for interspecies extrapolation (rat to human) and interindividual variability in sensitivity among bystanders. Factors that additionally determine the minimal value of the MOE are less-than-lifetime exposure for the PoD and the use of a LOAEL instead of a NOAEL as PoD. Further, a distinction is made between systemic effects and local effects on the respiratory tract. Default values for these factors are described in ECHA (2012).

The calculated MOE should be at or above the minimal value of the MOE in order to conclude that no adverse health effects are to be expected. The smaller the calculated MOE, the higher the risk for adverse health effects.

When evaluating the calculated MOE, differences in exposure profile between animal or human study on the one hand and (daily) exposure of the bystander on the other hand should be taken into consideration. The daily exposure duration in an experimental or epidemiologic setting is usually a continuous number of hours, for example 6h in an animal experiment. Exposure of a bystander will, in general, be shorter than 6h; this will among others depend on the vaping behaviour of the vaper. It should however be kept in mind that in an animal experiment, but also occupational epidemiologic studies, exposure is applied during 5 days per week, while vaping can be done daily.

Finally, specific for current evaluation, the fact that the risk assessment was based on assuming (worst-case) zero pulmonary absorption (in case of local effects on the respiratory tract) or using a default absorption fraction leading to a worst-case systemic exposure estimate (in case of systemic effects) and using worst-case the concentration of a chemical present in the room at the *end* of the vaping period should be accounted for when evaluating the MOE.

Also the fact that the concentration of a chemical in the room will also change (*i.e.*, decrease) depending on the total number of persons present in the room. The more persons present in a room that inhale (and absorb) the chemical the lower the concentration and exposure of a single bystander. In case a chemical is absorbed, the exposure can roughly be equally divided over the number of people present in the room. In the present MOE calculations, it is assumed that only a single bystander is exposed to the total amount of chemical exhaled by (a) vaper(s). The presence of multiple persons is considered in the evaluation of the MOE.

Evaluation of the MOE for carcinogenic chemicals without treshold.

For carcinogenic chemicals without threshold, preference is given to the use of a BMDL10 as PoD. EFSA states that in general a MOE of 10,000 or higher, if it is based on a BMDL10 from an animal study, would be "of low concern", *i.e.,* the tumour risk is very low. The factor of 10,000 considers interspecies differences, interindividual variability, the nature of the carcinogenic process, and the reference point on the dose-response curve. This approach will be applied in current risk assessment. In case the PoD is a parameter different than the BMDL10, this should be accounted for when evaluating the MOE.

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15 Appendix B. Overview of the available animal studies for nicotine

(based on ACGIH (1994) and The Health Council of the Netherlands (2005))

A- animal studies

Species + sex	Exposure route	Exposure duration	Dose or concentration	Effects observed
rat (f)	inhalation	20 h/d, 5d/wk, during 2 year	0.5 mg/m^3	Reduced bodyweight (5%); whether this is statistically s
rat (m)	oral (via drinking water)	34 wk	1.14 and 4.56 mg/kg bw/d	Minimal biochemical changes muscle a high dose level
rat (f) (young and adult animals tested)	oral (via drinking water)	131 d	1.5 and 3.8 mg/kg bw/d (young animals) 1.1 and 2.8 mg/kg bw/d (adult animals)	Reduced bodyweight Effect on behaviour (such as locomotor activity)
mouse (m)	oral (via drinking water)	50 d	60-65 mg/kg bw/d (the nicotine dosing was gradually increased during the first 3 weeks)	Effects on behaviour (such a locomotor activity), increase concentrations of monoamin were reversible within 12-24 dosing on day 50
rat (m)	Subcutaneous via mini-osmotic pump	3 wk	1 mg/kg bw/d	Immunological effects (inhib Concavalin A-induced prolife periferal blood and spleen ce
muis (f)	Subcutaneous	2^e or 3^e tremester of gestation	2.7 mg/kg bw/d	Reduced gestational period Maternal toxicity not describ
rat (f)	Subcutaneous	GD 4-20	6 mg/kg bw/d	Reduced bodyweight dams a Reduced brain development

Species + sex	Exposure route	Exposure duration	Dose or concentration	Effects observed
				Maternal toxicity not describ
rat (f)	dermal (via transdermal patches)	GD 2-19 or GD 2-7	0 – 1.75 – 3.5 mg/d	Abortus at both experimenta Fetuses not evaluated Maternal toxicity not describ
rat (f)	oral (via drinking water)	6 wk before gestation and during gestation	Nicotine dosing is gradually increased during the first 3 weeks up to 6 mg/kg bw/d	Reduced number of male pu Reduced body weight male p Reduced locomotor activity r No effects on female pups Maternal toxicity not describ
rat (v)	oral (via drinking water)	1 wk before gestation, during gestation, during lactation	0 – 2.4 – 4.5 mg/kg bw/d	Reduced body weight and wa consumption during lactation dams Reduced number of pups pe Reduced pup body weight du day 20, 30 and 40
rat (f)	oral (gavage)	GD1-21	0 and 3 mg/kg bw/d	Reduced body weight and fo consumption with dams Reduced birth weight pups a litters (not significant)
muis (f)	oral (via drinking water)	Minimal 2wk before gestation, during gestation	0 – 5.7 – 17.2 – 28.6 mg/kg bw/d	Reduced placental weight Reduced fetal weight Maternal toxicity not describ
muis (f)	oral (gavage)	GD8-12	0 and 35 mg/kg bw/d	Maternal toxicity: overt signs reduced bw, lethality ratio of Number of pups per litter, liv postnatal day 1-3, pup weigh postnatal day 1-3 were sligh though no statistically signifi
rat (f)	subcutaneous	GD1-20	0 and 0.5 mg/kg bw/d	Pups: reduced pup bw, delay opening postnatally, decreas motor reflexes stimulation

Species + sex	Exposure route	Exposure duration	Dose or concentration	Effects observed
				Early adulthood: stimulation activity
rat (f)	Subcutaneous via mini-osmotic pump	GD6-12	0 and 3.6 mg/d Both pair-fed as well as untreated control animals were included in this study	Increased incidence of ossifi sternae and skull when comp pair-fed controls Maternal toxicity not describ
rat (f)	Subcutaneous via mini-osmotic pump	GD6-12	0, 1.8 and 3.6 mg/d Both pair-fed as well as untreated control animals were included in this study	Delayed fetal development (brain, ear and eyes, hind lim nicotine dose Maternal toxicity not describ
Rhesus-monkey (f)	Subcutaneous via mini-osmotic pump	GD26-134	0 and 1 mg/kg bw/d	Fetal effects: Reduced bw (8%) Reduced organ weight (hear adrenals, kdiney, brain) Reduced biparietal, crown-ru Reduced bw Reduced lung volume (not si Lung development affected (hypoplasia, reduced surface developing lung)
Mouse (m)	oral (via drinking water)	7 wk before gestation 20 wk before gestation	0 and 2.7 mg/kg bw/d 0 and 2.3 mg/kg bw/d	Increased incidences of limb in fetuses resulting from ma treatment week 1 and 2 of m for 20 weeks premating
Mouse (m)	intraperitoneal	15 d	0, 2, 4, 6 mg/kg bw/d	Reduced relative weight of t epididymis, seminal vesicle, vas deferens. Reduced numb spermatocytes and spermati

B- human studies

Study subjects	Exposure route	Dose or concentration	Exposure duration	Effects observed
Non-smokers	inhalation	Single-breath inhalation of 0.1 ml of a	Single-breath inhalation	Concentration-dependent co response and broncho-const

Study subjects	Exposure route	Dose or concentration	Exposure duration	Effects observed
		nebulized nicotine solution (0, 1, 2, 4, 8, 16, 32 or 64 mg/mL)		
Non-smokers	inhalation	Single-breath inhalation of 0.01 ml of a nebulized nicotine solution (0, 2, 4, 8 mg/mL) per 15 sec during 5 min (21 single-breath inhalations, resulting in a dose of 0, 0.42, 0.84 or 1.68 mg nicotine/5 minutes)	Repeated single-breath inhalations, 1 inhalation/15 sec, during total period of 5 min	Dose-dependent increase in and systolic blood pressure
Not specified	intravenous	0,6 mg	-	Small to moderate increase breathing frequency, heart r pressure; nausea
Not specified	intravenous	3 mg	-	Increased blood pressure an rate in 8 volunteers; an initi of the heart was produced in volunteers; nausea

References appendix B:

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Health Council of the Netherlands (2005). Nicotine (CAS no: 54-11-5). Health-based Reassessment of Administrative Occupational Exposure Limits (Revised version). Committee on Updating of Occupatio